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Advances in Probiotics for Sustainable Food and Medicine

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Advances in Probiotics for Sustainable Food and Medicine

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Preface

Advances in Probiotics for Sustainable Food and Medicine is a blend of fundamentals as well as the applied aspects of probiotic science. As we all understand the complexity of human gastrointestinal tract whereby the microbial niches play various important roles in human physiology and health status, such as digestion of nutrients from food, protection from pathogens, etc. The reported and claimed health benefits of the beneficial group of microorganisms in the gut have seen a plethora of research activities in this area focusing on diet–microbe interaction and their role in maintaining gut homeostasis. In the past decades, the development of novel screening and analytical methods for the evaluation of probiotics and their safety necessitates the understanding of fundamental as well as applied aspects of probiotics for human consumption. The book entails the fundamental aspects of probiotics and their evaluation, selection of sources of probiotics such as human, animal, and plant followed by their critical evaluation using *in vitro* or *in vivo* studies in animal models. The applicability of probiotics at the commercial level and delivery mechanisms of the probiotics based on traditional or processed cereal or dairy-based fermented foods have also been presented. The reported health claims of probiotics for all age groups have also been described for their applications as therapeutics.

The editors of the book feel fortunate to have the input of renowned collaborators to provide unbiased and the latest information in the field of probiotics to its readers. We have compiled the chapters written by various experienced researchers working in the area of probiotics and related aspects. The information provided in this book is worth of interest in the areas of nutraceuticals and functional foods along with the traditional health-promoting probiotics. The book will serve as a suitable reference book for the food, pharmaceutical, nutraceutical industries, food regulatory authorities who are keen to get the most recent advancements in the area of probiotics and prebiotics. The book will serve as a unique reference for food science professionals pursuing their work in functional food development, marketing expansion of already designed functional foods, as well as nutritional dietary management. The readers will obtain sound scientific knowledge of the nutritional value and health benefits of the probiotic cultures and their products.

The first four chapters will introduce the various fundamental aspects of probiotics and their health benefits. Starting from the source and screening of probiotics, the authors have discussed the evaluation criteria in detail to classify any microorganism as a potential probiotic candidate. The next chapter details about the traditional indigenous foods which can serve as a potential reservoir for probiotics. Further to enhance the proliferation of probiotics, the role of prebiotics was also described in the fourth chapter. The next section of the chapters includes the application of probiotics as therapeutics. We have covered the applications of probiotics in humans as well as animals. The presented chapters described how probiotics can be valorized in the development of safer functional foods. The last section of the book details two chapters on AMPK-dependent health activity of probiotics and further high-level research in the metabolic engineering of probiotic strains for their use as therapeutics.

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Waknaghat, India Ashok Kumar

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About the Editors

Gunjan Goel is an Associate Professor at the Department of Microbiology, Central University of Haryana, Mahendergarh, India. He has completed his Ph.D. in Microbiology at the National Dairy Research Institute (ICAR), Karnal, India, focusing on the microbial degradation of polyphenols. During his postdoctoral research at the University of Hohenheim, Germany and the University of Ghent, Belgium, he studied the effects of plant secondary metabolites in enhancing the functions of rumen microbes, as well as metabolic pathways of fatty acids. He has published number of research papers in journals of international repute. His current research focuses on the microbial ecology of gut, bioaccessibility and bioavailability of food ingredients in in vitro simulated gut model systems, and the assessment of probiotics using in vitro and in vivo models. He is life member of the Association of Microbiologists of India (AMI) and the Probiotic Association of India (PAI).

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Chapter 1
Probiotics and Their Potential Applications: An Introduction

Sampan Attri, Niharika Singh, Ashok Kumar Nadda, and Gunjan Goel

Abstract Probiotics constitute beneficial microorganisms or their components reported for their health benefits to the hosts. A plethora of research conducted over the past three decades resulted in elucidating the association of probiotic strains under different clinical manifestations. Although several mechanisms have been reported behind these beneficial activities of these strains, the biological activity is still strain specific which needs further investigation using suitable animal models and clinical trials. This chapter reviews the details on different genera used as probiotic, their potential health benefits and mechanisms behind these effects. The formulation of different dairy or non-dairy products and pharmaceutical formulations has also been discussed.

Keywords Probiotics · Prebiotics · Probiotic products · Intestinal health

1.1 Introduction

In the year 2001, expert team members of the WHO (World Health Organization) and FAO (Food and Agriculture Organization) defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the

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host". The term "probiotics" was derived from the Greek word which means "for life" (Jobby et al. 2020). The most commonly used bacterial genera belong to the group of lactic acid producing bacteria (LAB), mainly the Lactobacillus, Bifidobacterium, Enterococcus and Streptococcus. The common reservoir of these beneficial probiotic microbes are reported to be the traditional fermented dairy products such as kefir, lassi, Maasai milk, curd, buttermilk or kurut, etc. (Ezzatpanah 2020). Probiotics have been reported for an array of therapeutic properties such as enhancement of immune defence system, lowering of serum cholesterol prevention of colonic cancer, gastrointestinal and urinary infections, treatment of atherosclerosis, arteriosclerosis, rheumatoid arthritis, atopic dermatitis and others (Diez-Gutiérrez et al. 2020).

The use of beneficial microorganisms to improve human health is in fact very ancient; several reports mentioned the use of fermented milk for treatment of gastrointestinal tract disorders. In India, live microorganisms and fermented food stuff were and are still used for their beneficial effects on human health since pre-*Vedic* and *Vedic* period. Moreover, Ayurvedic traditional system of Indian medicine recommended *Dahi* (curd, a fermented milk product) for its beneficial effect against diarrhoea much before the microorganism's existence was even accepted (Singhi and Baranwal 2008). During the twelfth century period, fermented milk was consumed as source of good health and strength by the people of Mongolia kingdom under Genghis Khan. At the beginning of the twentieth century, Noble Prize winner Elie Metchnikoff correlated the life span of Bulgarians to their high rate of consumption of fermented milk and suggested that maybe all microorganisms were not dangerous for human health. The benefits of these microbes were suggested as they were able to compete with pathogenic microbes due to production of lactic acid from sugars through fermentation by lactic bacteria. Metchnikoff also suggested autotoxins derived from putrefactive bacteria in the colon turn macrophages into phagocytes and these putrefactive microbes could be prevented by replacing them with good bacteria. In the year 1906, French paediatrician, Henry Tissier reported that "bifid" Y-shaped Gram +ve bacteria were dominant in the stool samples of healthy infants as compared to the stool samples of diarrhoeal infants. In fact, the first time term "probiotics" was coined by Lilly and Stillwell in 1965 and stated them as "substances produced by bacteria that promote the growth of other bacteria" (Setta et al. 2020; Lilly and Stillwell 1965).

Probiotic products are categorized under different categories in different countries such as natural health promoting products (Canada), dietary supplements, drugs, medical food, live biotherapeutic agent, biological agent (United States of America), functional foods (Japan, India, China and Malaysia), food supplement (Sweden, Finland and Denmark) and biotherapeutic/pharmaceuticals (European countries like Belgium and Germany). The development and maintenance of each of these probiotic products is complex depending on the application of the product, which requires special protocols for manufacturing, labelling and safe delivery. Besides these potential technological hurdles, legislative and legal aspects, as well as consumer demands need to be taken into consideration while development of probiotic based functional food or pharmaceuticals. For example, in case of developing country such as India, a Task Force was constituted by DBT (Department of Biotechnology) and ICMR (Indian Council of Medical Research) Government of India, New Delhi, comprising of experts from concerned fields to develop guidelines for assessment of probiotics products in India. An attempt has been made by ICMR/DBT to frame the guidelines for probiotic ingredients in food. The guidelines provide update information on requirements for evaluation of safety and efficacy of the probiotic culture, health claims and labelling of probiotic products (Mallappa et al. 2019).

1.2 Classification: Probiotics, Prebiotics, Synbiotics and Postbiotics

During the last two decades, a plethora of research articles have appeared in journals targeting metabolic capabilities and probiotic attributes of bacteria belonging to group of Lactic acid bacteria. The ideal characteristics of probiotic microorganisms are depicted in Fig. 1.1.

1.2.1 Probiotic

The probiotic was first defined by Lilly and Stillwell in 1965 to describe “substances secreted by one microorganism which stimulates the growth of another microorganism”. This generalized claim was used to define probiotics later on. Further in 1971, the term was used by Sperti to the tissue extracts that stimulate microbial growth

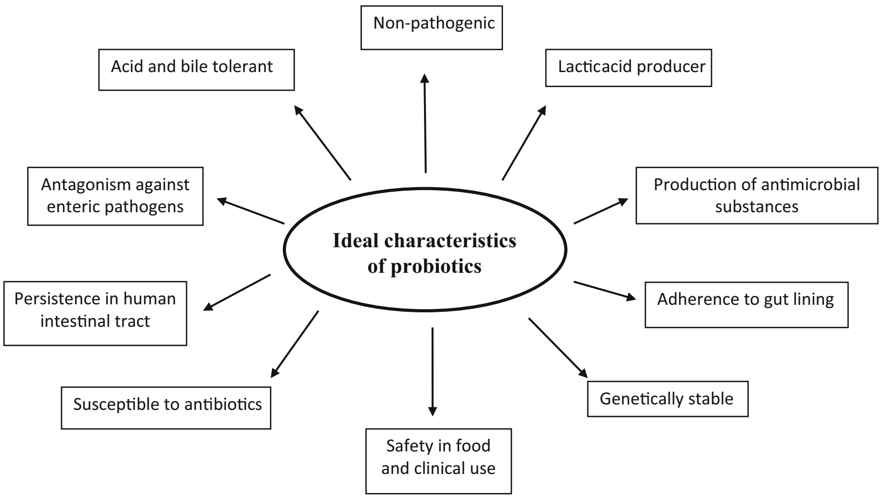

Fig. 1.1 Prerequisites of an ideal probiotic organism

(Sperti 1971). However, it was Parker who used the term probiotic as live microbes or their substances which lead to intestinal microbial balance (Parker 1974). The fundamental characteristic of probiotics is to deliver "balance" to the growth and bioactivity of beneficial intestinal microbiota, whilst reducing adverse ones. Probiotics mainly focus on improving the host digestive and immune systems as their essential characteristics but the distinctive health benefits, such as the production of bioactives, are strain-specific (Mohan et al. 2017; Hill et al. 2014). To be an active probiotic, a daily dose of approximately 10^8–10^9 colony-forming units (CFU) is recommended during passage through GI tract. The major issue with the probiotics is the viability of the strain during the different production stages. The first generation probiotics used were in the form of live and/or lyophilized bacterial cells only without any protection, therefore, those probiotics were having lesser viability of 7–30%. Further the lyophilized probiotics were encapsulated to increase their survival up to 80% which were often referred as second generation probiotics; however, the release of probiotic strain was the major issue in having the biological activity. Therefore, the process was further improved to get third generation probiotics where the encapsulated probiotic strains are released in metabolic active stage when the microencapsule are destroyed. These probiotics were further improved to produce fourth generation probiotics, whereby bacterial cells exist in the form of biofilms enhancing its survival and viability.

Presently, different groups of microorganisms are used as probiotics possessing beneficial effects on the human health (Tables 1.1 and 1.2). Most of these bacterial groups belong to Lactic acid bacteria.

1.2.1.1 *Lactobacillus* Species

Lactobacilli are Gram-positive, non-spore forming and are the most commonly fermentative resulting in production of lactic acid as a major product. Based on

Table 1.1 Major group of microorganisms used as probiotic agents

Probiotic bacteria	Species
Lactobacillus	*L. rhamnosus, L. acidophilus, L. casei, L. bulgaricus, L plantarum, L. reuteri, L. paracasei, L. gasseri, L. amylovorus, L. helveticus, L. pentosus, L. johnsonii*
Bifidobacterium	*B. bifidum, B. lactis, B. longum, B. breve, B. infantis, B. adolescentis, B. animalis, B. catenulatum*
Bacillus	*Bacillus coagulans, B. subtilis, B. laterosporus, B. clausii*
Streptococcus	*S. thermophilus, S. faecium, S. oralis, S. salivarius, S oralis, S. sanguis, S. mitis*
Saccharomyces	*S. boulardii*
Others microorganisms	*Lactococcus lactis, Enterococcus faecium, Propionibacterium jensenii, Propionibacterium jensenii, Propionibacterium freudenreichii, Pediococcus acidilactici, Pediococcus pentosaceus, Bacteroides uniformis, Escherichia coli* Nissle 1917

Table 1.2 Commercial probiotic products and their functional properties (this is not an exhaustive list)

Name	Company name	Type	Strain	Functional properties
Yakult	Yakult Honsha	Dairy	*Lactobacillus paracasei* Shirota	Improve bowel and maintain a healthy population of gut flora
Activia	Danone	Dairy	*Bifidobacterium animalis* DN 173010	Improve digestive health
Bifiene	Yakult Honsha	Dairy	*B. breve*	Improve digestive system/gut health
Align	Procter and Gamble	Capsule and tablet	*B. infantis* 35624	Improve digestive system
Actimel	Danone	Dairy	*L. bulgaricus*, *S. thermophilus* and *L. paracasei* ssp. paracasei CNCM I-1518	Reducing the incidence of diarrhoea and rhinitis and improvement of the immune function
Bioflorin	Cerbios pharma	Capsule	*Enterococcus faecium* SF68	Prevention and treatment of gastrointestinal disorders
LC1	Nestle	Dairy	*L. acidophilus* La 1	Improve digestive health
GoodBelly multi probiotic	NextFoods	Fruit drink	*L. plantarum* 299 V	Promote digestive health
Mucilon	Nestle	Cereal based powder	*Bifidus BL*	Protection from pathogens in children digestive tract
Rela caps	BioGaia	Capsule	*L. reuteri* protectis	Prevention and amelioration in gut
Attune bar	Attune Foods	Food bar	*B. lactis* (LAFTI®B94), *L. acidophilus* (LAFTI® L10) and *L. casei* (LAFTI® L26) and	Support digestive health and immunity
Nexabiotics advanced multi probiotics	DrFormulas	Capsule	*L. brevis, L. bulgaricu, L. Casei, L. helveticus, L. acidophilus, L. paracasei. L. plantarum, L. rhamnosus, L. salivarius, L. lactis, Bacillus coagulans, B. breve, B. bifidum, B. infantis, B. lactis, B. longum, Pediococcus acidilactici* and *Saccharomyces boulardii*	Promote beneficial gut bacteria and immune system
Tropicana essential probiotics	PepsiCo	Fruit juice	*Bifidobacterium lactis* HN019	Healthy immune system and gut functionality

(continued)

Table 1.2 (continued)

Name	Company name	Type	Strain	Functional properties
LactoSpore	Sabinsa Corporation	Powder	*Bacillus coagulans* MTCC 5856	Beneficial in many gastrointestinal disorders
Kefir milk	Feel well	Dairy	*L. lactis*, *L. cremoris*, *L. diacetylactis* and *L. acidophilus*	Enhance gut health
ActiPlus probiotic dahi	Nestle	Dairy	*L. acidophilus*	Healthy digestive system
Vita biosa	Vital health	Herbal drink	*L. acidophilus, L. casei, L. rhamnosus, L. salivarius, Lactococcus lactis, B. lactis, B. longum* and *S. thermophilus*	Enhanced gut health and immunity
Koji	Graindrops	Dairy free beverage	*L. acidophilus, L. rhamnosus, L. casei, L. bulgaricus, S. thermophilus, B. bifidum* and *B. lactis*	Improve digestion and absorption of nutrients

the metabolic capabilities the species are classified as homofermentative or heterofermentative. The species of this genus are largely discovered in the human digestive and urogenital tracts, while so far there have been studies that their existence may fluctuate within a species, age of the host or the site within the gut. Presently, research data suggest that the Lactobacilli, which are used therapeutically, are considered as probiotics, the reverse of antibiotics. Moreover, they are looked as "friendly" microbes and have prospective implications in enhancing the bioavailability and uptake of minerals, reducing risk of cardiovascular disease and promoting balanced metabolism and proper weight (da Silva et al. 2015).

1.2.1.2 *Bifidobacterium* Species

Bifidobacteria are Gram-positive, non-spore forming bacteria belonging to the family Bifidobacteriaceae which metabolize glucose to generate lactic and acetic acids (Milani et al. 2017). Bifidobacteria spp. generally reside in the human gastrointestinal tract, insect and bird's intestine. Their utmost profusion during infancy of mammals is associated with their suggested character as an imperative microbial modulator of the immune response as well as the gut microbiota of the host (Turroni et al. 2018).

1.2.1.3 *Bacillus* Species

Bacillus coagulans belonging to the genus Bacillus is a Gram-positive spore forming bacterium, which blends both Bacillus and LAB attributes in its mechanisms. It is able to survive in high temperature of heat treatment and physiological conditions such as very low pH of the stomach and bile salts and directly affecting enteropathogens. Predominantly, this species is also recognized to maintain health and therapy by posing antagonistic effects on the pathogens (Wang et al. 2012). Apart from this species, *B. clausii* is also well documented for its probiotic attributes.

1.2.1.4 *Saccharomyces* Species

The non-pathogenic yeast strain *Saccharomyces boulardii* has also received attention worldwide as both therapeutic agent for gastrointestinal and other diarrhoea disorders caused by the administration of antimicrobial agents. *S. boulardii* possesses many therapeutic properties that make it as a potential probiotic agent, i.e., its survival during transit through the gut lumen, its optimum temperature is 37 °C, both *in vitro* and *in vivo* conditions and antimicrobial action.

1.2.2 Prebiotic

In 1995, the first published definition of prebiotic was provided by Glenn Gibson and Marcel Roberfroid as a "non-digestible food component that beneficially affects the host by selectively activating the growth and/or activity of one or a partial number of microorganisms in the colon, and consequently improves host health" (Gibson and Roberfroid 1995). Bindels et al. (2015) suggested another explanation of a prebiotic as "a non-digestible compound that confer a beneficial physiological effect on the host, through its utilization by gut microbiota, modifies composition and/or activity of the gut microbiota". They also recommended that non-carbohydrate compounds may serve as prebiotics and supplemented the subsequent prebiotics to the normal list: pectin, resistant starch, whole grains, arabinoxylan, several dietary fibres and non-carbohydrates that employ their act through a modulation of the microorganisms in the gut. Further in the year 2016, an expert advisory panel member convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP) customized this to "a substrate that is selectively used by host microbiota conferring health benefits" (Gibson et al. 2017). A clear definition of selectivity is viewed as fundamental to the prebiotic approach; in disparity to fibres, that is, pectins, cellulose and xylans, which promote the proliferation of numerous microbes in the gut principally *Bifidobacterium* and *Lactobacillus* and prebiotics such as galacto-oligosaccharides and fructooligosaccharides.

An ideal prerequisite for a substrate to be a prebiotic should include the following:

- Resistance to gastric environment.
- It should not be absorbed in the upper gastrointestinal tract.
- Resistance to action of mammalian enzymes.
- Fermentation by the intestinal microflora.

1.2.2.1 Types of Prebiotics

The prebiotics are group of diverse nutrients of various molecular structures that naturally exist in different dietary products consumed in our everyday life.

They are generally composed of linked sugars like short and long chain β-fructans, galacto-oligosaccharides and xylo-oligosaccharides (Tables 1.3 and 1.4). These polysaccharides have the chemical characteristic of being non-digestible by the enzymes present in the gastrointestinal tract. (Brosseau et al. 2019)

Fructans Fructans such as fructooligosaccharides (FOS) and inulin are reported widely for prebiotic activities. These substrates possess linear chain of fructose with β-2,1 bond (Brosseau et al. 2019). The literature reported on fructans indicates that these can selectively stimulate the proliferation of probiotic bacteria. These fructans are found naturally in foods, such as asparagus, artichoke, leeks, wheat, chicory, onions, bananas, honey and garlic.

Galacto-Oligosaccharides Galacto-oligosaccharides (GOS) are enzymatic product of lactose formed by transglycosylation. The GOS generally consist of primarily a combination of oligosaccharides from tri- to pentasaccharide with galactose in β-1,6, β-1,3 and β-1,4 linkages (Ranucci et al. 2018). This kind of GOS is also known as trans-galacto-oligosaccharides or TOS. GOSs are reported for their prebiotic activity for Bifidobacteria and Lactobacilli with a lesser activity towards Bacteroidetes and

Table 1.3 Commonly used prebiotics and their natural sources

Prebiotics	Natural sources
Fructooligosaccharides	Asparagus, chicory, onion, garlic, wheat, leek, Jerusalem artichoke, oat
Inulin	Burdock camas, Globe artichoke, banana, elecampane, costus, agave, chicory, coneflower, dandelion, garlic, Jerusalem artichoke, jicama, onion, wild yam
Isomalto-oligosaccharides	Soy, sauce, miso, sake, honey
Galacto-oligosaccharides	Human milk, kidney bean, chickpea, lentil, lima bean, green pea
Xylose-oligosaccharides	Fruits and vegetables, bamboo shoot, milk, honey
Lactulose	Skimmed milk

Table 1.4 Commercial prebiotic products and their functional properties (this is not an exhaustive list)

Name	Type	Ingredients	Functional properties
Natural stacks prebiotic+	Powder	Potato starch, green banana flour and inulin	Improves your digestion, metabolism and gut health
Hyperbiotics prebiotic	Powder	Acacia fibre, Jerusalem artichoke and green Banana flour	Improve gut health
DrTobias prebiotics	Capsule	LH01-*Myoviridae*, LL5-*Siphoviridae*, T4D-*Myoviridae* and LL12-*Myoviridae*	Support beneficial bacteria
DrFormulas nexabiotic prebiotic	Tablet	Organic blend of Alfalfa grass, barley grass, broccoli, spinach, kale, cabbage cauliflower, brussel sprouts, apple, carrot, beet, tomato, strawberry, tart cherry and garlic	Enhance gut bacterial composition, nutrient absorption, immune system and reduces constipation
Prebiotin prebiotic	Tablet and powder	Oligofructose and inulin derived from chicory root	Support a healthy microbiome and immunity
Goodgut balance	Capsule	Japanese honeysuckle, pomegranate, grape, kiwi, green tea, apple, bilberry, blueberry, chokeberry, clove, cranberry goji berry and mangosteen	Maintain digestive balance and nourish gut bacteria
Enzymedica prebiotic	Powder	Dietary fibre, guar fibre, tapioca fibre, acacia gum, galacto-oligosaccharides, isomalto-oligosaccharides and fermented barley grass	Improve digestion and nourishes gut microbiota
Benefiber	Powder	Wheat dextrin	Nourishes healthy gut bacteria
Sanitarium so Good Soy prebiotic	Soy milk based drink	Corn maltodextrin and prebiotic fibre (chicory root extract)	Enhances gut health

other probiotics. Lactulose, an isomer of lactose, is also known GOS reported as potential prebiotics used in the therapy of constipation and hepatic encephalopathy.

Other Saccharides Resistant starch (RS) is a category of starch which is resistant to digestion in the upper gut. RS is generally fermented by colonic bacteria and results in production of prominent amount of butyrate; therefore referred as a potential prebiotic substrate. Various studies reported the degradation of RS by fibre utilizing bacteria such as *Bifidobacterium adolescentis* and *Ruminococcus bromii*, and also to a lesser extent by *Eubacterium rectale* and *Bacteroides thetaiotaomicron*.

Polydextrose, polymer of glucose-derived oligosaccharide, is a food ingredient that is indigestible by intestinal enzymes and thus possibly affects colonic function (Duncan et al. 2018).

Non-carbohydrate Compounds Although carbohydrates are more likely to conform to the criteria of prebiotics definition, there are few compounds that are not classified as carbohydrates, but are recommended to be classified as prebiotics, such as cocoa-based flavanols, polyphenols in berries.

1.2.2.2 Mechanisms of Action of Prebiotics

Prebiotics are reported to stimulate the growth of beneficial bacteria and antagonistic to pathogenic group of bacteria. Prebiotics not only aid as an energy source via production of short chain fatty acids (SCFAs) but also have various health benefits such as decreasing the occurrence and extent of diarrhoea, employing protective effects to avert colon cancer, decreasing gut inflammation and other problems related with intestinal bowel disorder. Moreover, prebiotics are also involved in boosting the bioavailability of minerals, dropping some risk issues for cardiovascular disease and endorsing weight loss and satiety.

1.2.3 Synbiotic

The development in research related to probiotic and prebiotics has driven to formation of term synbiotics which is a selective fusion of both probiotics and prebiotics. The synbiotic action of probiotic or prebiotic on the beneficial effect on human health is supposed to be more dynamic as comparison to the effect of probiotic or prebiotic alone (Kerry et al. 2018). The synbiotic strategy positively enhances the implantation and survival of live bacterial dietary supplements in to gastrointestinal tract.

Most frequently used synbiotic blends comprise of Bifidobacterium, Lactobacilli, *B. coagulans*, *S. boulardii*, as probiotic constituent, and oligosaccharides and inulin as prebiotic constituent (Tables 1.5 and 1.6) (Pandey et al. 2015). The health promoting effects of synbiotic preparation include the following:

- Improved proliferation of bifidobacteria and lactobacilli besides balanced gut microbiota.
- Prevent microbial translocation and limiting pathogenic colonization in surgical patients.
- Improved functioning of liver in cirrhotic patients.
- Enhancement of immunomodulating capability (Zhang et al. 2010).

Table 1.5 Commonly used Synbiotic preparations for human nutrition

Prebiotics	Probiotics
Fructooligosaccharides	*Bacteroides fragilis, Peptostreptococcaceae,* Bifidobacteria, *Klebsiellae*
Inulin	*L. acidophilus, B. animalis, L. paracasei*
Isomalto-oligosaccharides	*Bacteroides fragilis,* Bifidobacteria
Galacto-oligosaccharides	*B. longum, B. catenulatum*
Xylose-oligosaccharides	*B. adolescentis, L. plantarum*
Lactulose	*L. bulgaricus, L. acidophilus, B. lactis, L. rhamnosus*

1.2.4 Postbiotics

Postbiotics are defined as functional bioactive components generated during microbial fermentation process that trigger the beneficial effects on the host (Collado et al. 2019). Postbiotics consist of peptides, bacterial enzymes, peptidoglycan-derived neuropeptides and organic acids, for instance, acetic acid and lactic acid. The postbiotics exhibit therapeutic effects, *viz.* anti-inflammatory, immunomodulatory and anti-bacterial properties, anti-hypersensitive, anti-proliferative and hypocholesterolemic activity (Tomasik and Tomasik 2020). The metabolic by-products from postbiotic, like lipoteichoic acid, could have an anti-inflammatory effect, prompting the production of cytokines IL-4, IL-6 and IL-10, and reducing the production of reactive oxygen species (ROS) (Nakagawa and Miyazaki 2017). In addition, heat-killed probiotic preparations possessing vital bacterial structures are also reported for biological activity in the host.

The prerequisites for a postbiotic include all the characteristics of probiotics and prebiotics with similar functions as reported for probiotics. Generally the postbiotics include bacteriocins, SCFAs, heat-killed probiotics, polyphosphates, exopolysaccharides, cell wall components.

1.3 Colonization and Alterations of Gut Microflora

The human intestinal tract is colonized by a large number of microbes beginning by birth and is influenced by number of factors such as mode of delivery, feeding practices, environmental factors, etc. (Attri et al. 2018). This region is an extremely complex micro-ecosystem with an interaction between nutrients, gut microbial communities and host intestinal cells. Various studies have reported that the human gut contains approximately 1000 different species of microbes and around 10^{14} in numbers, which are nearly 10 times more microbial cells as compared to number of human cells and over 100 times the amount of genomic material as the human genome. Therefore, the gastrointestinal region is often regarded as “Super

Table 1.6 Commercial synbiotic products and their functional properties (this is not an exhaustive list)

Name	Type	Prebiotic	Probiotic	Functional properties
Zenwise digestive enzyme with probiotic and prebiotic	Powder	Inulin, apple pectin, fennel seed powder, turmeric root powder, papaya fruit powder, ginger root powder, sea vegetable, peppermint leaf extract	*L. plantarum, L. acidophilus, L. casei, L. rhamnosus, L. salivarius, B. breve, B. bifidum, B. longum, B. animalis lactis* and *Bacillus subtilis DE111*	Supports digestion and promotes proper nutrient uptake
Earth's Pearl probiotic and prebiotic	Pearl shaped tablet	Fructooligosaccharides	*L. plantarum, L. acidophilus, L. reuteri, B. lactis* and *B. infantis*	Improve gut health and immune system
Onnit total gut health	Capsule	Jerusalem artichoke and dandelion root powder	*L. rhamnosus, L. plantarum, L. acidophilus DDS-1, B. lactis, B. infantis* and *Saccharomyces Boulardii*	Immune system support and promote healthy gut flora
Ora organic probiotics with prebiotics	Capsule and Powder	Jerusalem artichoke inulin, Tapioca oligosaccharide, apple, raspberry, rice hull powder	*L. acidophilus DDS-1, L. reuteri, B. bifidum, B. longum* and *B. lactis*	Enhance optimal digestive health and immune function
Activa well-being synbiotic	Capsules	Fructooligosaccharides	*B. longum, B. infantis, L. acidophilus* and *L. rhamnosus*	Enhanced immunity and balanced gut
LactoWise	Capsule	Galactomannan	*Bacillus coagulans MTCC 5856*	Regulate growth of beneficial gut microbiota
Essential-biotic synbiotic	Powder	Xylo-oligosaccharides	*L. acidophilus* DDS-1 and *B. lactis* UABla-12	Enhanced digestive health
Now probiotic-10™ + Inulin	Powder	Organic Inulin (fructooligosaccharides)	*L. plantarum* Lp-115, *L. paracasei* Lpc-37, *L. rhamnosus* Lr-32, *L. acidophilus* La-14, *L. casei* Lc-11, *L. salivarius* Ls-33, *S. thermophilus* St-21, *B. lactis* BI-04, *B. breve* Bb-03 and *B. longum* BI-05	Healthy intestinal flora

organ". Due to this large genomic content and a number of metabolic activities in gut region, the microbe-nutrient interaction imparts a range of beneficial effects to the host. The major roles of these microorganisms in gastrointestinal tract are to maintain the gut homeostasis, immune system, to provide nutrients such as vitamins, protection from pathogenic microbes and maintaining integrity of the mucosal barrier (Attri and Goel 2018). The antimicrobial action of beneficial bacteria in gut include: Reduction of the adhesion of pathogens to epithelial cells by colonizing the available intestinal sites, competition for nutrients and release of anti-bacterial substances such as bacteriocins, lactic acid and short chain fatty acids which makes the colonic region unfavourable for survival of potential pathogenic microbes. Apart from this, gut microbiota also play a crucial part in production of many important vitamins such as vitamin B_{12} and K, and in enhanced bioavailability of iron, magnesium and calcium ions (Thursby and Juge 2017).

Alterations in the composition of microbiota or dysbiosis can be due to environmental factors, including diet, toxins, drugs and pathogens. Managing dysbiosis and manipulating the microbial environment in the colonic region using probiotics is a promising area for probiotic based therapies. Consumptions of probiotics may result in beneficial effects in the colonic region or enhance the functionality of existing gut microbiota by maintaining the gut homeostasis. Study conducted by Kuugbee and co-workers (Kuugbee et al. 2016) reported alteration in gut microbiota composition in animal model after treatment with probiotics. The relative abundance potential pathogenic bacteria belonging to genera *Clostridium, Helicobacter, Congregibacter, Escherichia, Candidatus, Phaeobacter* and *Pseudomonas* was decreased, whereas an increase in content of species of *Lactobacillus* was observed. However, to extend these beneficial effects, the efficacy is dependent on its ability to survive the conditions of gastrointestinal tract, commonly encountered conditions include: acidic conditions of stomach and alkaline environment small intestinal region. The prerequisite for probiotics also includes their capacity to attach to the intestinal mucous membrane and to colonize in this region. This adhesion by probiotics is considered as important property for colonization, and is one of the main selection criteria for novel probiotic strains. In the colonic region, the mucous membrane covering the intestinal epithelium layer is rich in glycolipids and glycoproteins which act as suitable receptors and carbohydrate moieties for bacterial adhesion. Gastrointestinal conditions such as pH, bile, digestive enzymes also influence adhesion of probiotic bacteria. In addition, due to the presence of different substances such as exopolysaccharides and calcium ions released by probiotic microbes can also alter their adhesion to colonic mucous membrane. The mechanism of adhesion of probiotics involves passive forces, electrostatic and hydrophobic. Presence of complex surface exposed polymer lipoteichoic acids in the cell wall of Gram +ve bacteria are also reported to be involved in adhesion process. Also prebiotics such as oligosaccharides could also enhance the adhesion properties of probiotic cultures (Monteagudo-Mera et al. 2019; Chua et al. 2019). Several reports also hinted that proteinaceous components such as mucus binding protein, mannose-specific adhesion protein, collagen binding proteins, fibronectin, lectin like protein also play role in the adhesion of microbes to intestinal cells in animal system and *in vitro* human studies (Collado et al. 2010).

1.4 Immune System and Gastrointestinal Response to Probiotics

The immune response in the host body is initiated by innate immunity after the entry of foreign particles such as pathogens or any tissue injury**.** However, the uneven immune response may lead to acute inflammation and unrestrained tissue injury and disorders. In the gastrointestinal tract, epithelial cells represent the first barrier against pathogens, followed by mucosal immune system response. Microbes can interact with pattern recognition receptors of intestinal antigen presenting cells such as macrophages and dendritic cells through microorganism associated molecular patterns present on the bacterial cell surface. Sensing of the gut microbiota by the mucosal immune system plays significant roles in maintaining intestinal homeostasis and generation of protective responses (Monteagudo-Mera et al. 2019). Study conducted by Galdeano and Perdigon (2004)) demonstrated that after adherence of a potential probiotic strain to the epithelial cells, immune response is stimulated through formation of toll-like receptor. Following this adherence, there is an enhanced production on the cytokines like IL-6 and macrophage chemo-attractant protein-I, without altering the intestinal barrier. Probiotics also stimulate dendritic cells/macrophages which result in an increased antibody immunoglobulin A (IgA) and cytokines secretion Molecules derived from these bacteria also bind to receptors present on dendritic cells which further activate different signalling pathways. Some probiotic strains such as *L. rhamnosus* can induce heme oxygenase activity in dendritic cells which result in mucosal T regulatory (Treg) cells within the gut associated lymphoid tissues (Delgado et al. 2020).

These probiotics also promote human health by production of low molecular weight compounds such as organic acid and antimicrobial high molecular weight compounds like bacteriocins which further inhibits the growth of pathogenic bacteria. Organic acids such as butyric, acetic and lactic acids have been reported to show strong anti-pathogenic activity against Gram –ve bacteria, such as *E. coli*, *P. aeruginosa, H. pylori,* etc., by disrupting their cell wall. Some reported bacteriocins produced by probiotics are bifidocin B produced by *B. bifidum,* nisin from *Lactococcus lactis,* plantaricin from *L. plantarum* and lactacin B from *L. acidophilus* (Bermudez-Brito et al. 2012).

It is well known that natural killer cells are involved in both innate and adaptive immunity. Natural killer cells also interact with gut microbiota and influence the adaptive T cell-mediated immune response by acting on antigen presenting cells. These natural killer T cells are also effected by lipid antigens present on probiotic cells. Probiotic bacteria *B. polyfermenticus* increase $CD56^+$ T cells, whereas *B. lactis* HN019 consumption improves natural killer cells tumoricidal properties in old-aged person. Intake of probiotic *L. casei* Shirota simulates the surface expression of CD25 and CD69 on both $CD56^+$ and $CD56^+$ cells. Also, regular consumption of probiotics products containing *L. paracasei*, *B. animalis* ssp. lactis and heat-treated *L. plantarum* has been reported to enhance the functionality of natural killer cells (Lee et al. 2017).

Probiotics beneficial effects against certain disorders such as allergies and colitis can be ascribed to their potential to enhance the production of T lymphocytes. Probiotic strain *B. longum* improved colorectal colitis in animal model by regulating the proportion of Treg cells, whereas IL-10 and the ratio of IL-10/IL-12 were enhanced in the serum, while the proinflammatory cytokines IL-12, IL-17 and IL23 were downregulated. After consumption of *B. infantis* 35,624 in healthy humans, there is an upregulation of Foxp3 lymphocytes, which further results in low level of proinflammatory cytokines in patients with ulcerative colitis or psoriasis. Probiotics can modulate T cells response by their metabolites like short chain fatty acids. In fact, the short fatty acid such as butyric acid enhances extrathymic generation of Treg cells, while de novo Treg cell generation is simulated by propionic acid (Zhang et al. 2017; Alexander et al. 2014).

1.5 Potential Applications of Probiotics

The promising effects of probiotics attract considerable attention of microbiologists, clinicians, nutritionists as well as food technologists to develop probiotics as such as or probiotic based foods or supplements for consumer applications. Some of the elite properties of probiotics, such as anti-pathogenicity, anti-inflammatory, anti-cancer, anti-allergic and anti-obesity activities, and their consequence on the mind and central nervous system (CNS), are briefly discussed below.

1.5.1 Antimicrobial Activities of Probiotics

One of the most studied effects of probiotics is modulating the intestinal microbiome against pathogenic microorganism. The probiotic microorganisms generate various metabolites such as short chain fatty acids (lactic, acetic, formic, butyric and propionic acids), which are reported to inhibit pathogens such as *Salmonella enterica* serovar Typhimurium and *Clostridium difficile* (Bermudez-Brito et al. 2015; Goldenberg et al. 2017). SCFAs are reported to maintain suitable pH conditions in the colonic lumen, which significantly boosts the metabolism of foreign compounds and carcinogen residues in the gut. In addition, probiotics produce various inhibitory compounds to reduce pathogens, *viz.* peptides, bacteriocins, organic acids, hydrogen peroxide, diacetyl, and hydrogen peroxide. Predominantly components of the probiotic metabolome are engrossed in some metabolic pathways that regulate cellular pathways for proliferation, differentiation, apoptosis (Kumar et al. 2013). Probiotics also prompt host anti-pathogenic immunomodulatory pathways such as secretion of anti-inflammatory compounds and others.

1.5.2 Anti-inflammatory Intestinal Activity of Probiotics

Ulcerative colitis (UC) and Crohn's disease (CD) are among the most frequent types of chronic inflammatory disorders and are classified as inflammatory bowel diseases (IBD) that cause digestive disorders and inflammation in the intestinal tract accompanied by abdominal pain (Seyedian et al. 2019).

Crohn's disease commonly affects the intestine, but may exist anywhere in GI tract. The disease is manifested with ulceration and inflammation that influence the digestive health of the body with limited absorption of food nutrients and elimination of wastes. The main causative agents identified are *Salmonella*, *Campylobacter jejuni*, *Clostridium difficile*, Adenovirus. In overcoming the problems related to CD in humans, *L. rhamnosus* strain GG, VSL#3, *E. coli* Nissle 1917, *S. boulardii* are reported as potential therapeutic probiotic strain (Fedorak et al. 2015; Petersen et al. 2014). In contrast, UC mainly affects the lining of the colon and rectum. The supplementation of various probiotic species like *S. boulardii*, *L. casei* and *B. bifidum* has shown to manipulate the intestinal microbial balance, which can be an alternative therapy for IBD (Kelesidis and Pothoulakis 2012). The probiotic intervention in IBD has been suggested due to the competitive action with commensal, pathogenic flora and an impact on overall immune response system (Sivamaruthi 2018).

1.5.3 Anti-cancer Activity of Probiotics

Presently, profound research on application of proteomics, genomics and molecular pathology as technological tools to detect the mechanisms of cancer has elevated the awareness about cancer and public consciousness. Simultaneously, new drugs or molecules with attractive anti-cancer properties have been identified using biotechnology and nanotechnology; however, still tolerance to their load and side effect has been a major restriction in the use of most of the drugs. In this context, probiotics with anti-carcinogenic effects have drawn great interest to prepare effective anti-cancerous targets with minimal or no side effects.

Various modes of actions have been suggested for anti-tumour activities of probiotics, however, a specific mechanism to state is still unclear. Probiotic bacteria play an essential role in the preservation of homeostasis, maintaining sustainable physicochemical conditions in the colon. Reduced pH caused inter alia by the excessive presence of bile acids in faeces may be a direct cytotoxic factor affecting colonic epithelium leading to colon carcinogenesis which include: maintaining the gut homeostasis, modulation of pH and bile acid profile, binding and degradation of potential carcinogens, production of SCFAs which may act as signalling molecules affecting the immune system, cell proliferation and apoptosis. There are plenty of new reports on the anti-tumour activities of different probiotic strains, nevertheless, the in vitro studies reported that probiotics could perform a substantial role in neutralizing cancer which needs to be validated in *in vivo* models and progress towards animal and clinical trials.

1.5.4 Anti-allergic Activities of Probiotics

The prevalence of allergic responses due to different food components due to immune disorders has been increasing worldwide resulting in severe economic and societal burden. Embracing the indispensable molecular mechanism that leads to the aetiology of allergic diseases, along with new treatment strategies, is vital in preventing the allergic responses of food components. In this context, the role of the microbiota in allergies is now widely studied; however, the role of probiotics as therapy was only recently noted. The beneficial effects of probiotics have become popular approach for health maintenance and allergic-disease prevention. Different strains have been tested for their anti-allergic responses. The common mechanisms by which these probiotics exhibit anti-allergic responses include modulation of innate lymphoid cells through toll-like receptors (TLRs), production of butyrate as one of the SCFA which protect from intestinal inflammation; act as histone deacetylase inhibitor; increase Foxp3 protein acetylation and immunomodulatory activity such as enhanced secretions of interleukin-4 and interferon-γ (Takeda et al. 2014).

1.5.5 Anti-obesity Activity of Probiotics

The accumulation of excessive fat (obesity) on the body directly deteriorates health which is related to an increase in energy availability, sedentariness and maintenance of environment temperature, resulting in imbalance between energy intake and expenditure. Probiotics possess physiological attributes that devote to the health of host environment regulating microbes. In most citing, weight loss is eased by lipolytic and thermogenic responses through activating the sympathetic nervous system. The mechanisms by which probiotics exert anti-obesity effects include maintaining the gut homeostasis, strengthening the functions of the gut epithelial barrier, production of health promoting substances such as Conjugated linoleic acid (CLA), competitive adherence to the gut mucosa, modulation of the immune system via release of anti-inflammatory compounds and modulation of energy homeostasis.

1.5.6 Effect of Probiotics on Brain and Central Nervous System

The gut microbiota composition is linked with both neurological and gastrointestinal disorders. Moreover, in recent years, new insights have been dedicated towards elucidating promising therapy of probiotics on the central nervous system (CNS) disorders. Probiotic studies in humans are still scarce, but the available data using probiotics against neurological disorders are promising. A study conducted in 2005 suggested that probiotic cultures might be used as an adjuvant therapy for the treatment of major depressive disorder (MDD) (Logan and Katzman 2005). Since stress (a significant factor contributing to MDD) is known to alter the composition of gut microflora, resulting in reduction of lactobacilli and Bifidobacterium spp., it would be conceivable that gut microbiota has significance in chronic stress. Evidently, *Bifidobacterium infantis* showed antidepressant-like properties by normalizing the levels of cytokines and tryptophan (Desbonnet et al. 2008, 2010), an increase in levels of which is associated with depression. Probiotics such as *L. rhamnosus* JB-1 and *L. plantarum* PS128 are known for their anxiolytic and anti-depressive effects on mice, which are some early examples of psychobiotics. The reported psychobiotics modulated the levels of neurotransmitters, serotonin, dopamine and their metabolites, in animal studies which contributed to the psychotropic effects (Liu et al. 2019).

1.6 Commercial Potential of Probiotics

Awareness towards health has grown to a greater extent as the consumers demand healthy diet and functional food products. Therefore, there is a high demand of food products fortified with prebiotics and probiotics in the market. As per the global market trend, probiotic business is projected to reach a turnover value of US$ 46.55 billion by 2020. The most commercial probiotics products available in the foods market belong to the genera of *Bifidobacterium, Lactobacillus, Lactococcus, Streptococcus* and some species of *Enterococcus*. Some non-pathogenic yeasts such as *Saccharomyces boulardii* are also used as probiotics.

Commercially probiotic products are being sold out in the global market mainly in the form of pharmaceutical formulations, fermented dairy products and non-dairy based probiotics (Fig. 1.2). However, since consumers who are allergic to milk proteins, lactose intolerant or due to high content of cholesterol and saturated fatty acids, they are shifting from dairy based products to non-dairy based products. Therefore, nowadays such non-dairy based products like fermented probiotic fruits, herbal, cereals, soy, vegetables and tea derived beverages are in high demand. Also, addition of probiotic strains into various food matrices-based product can alter its properties. Therefore, while developing probiotic food product, selection of an appropriate food system is a very important factor. Also, different types of probiotic

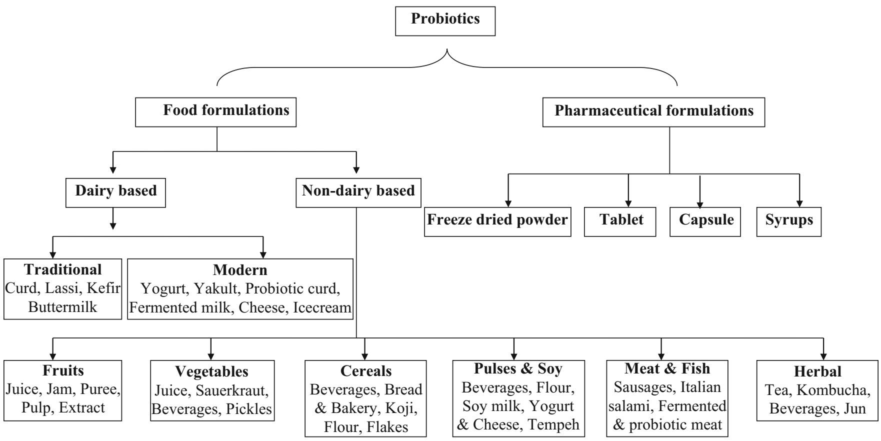

Fig. 1.2 Types and classifications of probiotics available in market (Modified from Kumar et al. 2015)

cultures show different properties towards the acidity of the food substrate, dissolved oxygen content, post-acidification after processing and metabolism in products. Also, processing conditions such as heat, mechanical or osmotic stress during preparation of probiotic products can negatively impact on viability of probiotic cells. Most common fermented probiotic food products which are consumed throughout the world help to preserve the quality of food, enhance its nutritional values and improve its sensory qualities (Kumar et al. 2015).

1.6.1 Dairy Based Probiotic Products

Milk is a rich source of vital growth factors and essential components required by the probiotic cultures. The most common probiotic dairy products consumed are various kinds of yogurt, products fermented with lactic acid bacteria such as curd, buttermilk, etc. Yogurt which is widely consumed as healthy and nutritious dairy food product is a complex gel based system containing polysaccharides, lipids and proteins. Yogurt is produced using a culture of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Probiotic cheese, both fresh and ripened, has been developed and is available in market which mainly contains *Lactobacillus* sp. and *Bifidobacterium* sp. Probiotic ice creams are also commercially available around the different parts of world which are manufactured using *B. lactis* and *L. acidophilus* mainly. Another fermentation based dairy probiotic products widely consumed in Indian subcontinent is curd/buttermilk which contains wide range of lactic acid bacteria mainly *L. acidophilus*, *L. lactis*, *L. bulgaricus* and *Leuconostoc citrovorum*. The cheese whey supplemented with probiotic bacteria production for commercial use is extremely attractive section in food biotechnology industry nowadays. Another probiotic product was prepared by fermenting whey based goat milk beverage with probiotic culture *L. rhamnosus* Lr-32, *B. animalis* BB-12 and *S. thermophilus* TA-40. Also, addition of whey protein concentrate in dairy beverages enhances the viability of *Lactobacillus* sp. and *Bifidobacterium* sp. during cold storage. This property of whey protein is due to higher buffering capacity and caseins which delay the post acidification of beverage during cold storage. Another study concluded that food matrix of whey protein beverage is suitable for viability of probiotic bacteria (Buriti and Saad 2014; Shori 2016).

1.6.2 Non-dairy Based Probiotic Products

Few years back most of the probiotic products in the market were dairy based but now many non-dairy based products are also available for people with milk allergies and lactose intolerance.

1.6.2.1 Fruit Based Probiotic Products

Fruit are a rich source of phytochemicals, minerals, vitamins and antioxidants as compared to dairy products. Fruit juices also rich source of oligosaccharides which support the growth of probiotic cultures and their good refreshing taste is suitable for people of all age groups. Also, these juices stay very less time in the stomach due to which these probiotic microbes spend very less time in the harsh acidic conditions of the stomach. Generally, fruit products possess low pH, organic acids like ascorbic acid enhance viability of probiotic cultures. Also, presence of polysaccharides such as dietary fibre and cellulose can protect probiotics during processing and storage processes. Several fruits such as apples, cranberry, oranges, litchi, pomegranate, raspberry, cantaloupe melon, blueberry, pineapple, blackcurrant, banana, cashew apple, etc. are used for manufacturing of probiotic products. Currently, the development of probiotic fruit juice based beverages has increased and is in high demand as they provide a large number of health benefit beyond the traditional nutrients as well as they are showing good satisfactoriness among consumers (Aspri et al. 2020).

1.6.2.2 Vegetable Based Probiotic Products

Vegetables are also rich in functional polyphenols and antioxidants that synergistically provide many health benefits to human. Vegetable can also provide probiotics–prebiotics benefits to human through direct consumption of vegetable based products. Numerous indigenous lactic acid bacteria such as *L. brevis, L. delbrueckii, L. casei, L. plantarum* and *L. paracasei* have been isolated from the raw vegetables. Peeling and cutting of vegetables increase the sugar, minerals, vitamins and other nutrients availability from cellular matrix which further creates favourable environment for microbial growth. Study by Yoon and co-workers (Yoon et al. 2005) reported that red beet can act as a good prebiotic for the manufacturing of probiotics beet juice. The survival of the probiotic strains in fermented tomato juice was enhanced during 30 days storage at 4 °C. Probiotic carrot and cabbage juice also seemed to be a growth medium for probiotic bacteria for production of functional beverage. Many traditionally fermented vegetable probiotic products such as Sauerkraut, cucumber and olives pickles in Europe; *kalpi* in India and Nepal; *pao-cai, inziangsang, sufu* and *hum-coy* in China; Turkish *hardaliye*; *kimchi* in Korea and Brazilian cashew apple juice are also commercially available now (James and Wang 2019).

1.6.2.3 Cereal Based Probiotic Product

Cereals have complex nutrient composition and are being consumed regularly as one of the major staple foods. Cereal based drinks are one of the major classes of fermented beverages all over the world. It also consists of non-digestible polysaccharides which have beneficial physiologic effects towards host such as enhanced viability of Bifidobacteria and Lactobacilli in the colonic region. Different LAB are used to ferment cereals such as rice, wheat, rye, oats, barley, maize and millet for preparations of probiotic beverages. Also, there are wide range of traditional cereal based probiotics such as *boza, togwa, kvass, amazake* and *pozol* produced around the different parts of world (Fernandesa et al. 2020).

1.6.2.4 Soy Based Probiotic Products

Soybean is rich source of proteins and consumption of its products can help in the prevention of many chronic diseases. Fermentation of soy products increases its digestibility of soy products and makes them more flavoured. In Japanese traditional appetizer *natto* soybean sprouts are fermented with *B. subtilis*. Experimental studies have shown the survival of probiotic cells which indicate that soy is a good substrate for viability of bacteria (González-Ferrero et al. 2018; Tomasik and Tomasik 2020).

1.6.2.5 Meat Based Probiotic Products

The preservation of meat through fermentation technology using native microbial cultures has been used for thousands of years. Although, the concept of probiotic meat product is quite new, therefore, it is not very well established in meat industry. Dry fermented meat products are usually not heated or only mildly heated, which is necessary for the transfer of probiotics into the human colonic region through normal consumption of product. Meat mainly in the form of sausages and salami is being used as potential probiotic foods. The probiotic strains belonging to LAB such as *L. plantarum, L. casei, L. sakei, L. paracasei* and *L. rhamnosus* are most commonly used in fermented meat products. Use of alginate microencapsulated of probiotic strains in the preparation of meat based sausages is also done (Bis-Souza et al. 2019).

1.6.2.6 Herbal Probiotic Formulations

Herbal probiotic drinks are generally referred as functional food that not only provides probiotics but also beneficial phytochemicals and highly bioavailable nutrients from some exotic herbs. These products are generally made in combination with honey and extracts derived from herbs such as lemon, anise, basil, fenugreek, dill, juniper, fennel, cinnamon, cardamom, ginger, basil, cloves, black pepper, etc. Also, many herbal probiotic teas such as *Kombucha* are also now commercialized due to many reported health benefits. *Kombucha* is prepared by fermentation of tea leaves by bacteria and yeast (Afsharmanesh and Sadaghi 2014).

1.6.3 Pharmaceutical Probiotic Formations

Traditionally, probiotic cultures have been incorporated in fermented food products, with limited shelf life and refrigerated storage. To overcome this issue, lyophilized probiotic bacteria are also available in market in form of capsules, powder, sachets and tablets at health care stores with enhanced shelf life. Chewable tablets and mixing probiotic powder with other active ingredients are also in trends among commercially available probiotic products. The most commonly used microbial genera in probiotic pharmaceutical formulations are *Bifidobacterium, Lactobacillus, Enterococcus, Streptococcus, Bacillus and Saccharomyces*. Medical commerce sector recommends these products to treat various kinds of gastrointestinal tract disorders (Fenster et al. 2019).

1.7 Conclusions

In conclusion it is confirmed that probiotic strains or their components are future therapeutics in treating and preventing several diseases. The diverse nature of probiotic strains reported and their further application in treatment needs comprehensive understanding of the genome level organizations. Limited studies have been conducted to determine the complexity of gut microbiota which presents a challenge to explain the underlying mechanisms of probiotic strains. Although significant research based on experiments of clinical evidences states their potential in extending human gut homeostasis and well-being, yet there is limited use of probiotics in clinical practice except in gastric infections. Once the genome organization is elucidated, further application of synthetic biology and genome editing tools will make it easier to design probiotics with enhanced activity as therapeutics.

References

Afsharmanesh M, Sadaghi B (2014) Effects of dietary alternatives (probiotic, green tea powder, and Kombucha tea) as antimicrobial growth promoters on growth, ileal nutrient digestibility, blood parameters, and immune response of broiler chickens. Comp Clin Pathol 23(3):717–724

Alexander KL, Targan SR, Elson CO III (2014) Microbiota activation and regulation of innate and adaptive immunity. Immunol Rev 260(1):206–220

Aspri M, Papademas P, Tsaltas D (2020) Review on non-dairy probiotics and their use in non-dairy based products. Fermentation 6(1):30

Attri S, Goel G (2018) Influence of polyphenol rich seabuckthorn berries juice on release of polyphenols and colonic microbiota on exposure to simulated human digestion model. Food Res Int 111:314–323

Attri S, Mahajan R, Goel G (2018) Development and diversity of lactic acid producing bacteria and bifidobacteria in healthy full term Indian infants from Himachal Pradesh. Intest Res 16(4):529

Bermudez-Brito M, Plaza-Díaz J, Munoz-Quezada S, Gomez-Llorente C, Gil A (2012) Probiotic mechanisms of action. Ann Nutr Metab 61(2):160–174

Bermudez-Brito M, Muñoz-Quezada S, Gómez-Llorente C, Matencio E, Romero F, Gil A (2015) Lactobacillus paracasei CNCM I-4034 and its culture supernatant modulate Salmonella-induced inflammation in a novel transwell co-culture of human intestinal-like dendritic and Caco-2 cells. BMC Microbiol 15(1):79

Bindels LB, Delzenne NM, Cani PD, Walter J (2015) Towards a more comprehensive concept for prebiotics. Nat Rev Gastroenterol Hepatol 12(5):303

Bis-Souza CV, Barba FJ, Lorenzo JM, Penna AB, Barretto ACS (2019) New strategies for the development of innovative fermented meat products: a review regarding the incorporation of probiotics and dietary fibers. Food Rev Intl 35(5):467–484

Brosseau C, Selle A, Palmer DJ, Prescott SL, Barbarot S, Bodinier M (2019) Prebiotics: mechanisms and preventive effects in allergy. Nutrients 11(8):1841

Buriti FC, Saad SM (2014) Chilled milk-based desserts as emerging probiotic and prebiotic products. Crit Rev Food Sci Nutr 54(2):139–150

Chua JC, Hale JD, Silcock P, Bremer PJ (2019) Bacterial survival and adhesion for formulating new oral probiotic foods. Crit Rev Food Sci Nutr 26:1–12

Collado MC, Gueimonde M, Salminen S (2010) Probiotics in adhesion of pathogens: mechanisms of action. In: Bioactive foods in promoting health. Academic Press, Amsterdam, pp 353–370

Collado MC, Vinderola G, Salminen S (2019) Postbiotics: facts and open questions. A position paper on the need for a consensus definition. Benefic Microbes 10(7):711–719

da Silva JFMD, Peluzio JM, Prado G, Madeira JEGC, Silva MO, de Morais PB, Rosa CA, Pimenta RS, Nicoli JR (2015) Use of probiotics to control aflatoxin production in peanut grains. Sci World J 2015:959138

Delgado S, Sánchez B, Margolles A, Ruas-Madiedo P, Ruiz L (2020) Molecules produced by probiotics and intestinal microorganisms with immunomodulatory activity. Nutrients 12(2):391

Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG (2008) The probiotic Bifidobacteria infantis: an assessment of potential antidepressant properties in the rat. J Psychiatr Res 43 (2):164–174

Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG (2010) Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 170 (4):1179–1188

Diez-Gutiérrez L, San Vicente L, Barrón LJR, del Carmen Villarán M, Chávarri M (2020) Gamma-aminobutyric acid and probiotics: multiple health benefits and their future in the global functional food and nutraceuticals market. J Funct Foods 64:103669

Duncan PI, Enters-Weijnen CF, Emami N, McLean P, Nunes T, Beaumont M, Crabbe R, Whelan K, Scott SM, DeWit NJ, Weits T (2018) Short-term daily intake of polydextrose fiber does not shorten intestinal transit time in constipated adults: a randomized controlled trial. Nutrients 10(7):920

Ezzatpanah H (2020) Traditional food and practices for health: Iranian dairy foods. In: Nutritional and health aspects of food in South Asian countries. Academic Press, London, pp 275–287

Fedorak RN, Feagan BG, Hotte N, Leddin D, Dieleman LA, Petrunia DM, Enns R, Bitton A, Chiba N, Paré P, Rostom A (2015) The probiotic VSL# 3 has anti-inflammatory effects and could reduce endoscopic recurrence after surgery for Crohn's disease. Clin Gastroenterol Hepatol 13(5):928–935

Fenster K, Freeburg B, Hollard C, Wong C, Rønhave Laursen R, Ouwehand AC (2019) The production and delivery of probiotics: a review of a practical approach. Microorganisms 7(3):83

Fernandesa CG, Sonawaneb SK, Arya SS (2020) Cereal based functional beverages: a review. J Microbiol Biotechnol Food Sci 9(4):914–919

Galdeano CM, Perdigon G (2004) Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. J Appl Microbiol 97(4):673–681

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125(6):1401–1412

Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K (2017) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 14(8):491

Goldenberg JZ, Yap C, Lytvyn L, Lo CKF, Beardsley J, Mertz D, Johnston BC (2017) Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev 12:CD006095

González-Ferrero C, Irache JM, González-Navarro CJ (2018) Soybean protein-based microparticles for oral delivery of probiotics with improved stability during storage and gut resistance. Food Chem 239:879–888

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC (2014) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11(8):506–514

James A, Wang Y (2019) Characterization, health benefits and applications of fruits and vegetable probiotics. J Food 17(1):770–780

Jobby R, Flora Y, Bora A, Jha P, Kawalkar H, Desai N (2020) Exploring probiotic activity of *Lactobacillus* sp. isolated from indigenous breeds of cattle milk and fecal samples in Bhatan Village, MH., IN. Curr Microbiol 77(7):1184–1190

Kelesidis T, Pothoulakis C (2012) Efficacy and safety of the probiotic Saccharomyces boulardii for the prevention and therapy of gastrointestinal disorders. Therap Adv Gastroenterol 5 (2):111–125

Kerry RG, Patra JK, Gouda S, Park Y, Shin HS, Das G (2018) Benefaction of probiotics for human health: a review. J Food Drug Anal 26(3):927–939

Kumar M, Nagpal R, Verma V, Kumar A, Kaur N, Hemalatha R, Gautam SK, Singh B (2013) Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutr Rev 71 (1):23–34

Kumar BV, Vijayendra SVN, Reddy OVS (2015) Trends in dairy and non-dairy probiotic products—a review. J Food Sci Technol 52(10):6112–6124

Kuugbee ED, Shang X, Gamallat Y, Bamba D, Awadasseid A, Suliman MA, Zang S, Ma Y, Chiwala G, Xin Y, Shang D (2016) Structural change in microbiota by a probiotic cocktail enhances the gut barrier and reduces cancer via TLR2 signaling in a rat model of colon cancer. Dig Dis Sci 61(10):2908–2920

Lee A, Lee YJ, Yoo HJ, Kim M, Chang Y, Lee DS, Lee JH (2017) Consumption of dairy yogurt containing *Lactobacillus paracasei* ssp. paracasei, *Bifidobacterium animalis* ssp. lactis and heat-treated *Lactobacillus plantarum* improves immune function including natural killer cell activity. Nutrients 9(6):558

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

Liu YW, Ong JS, Gan CY, Khoo BY, Yahaya S, Choi SB, Low WY, Tsai YC, Liong MT (2019) Lactobacillus fermentum PS150 showed psychotropic properties by altering serotonergic pathway during stress. J Funct Foods 59:352–361

Logan AC, Katzman M (2005) Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 64(3):533–538

Mallappa RH, Singh DK, Rokana N, Pradhan D, Batish VK, Grover S (2019) Screening and selection of probiotic Lactobacillus strains of Indian gut origin based on assessment of desired probiotic attributes combined with principal component and heat map analysis. Food Sci Technol 105:272–281

Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, Belzer C, Palacio SD, Montes SA, Mancabelli L, Lugli GA (2017) The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev 81(4): e00036–e00017

Mohan A, Quek SY, Gutierrez-Maddox N, Gao Y, Shu Q (2017) Effect of honey in improving the gut microbial balance. Food Qual Saf 1(2):107–115

Monteagudo-Mera A, Rastall RA, Gibson GR, Charalampopoulos D, Chatzifragkou A (2019) Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Appl Microbiol Biotechnol 103(16):6463–6472

Nakagawa H, Miyazaki T (2017) Beneficial effects of antioxidative lactic acid bacteria. AIMS Microbiol 3:1–7

Pandey KR, Naik SR, Vakil BV (2015) Probiotics, prebiotics and synbiotics-a review. J Food Sci Technol 52(12):7577–7587

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

Petersen AM, Mirsepasi H, Halkjær SI, Mortensen EM, Nordgaard-Lassen I, Krogfelt KA (2014) Ciprofloxacin and probiotic Escherichia coli Nissle add-on treatment in active ulcerative colitis: a double-blind randomized placebo controlled clinical trial. J Crohns Colitis 8(11):1498–1505

Ranucci G, Buccigrossi V, Borgia E, Piacentini D, Visentin F, Cantarutti L, Baiardi P, Felisi M, Spagnuolo MI, Zanconato S, Baraldi E (2018) Galacto-oligosaccharide/polidextrose enriched formula protects against respiratory infections in infants at high risk of atopy: a randomized clinical trial. Nutrients 10(3):286

Setta MC, Matemu A, Mbega ER (2020) Potential of probiotics from fermented cereal-based beverages in improving health of poor people in Africa. J Food Sci Technol 56:1–12

Seyedian SS, Nokhostin F, Malamir MD (2019) A review of the diagnosis, prevention, and treatment methods of inflammatory bowel disease. J Med Life 12(2):113

Shori AB (2016) Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages. Food Biosci 13:1–8

Singhi SC, Baranwal A (2008) Probiotic use in the critically ill. Indian J Pediatr 75(6):621–627

Sivamaruthi BS (2018) A comprehensive review on clinical outcome of probiotic and synbiotic therapy for inflammatory bowel diseases. Asian Pac J Trop Biomed 8(3):179

Sperti GS (1971) Probiotics. Avi Publishing, West Point

Takeda S, Hidaka M, Yoshida H, Takeshita M, Kikuchi Y, Tsend-Ayush C, Dashnyam B, Kawahara S, Muguruma M, Watanabe W, Kurokawa M (2014) Antiallergic activity of probiotics from Mongolian dairy products on type I allergy in mice and mode of antiallergic action. J Funct Foods 9:60–69

Thursby E, Juge N (2017) Introduction to the human gut microbiota. Biochem J 474 (11):1823–1836

Tomasik P, Tomasik P (2020) Probiotics, non-dairy prebiotics and postbiotics in nutrition. Appl Sci 10(4):1470

Turroni F, Milani C, Duranti S, Ferrario C, Lugli GA, Mancabelli L, van Sinderen D, Ventura M (2018) Bifidobacteria and the infant gut: an example of co-evolution and natural selection. Cell Mol Life Sci 75(1):103–118

Wang Y, Fu L, Lin J (2012) Probiotic (*Bacillus coagulans*) cells in the diet benefit the white shrimp Litopenaeus vannamei. J Shellfish Res 31(3):855–860

Yoon KY, Woodams EE, Hang YD (2005) Fermentation of beet juice by beneficial lactic acid bacteria. Food Sci Technol 38(1):73–75

Zhang MM, Cheng JQ, Lu YR, Yi ZH, Yang P, Wu XT (2010) Use of pre-, pro-and synbiotics in patients with acute pancreatitis: a meta-analysis. World J Gastroenterol 16(31):3970

Zhang M, Zhang S, Hua Z, Zou X (2017) Long-term use of Bifidobacterium longum alleviates colorectal colitis in rats by regulating inflammatory cytokines and Treg cells. Int J Clin Exp Med 10:7543–7552

Chapter 2
Sources and Selection Criteria of Probiotics

Sukhminderjit Kaur, Rajinder Kaur, Nitu Rani, Swati Sharma, and Mahavir Joshi

Abstract Probiotics are the living microbial consortia that confer the health benefits to humans when incorporated in diet in adequate amount. These bacteria, referred to as "good bacteria," are present in human gut through various dietary sources and exhibit anti-inflammatory, anti-cancerous, immunity enhancing benefits. With enhancing knowledge about the field of probiotics, its potential sources are also been scrutinized so that cheap and easy availability of the probiotic product is produced. Traditionally fermented dairy products, fermented vegetables, fermented soy products are available throughout the world with different names. These traditional fermented products are conferring many health benefits owing to the presence of probiotic bacteria present in it. Presently, dairy companies are active producers of the probiotic rich yogurts, buttermilks, and tofu. These products are artificially supplemented with probiotics and are easy available options for the consumers. Also, many pharmaceutical companies are providing active probiotic supplements in the form of capsules and sachets which are recommended to patients with diarrhea, various infections, and allergies. However, with the fast emergence of the probiotic industry, it is imperative that the guidelines for bacteria to be termed as probiotic should be well defined so that the users can get a product with claimed benefits. These characteristics will ensure that the consumer is getting a standardized product with ensured efficiency.

Keywords Probiotics · Good bacteria · Health benefits · Sources · Probiotic supplements

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2.1 Introduction

Living microorganisms that can be incorporated into foods, dietary supplements, functional foods, and drugs in adequate amount for conferring health benefits to the host are called as probiotics. These live microorganisms provide health benefits to the host animal beyond their inherent nutrition. Earliest idea of the beneficial effects of bacteria to human health was provided by Nobel Laureate Metchnikoff, who tried to find out the possible effects of microbes on human health. He reported that the auto-intoxification caused by the intestinal microbes can be prevented by the intake of lactic acid producing bacteria present in yogurt. In his book, "The prolongation of life; optimistic studies," he suggested that the growth of proteolytic microbes can be prevented by intake of dairy products because they lower the colon pH by breaking lactose. He supported his hypothesis by providing the fact that villagers in Bulgaria who consumed fermented sour milk had longer life expectancy compared to general population. A pediatrician, Henry Tissier, from the Pasteur Institute reported the discovery of *Bifidobacterium* spp. in the gut of breast-fed infant. He reported that the gastroenteritis could be prevented by *Bifidobacterium* spp. Stamen Grigorov in 1905 identified Lactobacilli spp. in the starter culture of fermented Bulgarian diary product. Years later, in 1917, a German professor, Alfred Nissle, isolated non-pathogenic *E. coli* from the feces of two soldiers not affected by the epidemic of shigellosis. He reported that presence of non-pathogenic *E. coli* in these soldiers prevented the adherence of pathogenic bacteria by the secretion of bacteriocins (Sonnenborn and Schulze 2009).

Earlier the term probiotics was limited to the Lactobacillus and Bifidobacterium spp. With identification of new bacteria and increase in research in probiotics, bacterial spp. belonging to genera *Streptococcus, Leuconostoc, Pediococcus, Propionibacterium,* and *Enterococcus* are being used as probiotics. Yeast and molds like *Aspergillus niger, A. oryzae, Candida pintolopesii*, *Saccharomyces boulardii,* and *S. cerevisiae* are also regarded as probiotics.

Human gut is inhibited by both good (probiotics) and bad bacteria. Good bacteria confirm various health benefits to humans. They improve food digestion by fermenting the carbohydrates into simpler structures, producing vitamin-K, and compensating various deficiencies in the body. Probiotics play an important role in proliferation of epithelial cells, development of immune system (Cammarota et al. 2009). Bad bacteria, usually present, but are apparently non-existing, because they are under the pressure of good bacteria. Good bacteria are able to compete with these bad bacteria and prevent their colonization. In the conditions of stress, high alcohol consumption, high fats, genetic disorders, high chlorine and fluoride concentration in diet can cause decrease in the number of good bacteria and bad bacteria will take their position and produce toxins deteriorating human health. In that case, probiotics need to be administrated in higher dosage (Amara 2012).

2.2 Sources of Probiotics

Since ages humans are taking probiotics in diet through fermented food products such as beverages, yogurt, and cheese (Amara 2012). Intake of fermented foods or the dietary supplements are the two ways in which we can take probiotics. Fermented foods are regarded well over dietary supplements because their use is advocated to treat certain diseases and are not recommended for daily use. Fermented dairy products are one of the best sources of probiotics (Liong 2011). Kefir, sauerkraut, pickles, tempeh, miso, kimchi, sourdough bread, and some cheese are important fermented products which are rich sources of probiotics.

1. *Yogurt*—The "Yogurt" is the Turkish word which means to get thick. Turks are believed to be the earliest users of yogurt to treat gastrointestinal diseases (Atalay 2006). Yogurt, "the milk of eternal life" prepared from milk of goat was used to treat severe gastrointestinal diseases in France (Ozen and Dinleyici 2015). Yogurt is the fermented milk by the fermentation of milk by bacteria called as "Yogurt cultures." Yogurt culture is the combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *S. thermophile* is responsible for the lactic acid fermentation and consequent cuddling of the milk to give it a gel like consistency. *L. bulgaricus,* on the other hand imparts flavor, hydrolyses the casein proteins into smaller peptides. Yogurt is considered as the best carrier food that can be used to deliver *L. acidophilus* and *Bifidobacterium bifidum* to human gut, the most common probiotics present in the dairy products. Many other probiotic bacteria are also present in the yogurt. These include *B. animalis, B. lactis, B. longum, Enterococcus faecium*, *Lactobacillus casei, L. delbrueckii*, *L. johnsonii*, *L. gasseri*, *L. plantarum*, *L. rhamnosus*, and *S. boulardii.* Probiotics bacteria present in yogurt are associated with the prevention of acute diarrhea, allergies, inflammation, genitourinary infections, cardiovascular diseases, vaginitis. The yogurt has attracted the interest of scientific communities due to its antimicrobial, antihypersensitivity, immunomodulatory, lipid-lowering properties, anti-inflammatory properties (Ebringer et al. 2008; Zemel et al. 2005; Ricci-Cabello et al. 2012). Clinical trials have shown the lower risk of antibiotic induced diarrhea with the consumption of yogurt (Beniwal et al. 2003). Lactoferrin, the peptide formed by the digestion of proteins by the probiotic bacteria, present in the yogurt is associated with eradication of *Helicobacter pylori* infections (Sachdeva et al. 2014).
2. *Kefir*—It is a fermented milk product similar to yogurt. There has been an increased interest in the commercial use of kefir as a health promoting beverage due to the probiotics present in it. Kefir has a long history of being recommended for health benefits in Soviet nations. The health benefits of kefir are linked both to the consortia of probiotics in it and also due to the presence of organic acids. In addition to being a rich resource of probiotics, it can also be effectively used as a matrix for the delivery of beneficiary microorganisms to the human body (Oliveira et al. 2013; Medrano et al. 2008). Lactose fermenting yeast, non-lactose fermenting yeast, heterofermentative and homofermentative lactic

acid bacteria, lactic acid bacteria such as *L. kefiri, L. kefiranofaciens, L. paracasei, L. acidophilus, L. parabuchneri*, *L. delbrueckii, L. bulgaricus, L. plantarum, Acetobacter aceti, A. lovaniensis, A. rasens, A. syzygii, Leuconostoc mesenteroides, Lactococcus lactis, Enterococcus durans, the fungus such as Geotrichum candidum, S. cerevisiae, S. unisporus, Candida kefyr, and Kluyveromyces marxianus* (Zanirati et al. 2015; Witthuhn et al. 2004) are present in the kefir. The probiotic population in Kefir varies according to the kefir origin (Gao et al. 2012). Benefits of kefir like hypocholesterolemic effects, antiallergenic properties, food preservative properties, antitumor, and reduction in blood pressure are related to the biological properties of the probiotics present in Kefir (Liu et al. 2002; Chen et al. 2008; Yanping et al. 2009). Antibacterial compounds and antibiotics produced by probiotics present in kefir, inhibit the growth of pathogenic bacteria such as *Helicobacter, Shigella sonnei, Staphylococcus, Salmonella, Helicobacter, Escherichia coli, Enterobacter aerogenes, Micrococcus luteus, Listeria monocytogenes, Streptococcus pyogenes, Streptococcus faecalis* and fungus like *Candida albicans*, *Fusarium graminearum, Clostridium difficile* (Lopitz et al. 2006; Ismaiel et al. 2011).

3. *Sauerkraut*—It is one of the oldest fermented foods prepared from the cabbage and has a long history of human nutrition. It is regarded as a wonder food due to the presence of probiotics in it. Sauerkraut does not need any culture to ferment as the cabbage growing in a healthy soil has all the bacteria needed to start fermentation. The fermented end product is rich is Lactic acid bacteria (LAB). Predominant species of LAB found in it are identified as *L. brevis, L. plantarum, Leuconostoc mesenteroides, Leuconostoc fallax, Pediococcus pentosaceus, L. curvatus, L. sakei, Lactococcus lactis* (Björkroth et al. 2002; Murcia-Martinez and Collins 1990; Vogel et al. 1991).These bacteria have established therapeutic role in the treatment of constipation, diarrhea, irritable bowel syndrome, urinary tract infections, and certain cancers. Presence of LAB in sauerkraut has an established role to enhance immunity, prevent certain diseases, and promote lactose tolerance in human body (Orgeron II et al. 2016).
4. *Fermented Vegetables*—Fermentation is the oldest and the widest used biotechnological method to preserve the perishable vegetable products (Fleming et al. 1995). It is regarded as the natural method of enhancing the nutritional quality and organoleptic properties of the vegetables. Species of LAB such as *Lactobacillus, Lactococcus, Pediococcus, and Leuconostoc* are naturally present in the vegetables that carry out natural fermentation. Different fermented vegetables have different types of probiotic bacteria (Table 2.1). Probiotic LAB spp. isolated from vegetables are resistant to natural bile and gastric juices tolerating gut stress, therefore vegetables are considered as another important source of probiotics. These probiotic bacteria have found to have antimicrobial activity against Salmonella spp., against gram-positive and gram-negative pathogens (Wang et al. 2010).
5. *Kombucha*—Kombucha is a fermented black tea which is slightly carbonated and sweet in taste. The fermented tea was very popular in China, Germany, and Russia for preventing metabolic diseases, arthritis, hemorrhoids, and rheumatism,

Table 2.1 Different probiotics present in fermented vegetable products

Fermented product	Region	Source	Probiotic organisms	Reference
Gundruk	Nepal	Leafy vegetables	Pediococcus and Lactobacillus spp., *L. cellobiosus* *L. plantarum*	Karki et al. (1983)
Sinki	India, Nepal, Bhutan	Radish taproot	*L. fermentum* *L. brevis* *L. plantarum*	Tamang (2009)
Khalpi	Nepal	Cucumber	*L. plantarum* *L. brevis* *Leuconostoc fallax* *P. pentosaceus*	Tamang (2009), Dahal et al. (2005)
Inziangsang	North east India	Mustard leaves	*L. plantarum* *L. brevis* *Pediococcus*	Tamang (2009); Yan et al. (2008)
Soidon	Manipur	Bamboo shoots	*Lactobacillus brevis* *Leuconostoc fallax* *Lactococcus lactis*	Tamang (2009)
Goyang	Sikkim and Nepal	Magane saag (Brassicaceae)	*Lactobacillus plantarum* *Lactobacillus brevis* *Lactobacillus lactis* *Enterococcus faecium* *Pediococcus pentosus* *Yeast like candida spp.*	Tamang and Tamang (2007)
Mesu	Sikkim	Bamboo shoot, shoots of choya bans (Dendrocalamus hamiltonii), Karati bans (Bambusa tulda) and Bhalu bans (Dendrocalamus sikkimensis)	*Lactobacillus plantarum* *Lactobacillus brevis* *Lactobacillus curvatus* *Leuconostoc citreum* *Pediococcus pentosaceus*	Tamang and Sarkar (1993)
Soibum	Manipur	Bamboo shoots of Dendrocalamus hamiltonii, D. sikkimensis and D. giganteus, Bambusa tulda and B. balcona.	*L. plantarum,* *L. brevis,* *L. coryniformis,* *L. delburkii,* *Leuconostoc fallax* *Lactococcus lactis* *L. mesenteroides* *Enterococcus durans* *Streptococcus lactis* *Bacillus subtilis* *B. licheniformis* *B. coagulans* *Yeast like Candida* *Saccharomyces and Torulopsis*	Giri and Janmejay (1987) and Sarangthem and Singh (2003)

(continued)

Table 2.1 (continued)

Fermented product	Region	Source	Probiotic organisms	Reference
Yan-dong-gua	Taiwan	Wax gourd	*W. cibaria* *W. paramesenteroides*	Lan et al. (2009)
Yan-jiang	Taiwan	Ginger	*L. sakei* *Lactococcus lactis* *W. cibaria* *L. plantarum*	Chang et al. (2011)
Burong mustala	Philippines	Mustard Leaf	*L. brevis* *Pediococcus cerevisiae*	Karovicova et al. (2002)
Ca muoi	Vietnam	Eggplant	*L. fermentum* *L. pentosus* *L. brevis*	Nguyen et al. (2013) and Sesena et al. (2001)
Dhamuoi	Vietnam	Cabbage and leafy vegetables	*Leuconostoc mesenteroides* *L. plantarum*	Steinkraus (1997)
Kimchi	Korea	Cabbage, raddish, vegetables	*Leuconostoc mesenteroides* *L. brevis* *L. plantarum* *L. sakei*	Lee et al. (2005)
Olive	Spain and Italy	Olive	*L. plantarum* *L. brevis* *L. pentosus* *P. cerevisiae* *L. mesenteroides*	Argyri et al. (2013) and Nychas et al. (2002).

reducing blood pressure, increasing immune response, and curing cancer (Greenwalt et al. 2000). The health benefits of Kombucha are attributed to the presence of vitamins, polyphenols, organic acids produced during the process of fermentation and presence of various lactic acid, gluconic acid, and acetic acid producing bacteria with probiotic attributes (Greenwalt et al. 2000; Malbasa et al. 2008). The *Acetobacter xylinum, A. aceti, A. xylinoides, A. pasteurianus,* and Gluconobacter are the predominant bacteria present in Kombucha, while yeasts species includes Brettanomyces, Saccharomyces, Pichia, Zygosaccharomyces, and Candida species (Malbasa et al. 2008; Malbasa et al. 2011). However, the yeast population is found to be variable in the Kombucha from different regions (Table 2.2).

6. *Tempeh*—Tempeh is a fermented soy product, popularly found in Indonesia and is rich in fibers. Tempeh consumption enhances the human gut health by enhancing the number of *A. muciniphila* and *Bifidobacterium* significantly (Stephanie et al. 2019). Indonesian tempeh is rich in probiotic microbial consortia consisting of *Acetobacter indonesiensis, Flavobacterium sp., Bacillus subtilis, L. agilis,*

Table 2.2 Variable yeast species present in Kombucha

Place of origin	Yeast species found	Reference
German	Brettanomyces, Zygosaccharomyces, and Saccharomyces	Mayser and Fromme (1995)
Switzerland	Pichia and Zygosaccharomyces (NRRL Y-4810 and Y-4882)	Hesseltine (1965)
Taiwan	*Saccharomyces cerevisiae, Zygosaccharomyces bailii,* and *Brettanomyces bruxellensis*	Liu et al. (1996)
Mexico	*Brettanomyces intermedius, Candida famata, Pichia membranaefaciens, S. cerevisiae, S. cerevisiae*, *Torulaspora delbrueckii*, *Z. bailii*, and *Z. rouxii*	Herrera and Calderon-Villagomez (1989)
Yugoslavia	Saccharomyces, Torulopsis, Mycotorula, Schizosaccharomyces, Saccharomyces, Pichia, Torula, Mycoderma, and Candida	Jankovic and Stojanovic (1994)
North America	Zygosaccharomyces, and *S. cerevisiae*	Roussin (1996)

L. fermentum, and Enterobacter cecorum, Brevundimonas sp., Pseudomonas putida, and Acinetobacter spp. (Barus et al. 2008; Radita et al. 2017).

7. *Probiotic supplements*—WHO documented that most of the today's diseases are life style related diseases and advocated to switching over to the use of probiotics for treating these diseases. With enhancing health concerns and inclination towards the benefits of probiotics, the demand for "probiotic functional foods" and the probiotic drug supplements is increasing, especially among the younger generation. Probiotic-infused juices, yogurt-based drinks, liquid probiotics are the biggest probiotic supplements present in the market. Mother Diary, Amul, Nestle, Unique Biotech, Tablets India, Danone Yakult, Dr. Reddy Laboratories, Polchem Hygiene Laboratories are the major players in probiotic industry in India. Probiotic preparations are also sold in the form of capsules, syrups and pills. Some of the probiotics available in Indian markets is summarized in the Table 2.3.
8. *Human Breast milk*—Human breast milk is rich in prebiotics and probiotics, and is considered very important for the development of microbiota in the infant gut. Traditional breast milk was regarded to be sterile, but development of molecular and culture techniques confirms that breast milk continuously supply commensals and rarely, can also transfer infectious microbes to the infant gut from the mother's skin and gut.

   Approximately 600 different species belonging to Bifidobacteria, Lactobacilli, Enterococcus, Peptostreptococcus, Staphylococcus, Streptococcus, Corynebacterium, and/or an occasional Escherichia species are acknowledged to be present in breast milk that helps in the establishment of infant gut microbiota. Medically important probiotic strains present in human breast milk and their consequent effect on the neonatal growth are summarized in the Fig. 2.1.

Table 2.3 Probiotic functional food and Probiotic drug supplements available in market

Type of product	Company	Type of supplement	Probiotic present	Source
Probiotic functional food	Yakult Danone	Probiotic food	*L. casei*	https://www.yakult.co.in/india.php
	Amul	Drinking Yogurt	*L. acidophilus* La5 and *B. Lactis* Bb12	https://amul.com/products/amul-flaavyo-info.php
	Nestle	Nestle Actiplus Dahi Flavored milk	*L. acidophilus*	https://www.nestle.in/
	Mother Diary	B-Active Probiotic Curd Probiotic drink (Nutrifit) Fruit yogurt	*L. acidophilus* and Bifidobacterium	https://www.motherdairy.com/
Probiotic drug supplements	Unique Biotech	Probiotic blends	*B. coagulans, L. acidophilus, L. bulgaricus, L. casei, L. helveticus, L. reuteri, S. thermophilus*	http://www.uniquebiotech.com/
	Alpic Biotech Ltd	Abigut Capsules	*B. bifidum, B. longum, L. acidophilus, S. thermophilus*	https://www.alpicbiotech.com/
	Dr. Reddy Laboratories	Becelac Fortz Cap	Spores of Lactobacillus	https://www.drreddys.com/
	Tablets India Limited	Bifilac	Lactobacillus 50 million spores	http://www.tabletsindia.com/
	Tablets India Limited	Biors Sachet	*B. mesentericus, Clostridium butyricum,* Lactobacillus	http://www.tabletsindia.com/
	Merck	Ecobion Sachet	*B. bifidum, B. longum, L. acidophilus, L. rhamnosus, S. boulardii, and S. thermophilus*	https://www.merck.com/index.html

	Sanzyme	Sporlac	Sporolactobacilli, Lactobacilli solution	https://www.sanzyme.com/
	USV Limited	ViBact	Genetically modified *B. mesentericus*	https://www.usvindia.com/
	Elan Pharma Pvt Ltd.	Rinifol	Lactobacillus 50 million spores	https://www.zaubacorp.com/company/ELAN-PHARMA-INDIA-PRIVATE-LIMITED/U24230MH1997PTC108497
	Lupin Labs	Ubioz Powder	*B.longum, L. acidophilus, L. Casei, S. boulardii, S. thermophilus*	http://www.lupinpharmaceuticals.com/
	USV Limited	Vibact	*S. faecalis, C. butyricum, B. mesentericus and L. sporegenes*	https://www.usvindia.com/
	Herbs Nutriproducts Private Limited (Pure Nutrition)	Progut Capsules	*L. acidophilus, S. faecalis, Enterococcus faecalis, C. butyricum, L. plantarum, B. mesentericus, L. salivarius*	https://purenutrition.me/
	Promopharma	Lactoflora	*L. brevis, L. plantarum*	https://www.promopharma.it/en/

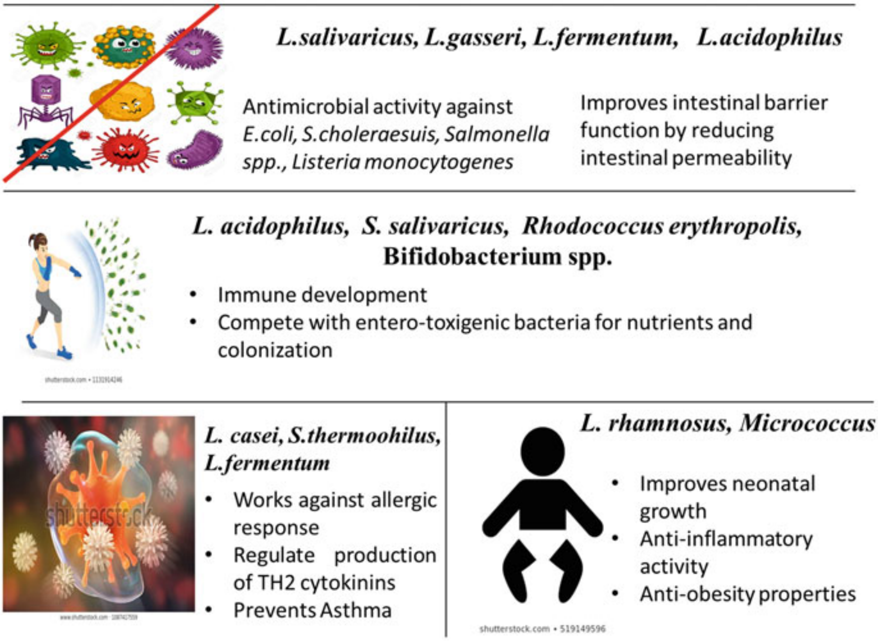

Fig. 2.1 Various probiotic bacteria present in Breast milk and their beneficial effects (Matsuzaki and Chin 2000; Donnet-Hughes et al. 2010; Gilliland and Speck 1977; Olivares et al. 2006; Vendt et al. 2006)

2.3 Selection Criteria for the Probiotics

The selection of a probiotic strain for human use should follow a systematic approach. This should be done with the objective that a safe and desired probiotic product is reached to the end users. General aspects like origin of the probiotic, genus and strain identification, biosafety, functional aspects like ability to sustain in gastrointestinal tract, health aspects and production aspects like acid production, proteolysis, and viability should be kept in mind before choosing a probiotic strain. Guidelines provided by ICMR-DBT, for the evaluation of probiotics in food, make it clear that it needs a "step-by-step" approach to test the functional ability of the microbe to be called as a "probiotic." It is recommended that the strain should be isolated from the targeted animal only, i.e., probiotics for human use should be ideally isolated from human intestine or breast milk. This is because they are better able to adhere to intestinal walls and proved to be safer for humans. Also, they can be isolated from various fermented food products discussed in the above section. The isolated strain should be tested for its probiotic potential and identified up to genus and strain level. The primary identification techniques should be followed by molecular and genetic techniques such as fatty acid methyl ester (FAME), 16SrRNA DNA Sequencing, Polymerase chain reaction (PCR) amplification and

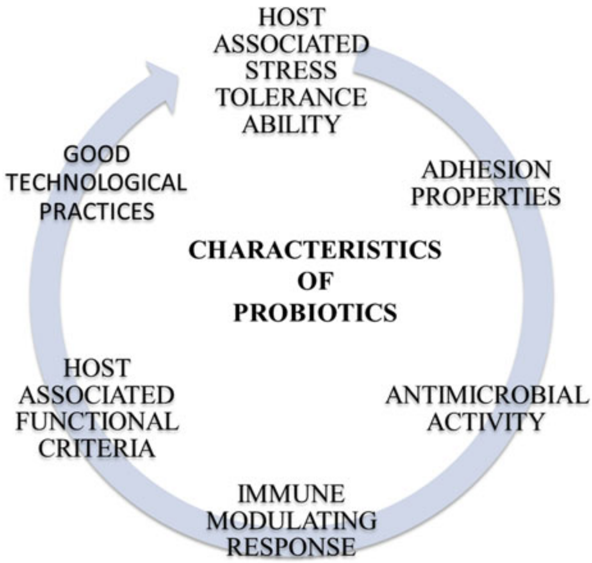

Fig. 2.2 Characteristics of probiotics

DNA and RNA hybridization, and DNA-DNA hybridization technique. For selecting a microbe to be used as probiotic, it should meet certain characteristics that are summarized in Fig. 2.2 and enlisted below:

1. *Host associated stress tolerance ability*—Upon ingestion the probiotic should be able to sustain through the different parts of digestive tract and tolerate various stress conditions in human body that includes the action of various digestive enzymes like lysozymes, amylase, pepsin, and chymotrypsin. The bacterial strain should have acid and bile tolerance, mild heat shock tolerance caused by internal body temperature. Different probiotic bacteria have found to have varying degree of resistance to the stress conditions. Ogunremi et al. 2015, Psani and Kotzekidou (2006) in their studies summarized that probiotic bacteria should be able to tolerate bile concentrations of 0.3–2.0% and pH range of 2–5.
2. *Adhesive properties*—The adhesion of the probiotic strain to the intestinal wall is very important in defining its probiotic potential because it ensures that the bacteria is not washed away, auto-aggregates to increase its cell density and biomass in the digestive tract. Adhesion is also important to ensure that the microbial cell forms a better interaction with human epithelial cells in order to produce various host associated functional effects on the host (defined below).
3. *Antimicrobial activity*—The probiotic strain should be able to fight and survive against the potential pathogenic microbes that may be present in the intestine. The prevention of pathogen survival in the gut is related to the competitive behavior of probiotic strain, where it prevent the adhesion of pathogens to the epithelial cells in the human body and due to the secretion of lacticins, alyteserin-1 a, bacteriocins Abp118, bacteriocin sakacin A, lactic acid, antibodies. Probiotic bacteria also employ increasing leukocyte phagocytic activity, increasing transepithelial

resistance, enhancement of cytoskeletal, and tight junctional protein phosphorylation activity to kill various pathogenic bacteria (Mathipa and Thantsha 2017).

4. *Immune modulating response*—Probiotic strain should be involved in production of metabolites that stimulate the maturation and functioning of the immune cells. These bacteria enhance the secretion of immunoglobulins, cytokine production (Rocha-Ramírez et al. 2017), IgA and IgM-secreting cells (Lammers et al. 2003). The probiotic strain should be selected depending upon the target host to stimulate the systemic immune response.
5. *Host Associated functional criteria*—One of the most important characteristics of the probiotic is the health benefits that it can provide to the host. The viability and colonization of the probiotic in the human gut should offer anticarcinogenic, anti-cholesterol activity, anti-depression, anti-anxiety, anti-obesity, anti-diabetic, and antioxidant activities to host besides protecting it against irritable bowel syndrome, gastroenteritis, and inflammatory bowel disease, diarrhea, infant allergies, cancer, lactase digestion, diabetes, and hyperlipidemia. Probiotic strains should be selected to inactivate enzymes (nitrate reductase, β-glucosidase, β-glucuronidase) involved in the activation of precarcinogens (Kumar et al. 2013). Additionally, the probiotic strain should be screened for secretion of conjugated linoleic acid production (dos Reis et al. 2017) and apoptosis induction activity (Ewaschuk et al. 2006) for possible anticarcinogenic activity.

   The efficiency of the probiotic strains for the reversal of depression and anxiety can also be studied in vivo on animal models. The anti-diabetic and anti-obesity activities of the potential strain can be analyzed by testing its ability to inhibit mast cell activation (Niers et al. 2005) and ability to break lipopolysaccharides (Alokail et al. 2013).

   Another major characteristic feature of the probiotic is the secretion of antioxidant enzymes (superoxide dismutase, glutathione dismutase), functional molecules (glutathione, melatonin, ascorbic acid), Vitamin-K, biotin, riboflavin, nicotinic acid, thiamine, pantothenic acid, and bioactive enzymes (lipases and amylases). The in vitro production of these components should be tested depending upon the objective of the product.
6. *Good technological properties*—The extent to which a probiotic can effectively deliver its benefits to the host will depend upon the capability of the strain to survive the storage period and maintain the same efficiency and viability. The probiotic bacteria should be able to grow quickly in different nutrient supplements (preferably cheap fermentation media), food matrices, and microaerophilic conditions. The stress adaptation of bacteria strain to withstand different physical handling techniques during food processing without losing the viability and efficiency is also important for its selection as the probiotic.

2.4 Conclusion

Fermented and non-fermented dairy products, fermented perishable vegetables and fruits, fermented cereals and soya products should be incorporated into the diet due to the presence of probiotic bacteria in it. The rise in the market potential of probiotics in India is driven by the rising lifestyle disorders and subsequently increasing the awareness of the consumers. The availability of the large number of commercial platforms providing probiotic functional foods and probiotic drug supplements has also contributed to the exponential increase in the use of probiotics in India. In the absence of any regulatory guidelines governing the probiotic industry, it is quite possible that the fake products with low or no health benefits are being marketed to the end users. To avoid such spurious products, the product must fulfill the above prerequisite conditions to be called or labeled as the "probiotic." Apart from this, efficacy studies should also be carried to prove its health benefits in humans. Manufacturing and handling practices should follow quality assurance so that the end users should get the product with claimed benefits.

References

Alokail MS, Sabico S, Al-Saleh Y, Al-Daghri NM, Alkharfy KM, Vanhoutte PM, McTernan PG (2013) Effects of probiotics in patients with diabetes mellitus type 2: Study protocol for a randomized double-blind placebo-controlled trial. Trials 14(1):195. https://doi.org/10.1186/1745-6215-14-195

Amara A (2012) Toward healthy genes. Schüling Verlage, Münster

Argyri AA, Zoumpopoulou G, Karatzas KAG, Tsakalidou E, Nychas GJE, Panagou EZ, Tassou CC (2013) Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food microbiol 33:282–291. https://doi.org/10.1016/jfm201210005

Atalay B (2006) Kaşgarlı Mahmut Divanü-Lügati't – Türk [translation]. Türk Tarih Kurumu Press, Ankara

Barus T, Suwanto A, Wahyudi AT, Wijaya H (2008) Role of bacteria in tempeh bitter taste formation: microbiological and molecular biological analysis based on 16S rRNA gene. Microbiol Indones 2(1):17–21. https://doi.org/10.5454/mi214

Beniwal RS, Arena VC, Thomas L, Narla S, Imperiale TF, Chaudhry RA, Ahmad UA (2003) A randomized trial of yogurt for prevention of antibiotic-associated diarrhea. Dig Dis Sci 48 (10):2077–2082. https://doi.org/10.1023/a:1026155328638

Björkroth KJ, Schillinger U, Geisen R, Weiss N, Hoste B, Holzapfel WH, Korkeala HJ, Vandamme P (2002) Taxonomic study of *Weissella confusa* and description of *Weissella cibaria sp.* nov. detected in food and clinical samples. Int J Syst Evol Microbiol 52:141–148. https://doi.org/10.1099/00207713-52-1-141

Cammarota M, De Rosa M, Stellavato A, Lamberti M, Marzaioli I, Giuliano M (2009) *In vitro* evaluation of *Lactobacillus plantarum* DSMZ 12028 as a probiotic: emphasis on innate immunity. Int J Food Microbiol 135:90–98. https://doi.org/10.1016/j.ijfoodmicro.2009.08.022

Chang CH, Chen YS, Yanagida F (2011) Isolation and characterization of lactic acid bacteria from yan-jiang (fermented ginger) a traditional fermented food in Taiwan. J Sci Food Agric 91 (10):1746–1750. https://doi.org/10.1002/jsfa.4364

Chen HC, Wang SY, Chen MJ (2008) Microbiological study of lactic acid bacteria in kefir grains by culture-dependent and culture-independent methods. Food Microbiol 25:492–501. https://doi.org/10.1016/j.fm.2008.01.003

Dahal NR, Karki TB, Swamylingappa B, Li Q, Gu G (2005) Traditional foods and beverages of Nepal-a review. Food Rev Int 21(1):1–25. https://doi.org/10.1081/FRI-200040579

Donnet-Hughes A, Perez PF, Dore J, Leclerc M, Levenez F, Benyacoub J, Serrant P, Segura-Roggero I, Schiffrin EJ (2010) Potential role of the intestinal microbiota of the mother in neonatal immune education. Proc Nutr Soc 69:407–415. https://doi.org/10.1017/S0029665110001898

dos Reis, S. A., da Conceição, L. L., Siqueira, N. P., Rosa, D. D., da Silva, L. L., & Maria do Carmo, G. P (2017) Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. Nutr Res. 37: 1–19. https://doi.org/10.1016/j.nutres.2016.11.009

Ebringer L, Ferenčík M, Krajčovič J (2008) Beneficial health effects of milk and fermented dairy products. Folia Microbiol 53(5):378–394. https://doi.org/10.1007/s12223-008-0059-1

Ewaschuk JB, Walker JW, Diaz H, Madsen KL (2006) Bioproduction of conjugated linoleic acid by probiotic bacteria occurs *in vitro* and *in vivo* in mice. J Nutr 136:1483–1487. https://doi.org/10.1093/jn/136.6.1483

Fleming HP, Kyung KH, Breidt F (1995) Vegetable fermentations In: Rehm HJ Reed G Biotechnology volume9 Enzymes biomass food and feed, 2nd edn. VCH, New York

Gao J, Gu F, Abdella NH, Ruan H, He G (2012) Investigation on culturable microflora in Tibetan kefir grains from different areas of China. J Food Sci. 77:425–433. https://doi.org/10.1111/j.1750-3841.2012.02805.x

Gilliland SE, Speck ML (1977) Antagonistic Action of Lactobacillus acidophilus Toward Intestinal and Foodborne Pathogens in Associative Cultures. J Food Prot 40:820–823. https://doi.org/10.4315/0362-028X-40.12.820

Giri SS, Janmejay LS (1987) Microbial and chemical contents of the fermented bamboo shoot "soibum". Frontier Bot 1:89–100

Greenwalt CJ, Steinkraus KH, Ledford RA (2000) Kombucha the fermented tea: microbiology composition and claimed health effects. J Food Prot 63(7):976–981

Herrera T, Calderon-Villagomez A (1989) Species of yeasts isolated in Mexico from the tea fungus. Rev Mex Micol 5:205–210

Hesseltine CW (1965) A millennium of fungi food and fermentation. Mycologia 57:149–197. https://doi.org/10.2307/3756821

Ismaiel AA, Ghaly MF, El-Naggar AK (2011) Milk kefir: ultrastructure antimicrobial activity and efficacy on aflatoxin B1 production by *Aspergillus flavus*. Curr Microbiol. 62:1602–1609. https://doi.org/10.1007/s00284-011-9901-9

Jankovic I, Stojanovic M (1994) Microbial and chemical composition growth therapeutical and anti-microbial characteristics of tea fungus. Microbiologija 31(1):35–43

Karki T, Okada S, Baba T, Itoh H, Kozaki M (1983) Studies on the microflora of Nepalese pickles gundruk. Nippon Shokuhin Kogyo Gakkaishi 30:357–367

Karovicova J, Kohajdova Z, Greif G (2002) The use of PCA CA FA for evaluation of vegetable juices processed by lactic acid fermentation. Czech J Food Sci 20(4):135–143

Kumar M, Nagpal R, Verma V, Kumar A, Kaur N, Hemalatha R, Gautam SK, Singh B (2013) Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutr Rev 71:23–34. https://doi.org/10.1111/j.1753-4887.2012.00542.x

Lammers KM, Brigidi P, Vitali B, Gionchetti P, Rizzello F, Caramelli E, Matteuzzi D, Campieri M (2003) Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. FEMS Immunol Med Microbiol 38:165–172. https://doi.org/10.1016/S0928-8244(03)00144-5

Lan WT, Chen YS, Yanagida F (2009) Isolation and characterization of lactic acid bacteria from Yan-dong-gua (fermented wax gourd) a traditional fermented food in Taiwan. J Biosci Bioeng 108(6):484–487. https://doi.org/10.1016/j.jbiosc.2009.06.009

Lee JS, Heo GY, Jun WL, Oh YJ, Park JA, Park YH, Pyun YR, Jong SA (2005) Analysis of kimchi microflora using denaturing gradient gel electrophoresis. Int J Food Microbiol 102(2):143–150. https://doi.org/10.1016/j.ijfoodmicro.2004.12.010

Liong MT (editor) (2011) Probiotics: biology genetics and health aspects Berlin: Springer-Verlag

Liu CH, Hsu SH, Lee FL, Liao CC (1996) The isolation and identification of microbes from a fermented tea beverage Haipao and their interactions during Haipao fermentation. Food Microbiol 13:407–415

Liu JR, Wang SY, Lin YY, Lin CW (2002) Antitumor activity of milk kefir and soya milk kefir in tumor bearing mice. Nutr Cancer 44:183–187. https://doi.org/10.1207/S15327914NC4402_10

Lopitz FO, Rementeria A, Elguezabal N, Garaizar J (2006) Kefir: una comunidad simbiótica de bacterias y levaduras con propiedades saludables. Rev Iberoam Micol 23:67–74. https://doi.org/10.1016/S1130-1406(06)70016-X

Malbasa R, Loncar E, Djuric M (2008) Comparison of the products of Kombucha fermentation on sucrose and molasses. Food Chemistry 106(3):1039–1045. https://doi.org/10.1016/j.foodchem.2007.07.020

Malbasa R, Loncar E, Vitas J, Canadanovic-Brunet JM (2011) Influence of starter cultures on the antioxidant activity of kombucha beverage. Food Chem 127(4):1727–1731. https://doi.org/10.1016/jfoodchem201102048

Mathipa MG, Thantsha MS (2017) Probiotic engineering: towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. Gut Pathogens 9(1):28. https://doi.org/10.1186/s13099-017-0178-9

Matsuzaki T, Chin J (2000) Modulating immune responses with probiotic bacteria. Immunol Cell Biol 78:67–73. https://doi.org/10.1046/j.1440-1711.2000.00887.x

Mayser PS, Fromme C, Leitzmann, Gruender K (1995) The yeast spectrum of the 'tea fungus Kombucha' Das Hefespektrum des 'Teepilzes Kombucha. Mycoses 38:289–295

Medrano M, Pérez PF, Abraham AG (2008) Kefiran antagonizes cytopathic effects of *Bacillus cereus* extracellular factors. Int J Food Microbiol 122:1–7. https://doi.org/10.1016/j.ijfoodmicro.2007.11.046

Murcia-Martinez AJ, Collins MD (1990) A phylogenetic analysis of the genus *Leuconostoc* based on reverse transcriptase sequencing of 16S rRNA. FEMS Microbiol Lett 58:73–83

Nguyen DTL, Van Hoorde K, Cnockaert M, De Brandt E, Aerts M, Vandamme PA (2013) Description of the lactic acid bacteria microbiota associated with the production of traditional fermented vegetables in Vietnam. Int J Food Microbiol 163(1):19–27. https://doi.org/10.1016/j.ijfoodmicro.2013.01.024

Niers LEM, Timmerman HM, Rijkers GT, Van Bleek GM, Van Uden NOP, Knol EF, Kapsenberg ML, Kimpen JLL, Hoekstra MO (2005) Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. Clin Exp Allergy 35:1481–1489. https://doi.org/10.1111/j.1365-2222.2005.02375.x

Nychas GJE, Panagou EZ, Parker ML, Waldron KW, Tassou CC (2002) Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. Lett Appl Microbiol 34(3):173–177. https://doi.org/10.1046/j.1472-765x.2002.01077.x

Ogunremi OR, Sanni AI, Agrawal R (2015) Probiotic potentials of yeasts isolated from some cereal-based Nigerian traditional fermented food products. J Appl Microbiol 119:797–808. https://doi.org/10.1111/jam12875

Olivares M, Diaz-Ropero MP, Martin R, Rodriguez JM, Xaus J (2006) Antimicrobial potential of four Lactobacillus strains isolated from breast milk. J Appl Microbiol 101:72–79

Oliveira A, Miguel ML, Peixoto MAL, Rosado RS, Silva AS, Paschoalin JT (2013) Microbiological technological and therapeutic properties of kefir: a natural probiotic beverage. Braz. J Microbiol 44:341–349. https://doi.org/10.1590/S1517-83822013000200001

Orgeron RP II, Corbin A, Scott B (2016) Sauerkraut: A probiotic superfood. Funct Foods Health Dis 6(8):536–543

Ozen M, Dinleyici EC (2015) The history of probiotics: the untold story. Benefic Microbes 6 (2):159–165

Psani M, Kotzekidou P (2006) Technological characteristics of yeast strains and their potential as starter adjuncts in Greek-style black olive fermentation. World J Microbiol Biotechnol 22:1329–1336. https://doi.org/10.1007/s11274-006-9180-y
Radita R, Suwanto A, Kurosawa N, Wahyudi AT, Rusmana I (2017) Metagenome analysis of tempeh production: Where did the bacterial community in tempeh come from? Malays J Microbiol 13(4):280–288
Ricci-Cabello I, Olalla Herrera M, Artacho R (2012) Possible role of milk-derived bioactive peptides in the treatment and prevention of metabolic syndrome. Nutr Rev 70(4):241–255
Rocha-Ramírez LM, Pérez-Solano RA, Castañón-Alonso SL, Moreno Guerrero SS, Ramírez Pacheco A, García Garibay M, Eslava C (2017) Probiotic Lactobacillus strains stimulate the inflammatory response and activate human macrophages. J Immunol Res. 2017:1. https://doi.org/10.1155/2017/4607491
Roussin MR (1996) Analyses of Kombucha ferments: report on growers Information Resources LC Salt Lake City Utah
Sachdeva A, Rawat S, Nagpal J (2014) Efficacy of fermented milk and whey proteins in Helicobacter pylori eradication: A review. World J Gastroenterol: WJG 20(3):724. https://doi.org/10.3748/wjg.v20.i3.724
Sarangthem K, Singh TN (2003) Microbial bioconversion of metabolites from fermented succulent bamboo shoots into phytosterols. Current Sci 84:1544–1547
Sesena S, Anchez-Hurtado IS, Gonz MA, Viñas MAG, Palop L (2001) Contribution of starter culture to the sensory characteristics of fermented Almagro eggplants. Int Food Microbiol 67 (3):197–205
Sonnenborn U, Schulze J (2009) The non-pathogenic Escherichia coli strain Nissle 1917 – features of a versatile probiotic. Microb Ecol Health Dis 21:122–158. https://doi.org/10.3109/08910600903444267
Steinkraus KH (1997) Classification of fermented foods: worldwide review of household fermentation techniques. Food Control 8:311–317
Tamang JP (2009) Himalayan Fermented Foods: Microbiology Nutrition and Ethnic Values. CRC Press, New Delhi
Tamang JP, Sarkar PK (1993) Sinki: A traditional lactic acid fermented radish tap root product. J Gen Appl Microbiol 39(4):395–408. https://doi.org/10.2323/jgam.39.395
Tamang B, Tamang JP (2007) Role of lactic acid bacteria and their functional properties in Goyang a fermented leafy vegetable product of the Sherpas. J Hill Res 20:53–61
Vendt N, Grünberg H, Tuure T, Malminiemi O, Wuolijoki E, Tillmann V, Sepp E, Korpela R (2006) Growth during the first 6 months of life in infants using formula enriched with Lactobacillus rhamnosus GG: double-blind randomized trial. J Human Nutr Diet 19:51–58. https://doi.org/10.1111/j.1365-277X.2006.00660.x
Vogel RF, Lohmann M, Weller AN, Hugas M, Hammer WP (1991) Structural similarity and distribution of small cryptic plasmids of *Lactobacillus curvatus* and *L sakei* FEMS. Microbiol Lett 84:183–190. https://doi.org/10.1016/0378-1097(91)90125-t
Wang CY, Lin PR, Ng CC, Shyu YT (2010) Probiotic properties of Lactobacillus strains isolated from the faeces of breast-fed infants and Taiwanese pickled cabbage. Anaerobe 16:578–585. https://doi.org/10.1016/j.anaerobe.2010.10.003
Witthuhn RC, Schoeman T, Britz TJ (2004) Isolation and characterisation of the microbial population of different South African kefir grains. Int J Dairy Technol 57:33–37. https://doi.org/10.1111/j1471-0307200400126x
Yan PM, Xue WT, Tan SS, Zhang H, Chang XH (2008) Effect of inoculating lactic acid bacteria starter cultures on the nitrite concentration of fermenting Chinese paocai. Food Control 19 (1):50–55. https://doi.org/10.1016/j.foodcont.2007.02.008
Yanping W, Nv X, Aodeng X, Zaheer A, Bin Z, Xiaojia B (2009) Effects of *Lactobacillus plantarum* MA2 isolated from Tibet kefir on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. Appl Microbiol Biotechnol 84:341–347. https://doi.org/10.1007/s00253-009-2012-x

Zanirati DF, Abatemarco M, Cicco Sandesb SH, Nicolia JR, Nunes AC, Neumanna E (2015) Selection of lactic acid bacteria from Brazilian kefir grains for potential use as starter or probiotic cultures. Anarobe 32:70–76. https://doi.org/10.1016/j.anaerobe.2014.12.007

Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E (2005) Dairy augmentation of total and central fat loss in obese subjects. Int J Obes 29(4):391. https://doi.org/10.1038/sj.ijo.0802880

Chapter 3
Indigenous Fermented Foods as a Potential Source of Probiotic Foods

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Abstract Food fermentation, known to preserve and improve the nutrition value, has been used traditionally since long back as a portion of human nutrition/diet throughout the world. Further, fermented foods provide health promoting benefits owing to the presence of functional properties that have been produced during the fermentation process by using the functional microorganisms present in the functional foods. These functional properties include probiotics properties, antimicrobial, antioxidant, peptide production, etc. These fermented foods can be exploited as a probiotic carrier due to the presence of lactic acid bacteria in them. This chapter is aimed to provide the detailed insight on fermented probiotic foods along with their benefits (nutritional and health) to the customers.

Keywords Food fermentation · Lactic acid bacteria · Probiotics · Human nutrition · Health benefits

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3.1 Introduction

The persisting evidence of science confirm the presence of nutritious and non-nutritious components in fermented food products. They bear the ability to conjugate the function of distinct targets of body which reflect upon health and welfare of consumers. It is estimated that almost 90% of the fermented foods and alcoholic drinks produced naturally all over the world are prepared at home traditionally. Various kinds of naturally functional microorganisms are observed in fermented foodstuffs and beverages (Tamang et al. 2016).

The whole period of human history has observed food fermentation as a mechanism which helped in ameliorating the dietetic value of foods. When food is being fermented, probiotic microorganisms alter the chemical composition elements of raw stuffs which are originated from plants or animals. It helps in enhancing the bioavailability of nutrients, upgrading sensory quality, bestow bio-conservative effects, and producing antioxidant and antimicrobial compounds which impact beneficially upon health (Nkhata et al. 2018).

Species of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Weissella* is linked with fermented foods and alcoholic drinks. Probiotics have been conferred as living microorganisms whose sufficient amount of dosage aids the health of the host (FAO/WHO 2002). The ultimate survival of a minimum count (10^9 colony forming units (CFU)/consumption) must be maintained till the expiry of the products and these counts depend upon the used probiotics and conferred health benefits (Forssten et al. 2011). The usage of probiotics as dietary supplements is widely viable. This chapter focuses on fermented probiotic foods along with their benefits (nutritional and health) to the customers.

3.2 Food Fermentation: Socio-Economic Importance

The usage of fermented products leaves a mark on the socio-economic aspects of a country's population which includes both the developing and developed countries. The production of fermented fruits and vegetables paves a way for earning and provides employment for millions of people around the world. Food processing plays a vital role for daily income and employment amongst the people residing in Europe, Africa, Asia, and Latin America (Anagnostopoulos and Tsaltas 2019). The traditional way of food production is one of the main factor of economic and social development which ultimately effects the progress of rural regions. The FAO has given clear notion on the rising utility of value addition and of their marketing and processing (Anon 1995). In sub-Saharan Africa in particular, a large part of the population is involved in the food processing sector and it is quite vital for a nation who are solely based on the primary production sector, and export a lot of products. Hence, the importance and popularity of fermented food and its output is increasing globally as it provides income, employment, and livelihood in rural areas. Asia has largely adopted the widespread tradition of fermentation which leaves its impact in socio-economic interests.

3.3 Probiotics: Concept and Health-Improving Properties

The composition of fermented food products may consist of probiotics, prebiotics, or their composite content. In 76 AD, the Roman historian Plinio suggested usage of fermented milk products in the aid of gastrointestinal disorders (Bottazzi 1983). Another scientific proposal was made by the French pediatrician Tissier in the beginning of 1900s, who advocated the beneficial effect of Bifidobacteria for preventing infections in infants (Ishibashi and Shimamura 1993).

The evolution of probiotics came from two words “pro” and “bios” which means “for” and “life,” respectively. Noble Prize winner Eli Metchnikoff proposed the concept of the positive effect of microorganisms in the health usage of human beings (Fuller 1992). Later his work showed the efficacy of *Lactobacillus bulgaricus* in putting off pathogenic bacteria from intestinal microflora that in turn exerts beneficial effect on human health (Metchnikoff 1907). His discovery suggested the longevity of the peasants of Bulgaria that has a connection with their increased intake of the fermented milk products consisting of lactic acid bacteria.

The probiotics concept became clearer with Lilly and Stillwell’s idea, which cited the probiotics as health promoting microbes which also trigger the growth of other beneficial bacteria (Lilly and Stillwell 1965). According to a definition proposed by Parker, probiotic and its byproducts/metabolites promote the microflora of the intestine (Parker 1974). Fuller described probiotics as live microbial food supplements which have a positive after-effect on the intestinal health of the host by aiding on its intestinal microflora (Fuller 1989). FAO and WHO introduced probiotics as the health-benefitting live microorganisms which on proper intake exert its beneficial application on gut microflora (FAO/WHO 2002).

The bacteria possessing the probiotic properties which are used frequently and originate from the microflora of the human beings generally belong to the group of Lactobacilli and Bifidobacteria. These genera of bacteria deploy positive properties in the human body, especially in their GI tract, which includes example like decreasing the level of serum cholesterol, slaking lactose intolerance, and various other beneficial factors like prevention of infection, toxicants, allergy, mutation, cancer are also being exerted. There are other beneficial effects of probiotic bacteria which cite its efficacy in producing inhibitory substances (organic acids/bacteriocins), which in return prevents the harmful microorganisms prevailing in the intestinal part of human beings. The mechanism is that the organic acids decreased the pH and hence the proliferation of pathogenic microorganisms gets hindered. The dairy products and its industries have increased the usage of probiotics as its action in alleviating allergy, diarrhea, malnutrition, etc., has got success and hence the dynamic dairy products are getting more familiar in the present scenario. The probiotic lactic acid bacteria and Bifidobacteria also play a role in the prevention and treatment of inflammatory bowel and other associated diseases.

3.4 Probiotics: Global Perspective on the Usages

Transformation of milk into yogurt, fermented milk, or cheese is quite common in western food culture. This indicates the higher usage of probiotic products in the form of fermented milk since a long period of time in Europe. Notably, industries in developed countries introduced other mechanisms for probiotics intake which includes milk and non-milk products**.** The global market for "functional foods" gained around 33 billion USD in 2003; while in Europe, the approximate trade gain surpassed 2 billion USD in that particular year only. The figure got a striking hike of 50 billion USD in 2005 (Franz et al. 2014). Western European market consumption of probiotic food is worth to 1.4 billion euros (Saxelin 2008). The maximum portion of the global functional food market is captured by probiotics and prebiotics (Figueroa-González et al. 2011), which effected their global market share that hiked to $ 15 billion (Bhadoria and Mahapatra, 2011). One of the vital sections of the fermented/functional food market in Europe is the food composed of probiotics, prebiotics, or synbiotics.

3.5 Probiotic: Application in Fermented Foods

Microorganisms are prospective sources of new enzymes. The enzymes so produced are quite stable than the enzymes parallelly accessible from other counterparts (Nguyen and Nguyen 2017). They find their usage in production of pectinases, alkaline proteases, esterases, high temperature proteases, lipases, cellulases, lignin, and amylase. The functional microorganisms' (probiotics) advantages include rapid growth, hike in food/feed productivity, hindering gastrointestinal ailments, and detoxification of toxic/antinutritional substances present in the food components. Many reports have suggested link of probiotics with elevated animal growth because of the production of several growth-related substances/enzymes by microorganisms (Seenivasan et al. 2016).

There have been reports suggesting the effect of probiotics in improving the taste of chickens and diversity intensification of intestinal flora. Probiotic bacillus showed efficacy in elevating chicken body weight. These scientific outcomes will increase the usage of probiotics in fermented foods. Information appropriate for each probiotic, including species, application areas, descriptions as well as conditions/characteristics related to storage, growth, and stress have been extracted from the BeNa Culture Collection (BNCC) database. There has been a rise in empirical proof which exhibits that intestinal microbiota is an inseparable part of the human physiological system. Dysfunctional bowel microbiota can be a causative agent of various disorders of inflammation, immunity, and metabolism. These outcomes have benefited the research on the consequences of certain probiotics on human health via intervention of their intestinal microbiota. The World Organization for Gastroenterology (WGO) presented proof and recommended the usage of probiotics in the prevention and cure of ailment. The application of probiotics is getting wider as their exploration suggested their therapeutic efficacy on human health and for the growth, protection, and nurturing of animal health (Dimitroglou et al. 2011; Ringø and Song 2016). Probiotic application in plants can also intensify their growth and yield.

3.6 Fermented Foods and Beverages

Various types of microorganisms like fungi, yeast, and bacteria dwell in fermented foods. Presence of microorganisms is found in materials, tools, vessels, and the atmosphere, and the particular microorganisms are selected/isolated depending on their capability to survive/adapt with the substrate (Hesseltine 1983; Steinkraus 1996). During the onset of fermentation, microorganisms have the ability to metabolize raw material constituents while increasing nutritional value and maintaining the integrity of the final product. Microorganisms regulate various features (acidity, taste, texture, and health beneficial properties) of fermented foods apart from basic nutrition (Vogel et al. 2011). They can be found as native food microbiota or intentional addition of microorganisms during the course of fermentation (Stevens and Nabors 2009). As a result, microbial culture has the ability to synthesize numerous components/compounds specifically for applications as food additives or as part of the food fermentation process (Longo and Sanromán 2006). It is widely recognized that out of the 5000 fermented food and beverage varieties, only a few uncommon products have been characterized properly; and even many more are having unclear understanding of biological and microbiological fermentation processes (Tamang 2010). Some data are available for identification and initial characterization of primary microbiota in the finished product. Probable existence of more results will require an additional comprehensive understanding of the microorganisms (types/activities) involved to make the fermentation process more consistent and foreseeable. Indeed, finding of these microorganisms will enhance the food fermentation process. Inoculation of raw materials with well-defined cultures makes it possible to manage the fermentation process with little variation. During the twentieth century, the wine, dairy, and meat products industries have guided production procedures for the use of starter cultures that were well marked and defined. Initially, starter culture was isolated from previous fermentation which was maintained and propagated at the production site. At present, starter culture is a product of broad characterization of strains/isolates through physiological, biochemical, and molecular methodologies, as well as extensive genetic characterization. In addition, the novice industries are currently adopting a breeding approach where possible and prepare genetic modification wherever possible, after it is accepted publicly. The application of microbiological technologies and processes has significantly improved the quality of fermented food products (Morgensen et al. 2002). Microorganisms used in fermented food products are categorized as bacteria (mainly the LAB group), yeast, and fungi (Table 3.1).

The existence of dominant microorganisms categorized the fermented milk in to several types. The first relates to lactate fermentation, which is dominated by LAB species and are of the types of thermophilic, probiotic, and mesophilic. The other type is fungal-lactic fermentation where the species of LAB and yeast works together to produce end products with alcoholic and moldy milk (Ghosh et al. 2019). In addition, there are two starter cultures used in milk fermentation depending upon the function/action, first culture participated in acidification and the another one in aroma development and maturation (Roos and Vuyst 2019).

Fermented cereal-based foods are well documented in Europe, America, and Australia (de Vuyst and Vancanneyt 2009). It is important to mention that grain fermentation is carried out by composite microbial ecosystems, mainly dominated by LAB and yeast species. Fermentation provides typical dough having characteristics features (palatability, high sensory quality, etc.) (Corsetti and Settanni, 2007).

Different methods have been adopted by the resident of coastal, lakes, and rivers areas to preserve fish and their byproducts. Methods like fermentation, sun drying, smoking, and salting have customarily been carried out to preserve fish products that are being utilized as a seasoning, condiments, and curry (Salampessy et al. 2010).

It is also suggested that, as in the products fermented milk, some strains of LAB have a high survival rate in plant material that is fermented, or even greater than the probiotic milk. The higher survivability might be linked to the microstructure of plants which creates favorable conditions and protects the survival of bacteria. In addition, cell walls are strong enough and resistant for the survival of bacteria.

Alcoholic drinks are typically formed by fermenting sugar through the action of yeast, which includes strains of *S. cerevisiae* species. The selection of the right yeast strain is important not only for maximizing the production limit but also for maintaining the aromatic/sensory excellence of drinks. *S. cerevisiae*, the important microorganism of alcoholic fermentation, significantly influences the aromatic properties of different drinks. Yeast brewing is also part of the genus *Saccharomyces*, but different species are utilized for brewing blonde beer. *S. cerevisiae* strains customarily carry out "top fermentation" where the yeast congregates on the surface fermenting wort. There are several types of ale yeast with complex genetic characteristics. This yeast culture performs "basic fermentation" that leads to flocculation. This facilitates yeast culture to get accumulated at the bottom of the fermenter vessel at the end of the fermentation process. Vinification involves extraction of grape juice followed by yeast fermentation. For whiskey and other grain-based spirit production, fermentation is carried out by specific *S. cerevisiae* strains that convert mashed sugar to ethanol, carbon dioxide, and many secondary metabolites that work collectively as flavor congeners in the final spirit (Russell and Stewart, 2014). Therefore, the right selection of yeast strains is very much crucial as it contributes to the organoleptic quality of the spirit. Maltose and maltotriose are the extracted sugars following cereal mashing in contrast to glucose, fructose, and sucrose which are liberated from grape crushing in must. In the production of malt whiskey and Scottish whiskey,

Table 3.1 Usage of functional microorganisms in fermented food products and their functional properties

Group	Genus	Microorganism	Morphological properties	Fermented products	Functional properties (General, nutritional, and health beneficial)	References
Bacteria	*Lactobacillus*	*Lb. acidophilus, Lb. alimentarius, Lb. brevis, Lb. buchneri, Lb. casei Lb. ghanensis, Lb. kefiri, Lb. kimchi, Lb. pentosus, Lb. plantarum*	Non-spore forming, organotroph, gram-positive, nonaerobic acid-tolerant. rod-shaped, fermentative	Ricotta cheese, fermented milks, fermented sausages, wine, sour-dough, kefir, kimchi	Production of organic acids. Inhibition of proliferation of harmful, decaying, and toxi-genic bacteria. Reduction of bitter taste in cit-rus juice. Bio-preservation of meat	Axelsson (1993), Holzapfel et al. (1995)
	Pediococcus	*Pediococcus acidilactici, P. pentosaceus*	Gram-positive, nitrate reduction, catalase activity, spore forming	Fermented meat	Beneficial in the treatment of constipation, diarrhea, reliev-ing stress, and enhancing immune response. Bio-preservation of meat	
	Bacillus	*Bacilllus acidopullululiticus, B. coagulans, B. licheniformis, B. subtilis*	Gram-positive, semi-anaerobic, endospore, rod-shaped, catalase posi-tive, and motile	Pullulanases; cocoa, fermented soybeans	Characteristic sticky texture of product	Gordon et al. (1973), Urushibata et al. (2002)
	Micrococcaceae	*Staphylococcus; Micrococcus*	Cocci gram-positive, aero-bic, non-spore, non-motile, and catalase positive	Fermented meat, fish products	Improvement of color stability of dry meat. Prevention of rancidity	Schleifer (1986), Villar et al. (2000), Wu et al. (2000), Papamanoli et al. (2002)
Yeast	*Candida*	*Candida famata, C. guilliermondii, C. krusei*	Small, oval, measuring 2–4 µm in diameter.	Blue vein fermented cheese	Production of secondary metabolites. Exerts enzymatic activity	Tamang and Tamang (2009)

(continued)

Table 3.1 (continued)

Group	Genus	Microorganism	Morphological properties	Fermented products	Functional properties (General, nutritional, and health beneficial)	References
			Unicellular, reproduce by budding	and meat. Citric acid, kefir, sourdough		
	Saccharomyces	*S. bayanus, S. cerevisiae, S. florentius, S. pastorianus, S. sake, S. unisporus,*	Prolate spheroids, smooth, glabrous	Kefir, juice, wine, bread, and sake	Production of secondary metabolites. Inhibition of the growth of mycotoxin-producing fungi. Exerts some enzymatic activities	Tamang and Tamang (2009)
Fungus	*Rhizopus*	*Rhizopus oligosporus, R. oryzae*	Non-motile, multinucleate spores	Tempe, soybean sauce, koji	Production of enzymes (maltase, invertase, pectinase, α-amylase, cellulase, hemicellulase, acid/alkaline protease, lipase). Degradation of antinutritional factors. Enhancement of mineral bioavailability	Nout and Aidoo (2002)

small branched maltodextrin molecules remain in necessity after brewing, and some types of whiskey yeast strains use these oligosaccharides because no exogenous enzymes are permitted, in accordance with the Scotch Whiskey Regulations of 2009. The overview of various fermented products of different regions has been depicted in Table 3.2.

3.7 Fermented Foods: Nutritional and Health Benefits

The fermentation can improve the nutritional quality of food. It can help in improvement of the digestibility and beneficial components of fermented foods. Some antimicrobial compounds (peroxides from hydrogen, diacetyl, bacteriocin, organic acids, etc.) are induced at the time of food fermentation. They have an impact on the growth of bacteria and conversely, increase the duration of food preservation. Lactic acid (produced during fermentation) content in food products can increase the utilization/adsorption of certain elements (vitamin D, calcium, phosphorus, iron, etc.). Foods obtained through fermentation possess various enzymes and each enzyme may act differently in improving the quality of the food. Lactase present in fermented food products reduces lactose to galactose (an important element of the cerebellum); therefore, helps in developments of brains during the growth phase of infants. Similarly, proteinases hydrolyse the casein chain into digestible peptides.

3.7.1 Fermented Milk Products

Probiotics obtained from fermented milk products have beneficial effect on humans. The probiotics contained in yogurt possess therapeutic properties and help in prevention of various diseases (Meydani and Ha 2000). The probiotics can decrease gastrointestinal problems, viz. constipation, diarrhea, inflammation of the intestine, dyspepsia, dysentery. Additionally, they have antibacterial effects on the intestine, antitumor properties, and can also help in improvement of the nutritional quality of food and can be used in treatment for pulmonary tuberculosis.

3.7.2 Fermented Soy/Cereal Products

Probiotics obtained from fermented soy/cereal-based products have beneficial effect on human health. Soybeans contain many compounds with various biological properties that have positive effect on human health (Choi et al. 2011). Soy products can improve the quality of nutrition and boost the immune system. By deploying beneficial microorganisms and their metabolites, value/functional addition has been

Table 3.2 Overview of various fermented products of different regions

Product category	Fermented products	Region/country	Microorganism	Functional properties	References
Fermented milk products	Cheese	Several countries	Lactobacillus sp., Lactococcus sp., Leuconostoc sp., Enterococcus sp., Penicillium sp., Debaryomyces sp.	Flavor and texture development. Creation of buffering against the high acidic environment	Quigley et al. (2011)
	Yogurt	Russia, Bulgaria	*Streptococcus thermophilus*, *Lactobacillus delbrueckii*	High in nutritious substances	Tamime and Robinson (2007)
	Kefir	Mainly in Eastern Europe	Lactobacillus sp., Lactococcus sp., Leuconostoc sp., Candida sp.	Rich in vitamins (water-soluble), protein, and minerals	Mayo et al. (2010), De Ramesh et al. (2006)
	Dahi	India, Nepal, Pakistan, Bhutan, and Bangladesh	Lactobacillus sp., Lactococcus sp., Streptococcus sp., Leuconostoc sp., Weissella sp., Saccharomycopsis sp., Candida sp.	Helpful in the prevention of various disorders related to gastro-intestine. Helpful in improving digestion and other ill effects in gastrointestinal tract	Harun-ur-Rashid et al. (2007)
	Koumiss	Turkey, Cyprus, Greece, Macedonia, Bulgaria	Lactobacillus sp., Saccharomyces sp.	Possess better healthy properties due to the presence of additional peptides and bactericide substances	De Ramesh et al. (2006)
	Cultured Buttermilk	Mainly in Eastern Europe	Lactococcus sp., Leuconostoc sp.	Helpful in the stimulation of the power of digestion and in the treatment of hemorrhoids, IBS, and other abdominal disorders	Mayo et al. (2010)
	Bulgarian buttermilk	Russia, Bulgaria, Hungary	Lactobacillus sp.	Rich in potassium, vitamin B12, calcium, riboflavin, and phosphorus	Mayo et al. (2010)
Cereal Products	Sourdough	Egypt	Lactobacillus sp., Issatchenkia sp.	Provides several minerals (iron and selenium)	Moroni et al. (2011)
	Tarhana	Turkish	Lactobacillus sp., Streptococcus sp., Saccharomyces sp.	Preventive/therapeutic effects on constipation and bacterial diarrhea	Sengün et al. (2009)

	Dosa	India and Sri Lanka	Leuconostoc sp., Enterococcus sp., Candida sp.	Helpful in the digestion. Rich in carbohydrates and protein	Soni et al. (1986)
	Boza	Kazakhstan, Turkey, Kyrgyzstan	Lactobacillus sp., Leuconostoc sp., Saccharomyces sp.	Helpful in maintaining blood pressure. Enhance milk production in lactating women as well as ease in digestion	Blandino et al. (2003)
Meat Products	Salami	Europe	Lactobacillus sp., Staphylococcus sp., Macrococcus sp.,	Rich in vitamin B12 and sodium	Toldra (2007)
	Kargyong	India and Nepal	LAB, Micrococci	Rich in dietary potassium. Low in calorie count and cholesterol & saturated fats	Rai et al. (2010)
	Alheira	Portugal	Lactobacillus sp., Enterococcus sp., Leuconostoc sp., Pediococcus sp.	Rich in antimicrobial end products (organic acids, ethanol, and bacteriocins)	Albano et al. (2009)
	Sausages	Spanish	Lactobacillus sp., Leuconostoc sp., Weissella sp.	Rich in sodium salt	Encinas et al. (2000)
Fish Products	Balao-balao	Philippines	Lactobacillus sp., Enterococcus sp., Leuconostoc sp., Pediococcus sp.	Rich in bacteriocin	Panda et al. (2011)
	Burong dalag	Philippines	Lactobacillus sp., Leuconostoc sp.	Inhibition of harmful bacteria	Panda et al. (2011)
	Ika-Shiokara	Japanese	Micrococcus sp., Staphylococcus sp., Debaryomyces sp.	Rich in vitamin E, vitamin B12, and taurine	Steinkraus (1995)
	Narezushi	Japan	Leuconostoc sp., Lactobacillus sp.	Prevention of constipation and help in lowering cholesterol	Steinkraus (1995)
	Sukuti	Nepal, Himalayan part of India and Tibet	Lactococcus sp., Lactobacillus sp., Enterococcus sp., Candida sp., Saccharomycopsis sp.	Helpful in mitigation of incidence of heart attacks. Keeps the body's circulation functioning well	Thapa and Tamang (2006)
	Plaa-som	Thai	Lactobacillus sp., Lactococcus sp., Streptococcus sp., Pediococcus sp.	Rich in protein, mineral, vitamins, and sodium	Saithong et al. (2010), Hwanhlem et al. (2011)

(continued)

Table 3.2 (continued)

Product category	Fermented products	Region/country	Microorganism	Functional properties	References
Fermented Vegetables	Sauerkraut	Germany	Lactobacillus sp., Leuconostoc sp.	Provide vitamins and fiber	Johanningsmeier et al. (2007)
	Kimchi	Korea	Lactobacillus sp., Leuconostoc sp., Saccharomyces sp., Pichia sp.	Possess antimicrobial and antitumor activity. Prevention of constipation	Shin et al. (2008), Jung et al. (2011)
	Olives	Japanese	Lactobacillus sp., Leuconostoc sp., Saccharomyces sp., Candida sp., Pichia sp.	Rich in vitamin E and antioxidants	Watanabe et al. (2009)
	Suan-tsai	Taiwan	*Lb. futsaii, P. pentosaceus, T. halophilus, Lb. farciminis, Lc. mesenteroides, Lc. pseudomesenteroides,*	Production of Plantaricin	Chao et al. (2009), Chen et al. (2006)
	Pao cai	China and Taiwan	Lactobacillus sp., Leuconostoc sp., Pediococcus sp.	Strengthening the spleen. Promoting digestive, antioxidative, antibacterial activity. Helpful in lowering cholesterol. Prevention of hypertension and tumors	Yan et al. (2008)

made possible in to cereals. Fermentation of cereals can be helpful in improving the digestion and reducing the blood cholesterol levels.

3.7.3 Fermented Fruits and Vegetables

Traditional probiotics food prepared from fermented vegetables or fruits exhibit health-benefitting effects on humans. Some fermented vegetable products have been used traditionally as a part of human nutrition since ancient time (Swain and Ray 2014). Health efficacies of fermented vegetables include against several diseases (cancer, obesity, constipation, etc.).

The toxin/anti-nutrients compounds present in most of the fruits and vegetables can be easily removed/detoxified through the use of microorganisms during the progression of fermentation. Plant's anti-nutrients compounds generally interfere with the absorbed nutrients. They can also adversely affect the physiological functions. It has been proven that many alternative processing/cooking procedures are allowed to diminish the anti-nutrients quantity and therefore to reduce the side effects.

Kimchi prepared from vegetables contributes to health in the same way like yogurt, which is a milk probiotic food. Kimchi is considered as the most renowned traditional Korean fermented food. There are almost 200 types of kimchi available in Korea and their variation is mainly dependent on the constituents used (vegetables, herbs, etc.) as well as preparation approaches. Its effective properties work quite well against inflammation, aging, etc. (Patra et al. 2016). The anti-cancer effect of LAB from kimchi is due to suppressed effect of enzyme activating carcinogenesis. Bioactive kimchi compounds, when given to hypercholesterolemia rabbit, reported a decrease in cholesterol (plasma/LDL) level (Kim et al. 2004). In addition, *L. plantarum* is evaluated as a probiotic with a potential to reduce the cholesterol.

3.7.4 Bio-Preservation

Currently, consumers are well acquainted with the health problems associated with food additives. On the contrary, health beneficial aspects associated with "natural" and "traditional" foods without any food additives are numerous and hence attract a lot of attention. LAB has been used significantly for thousands of years for the preservation of fermented food which eventually hiked the microbial stability of the fermented end product. LAB bacteriocin has been consumed by human civilization since long back as a LAB product. They can, therefore, be considered as natural food ingredients. The production of bacteriocin has been found in many bacterial species. It has been given "generally recognized as safe" status, which gives scientists the capability to enable the development of flora which is desirable in food fermentation or it may prevent the development of undesirable bacteria species in fermented and non-fermented foods by using broad and narrow host range bacteriocin, respectively (Swain et al. 2014).

3.7.5 Shelf Life of Probiotics

Fermentation is known to increase both preservation and safety of the product, and even enzymatic activity can bring desirable changes in the food in order to make them more digestible. In order to ensure process consistency and product quality, fermentation decreases the toxicity of the substrate and can improve the aroma and taste of a product, making it delicious (Blandino et al. 2003). Various fermented food microorganisms are able to degrade mycotoxins into less toxic or non-toxic products. Lactic acid/organic acids produced during food fermentation help in maintaining the pH of the large intestine to a satisfactory level, which in turn hinders the proliferation of unwanted/harmful bacteria (*H. pylori*, Clostridium spp., etc.), molds, and yeast, especially Candida (Lopez et al. 2002).

3.8 Conclusions and Future Prospects

Most fermented foods and drinks require extensive research for their microbiological, physical, and chemical properties. It will help to develop better organoleptic characteristics. The relationship between microorganisms, especially between bacteria and yeast populations, is an important factor that contributes to the final properties. It also helps in influencing the substrate and additives that can help in developing the efficacy of organoleptic and kinetics of fermentation. Microbiological metagenomic study will further provide a new knowledge-based approach that extends from the utilization of microorganisms in food fermentation to the engineering of microorganism's metabolism and in this way will help in production of antimicrobials or nutrients.

References

Albano H, Van-Reenen CA, Todorov SD, Cruz D, Fraga L, Hogg T et al (2009) Phenotypic and genetic heterogeneity of lactic acid bacteria isolated from "Alheira", a traditional fermented sausage produced in Portugal. Meat Sci 82:389–398

Anagnostopoulos DA, Tsaltas D (2019) Fermented Foods and Beverages. Innovations in traditional foods. https://doi.org/10.1016/B978-0-12-814887-7.00010–1

Anon (1995) Roads: a catalyst for development. Munic Eng 26(9):35–37

Axelsson LT (1993) Lactic acid bacteria: classification and physiology. In: Salminen S, Von Wright A (eds) Lactic acid bacteria. Springer, Dordrecht, p 164

Bhadoria PBS, Mahapatra SC (2011) Prospects, technological aspects and limitations of probiotics — a worldwide review. Eur J Food Res Rev 1:23–42

Blandino A, Al-Aseeri ME, Pandiella SS, Cantero D, Webb C (2003) Cereal-based fermented foods and beverages. Food Research International 36(2003):527–543

Bottazzi V (1983) Food and Feed Production with microorganisms. BioTechnol 5:315–363

Chao SH, Wu RJ, Watanabe K, Tsai YC (2009) Diversity of lactic acid bacteria in suan-tsai and fu-tsai, traditional fermented mustard products of Taiwan. Int J Food Microbiol 135:203–210

Chen YS, Yanagida F, Hsu JS (2006) Isolation and characterization of lactic acid bacteria from suan-tsai (fermented mustard), a traditional fermented food in Taiwan. J Appl Microbiol 101:125–130

Choi J, Park KY, Park K-Y, Yu BP, Kim ND, Jung JH, Chung HY (2011) The anti-inflammatory action of fermented soybean products in kidney of high-fat-fed rats. J Med Food 14(3):232–239

Corsetti A, Settanni L (2007) *Lactobacilli* in sourdough fermentation. Food Res Int 40(5):539–558

de Ramesh CC, White CH, Kilara A, Hui YH (2006) Manufacturing yogurt and fermented milks. Blackwell Publishing, Oxford

De Vuyst L, Vancanneyt M (2009) Biodiversity and identification of sourdough lactic acid bacteria. Food Microbiol 24:120–127

Dimitroglou A, Merrifield DL, Carnevali O, Picchietti S, Avella M, Daniels C, Güroy D, Davies SJ (2011) Microbial manipulations to improve fish health and production-a mediterranean perspective. Fish Shellfish Immunol 30(1):1–16

Encinas JP, Lopez-Diaz TM, Garcia-Lopez ML, Otero A, Moreno B (2000) Yeast populations on Spanish fermented sausages. Meat Sci 54:203–208

FAO/WHO (2002) Guidelines for the evaluation of probiotics in foods. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report. Food and Agricultural Organization of the United Nations and World Health Organization Working Group Report (online)

Figueroa-González I, Quijano G, Ramírez G, Cruz-Guerrero A (2011) Probiotics and prebiotics-perspectives and challenges. J Sci Food Agric 91:1341–1348

Forssten SD, Sindelar CW, Ouwehand AC (2011) Probiotics from an industrial perspective. Anaerobe 17:410–413

Franz CMAP, Huch M, Mathara JM, Abriouel H, Benomar N, Reid G, Galvez A, Holzapfel WH (2014) African fermented foods and probiotics. International Journal of Food Microbiology 190:84–96

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

Fuller R (1992) History and development of probiotics. In: Fuller R (ed) Probiotics - The Scientific Basis. Chapman and Hall, New York

Ghosh T, Beniwal A, Semwal A, Navani NK (2019) Mechanistic insights into probiotic properties of lactic acid bacteria associated with ethnic fermented dairy products. Front Microbiol 10:1–19

Gordon RE, Haynes WC, Pang CHN (1973) The Genus Bacillus, Handbook No. 427. United States Department of Agriculture, Washington

Harun-ur-Rashid M, Togo K, Useda M, Miyamoto T (2007) Probiotic characteristics of lactic acid bacteria isolated from traditional fermented milk "Dahi" in Bangladesh. Pak J Nutr 6:647–652

Hesseltine CW (1983) Microbiology of oriental fermented foods. Ann Rev Microbiol 37:575–601

Holzapfel WH, Giesen R, Schillinger U (1995) Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int J Food Microbiol 2:343–362

Hwanhlem N, Buradaleng S, Wattanachant S, Benjakul S, Tani A, Maneerat S (2011) Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (Plasom) and production of Plasom from selected strains. Food Control 22:401–407

Ishibashi N, Shimamura S (1993) Bifidobacteria research and development in Japan. Food Technol 47:126–127

Johanningsmeier S, McFeeters RF, Fleming HP, Thompson RL (2007) Effects of *Leuconostoc mesenteroides* starter culture on fermentation of cabbage with reduced salt concentrations. J Food Sci 72:166–172

Jung JY, Lee SH, Kim JM, Park MS, Bae JW, Hahn Y et al (2011) Metagenomic analysis of kimchi, a traditional Korean fermented food. Appl Environ Microbiol 77:2264–2274
Kim HJ, Kwon MJ, Seo JM (2004) The effect of 3 (4' hydroxyl 3'-5'dimethoxyphenyl) propionic acid in Chinese cabbage kimchi on lowering hypercholesterolemia. J Korean Soc Food Sci Nutr 33:52–58
Longo MA, Sanromán MA (2006) Production of food aroma compounds: microbial and enzymatic methodologies. Food Technol Biotechnol 44:335–353
Lopez HW, Leenhardt F, Coudray C, Remesy C (2002) Minerals and phytic acid interactions: is it a real problem for human nutrition. Int J Food Sci Technol 37:727–739
Lilly DM, Stillwell RH (1965) Probiotics: growth promoting factors produced by microorganisms. Science 147:747–748
Mayo B, Ammor MS, Delgado S, Alegria A (2010) Fermented milk products. In: Tamang JP, Kailasapathy K (eds) Fermented foods and beverages of the world. CRC Press, Taylor and Francis Group, New York, pp 263–288
Meydani SN, Ha WK (2000) Immunologic Effect of Yogurt. Am J Clin Nutr 71(4):861–872
Morgensen G, Salminen S, O'Brien J, Holzapfel A, Shortt C, Fonden R, et al. (2002) Inventory of microorganisms with a documented history of use in food, health benefits and safety evaluation of certain food components, pp. 377
Moroni AV, Arendt EK, Bello FD (2011) Biodiversity of lactic acid bacteria and yeasts in spontaneously fermented buckwheat and teff sourdoughs. Food Microbiol 28:497–502
Metchnikoff E (1907) Lactic acid as inhibiting intestinal putrefaction. In: Heinemann W (Eds) The prolongation of Life: optimistic studies. London, pp 161–201
Nguyen TH, Nguyen VD (2017) Characterization and applications of marine microbial enzymes in biotechnology and probiotics for animal health. Adv Food Nutr Res 80:37–74
Nkhata SG, Ayua E, Kamau EH, Shingiro JB (2018) Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci Nutr 6(8):2446–2458
Nout MJR, Aidoo KE (2002) Asian fungal fermented food. In: Osiewacz HD (ed) The Mycota. Springer, New York, p 2347
Panda RP, Barman HK, Mohapatra C (2011) Isolation of enriched carp spermatogonial stem cells from Labeo rohita testis for in vitro propagation. Theriogenology 76:241–251
Papamanoli E, Kotzekidou P, Tzanetakis N, Tzanetaki EL (2002) Characterization of Micrococcaceae isolated from dry fermented sausage. Food Microbiol 19:441–449
Patra JK, Das G, Paramithiotis S, Shin HS, Paramithiotis S (2016) Kimchi and other widely consumed traditional fermented foods of Korea: a review. Front Microbiol 7:1493. https://doi.org/10.3389/fmicb.2016.01493
Parker RB (1974) Probiotics, the other half of the antibiotics story. Anim Nutr Health 29:4–8
Quigley L, O'Sullivan O, Beresford TP, Ross RP, Fitzgerald GF, Cotter PD (2011) Molecular approaches to analysing the microbial composition of raw milk and raw milk cheese. Int J Food Microbiol 150:81–94
Rai AK, Palni U, Tamang JP (2010) Microbiological studies of ethnic meat products of the Eastern Himalayas. Meat Sci 85:560–567
Ringø E, Song SK (2016) Application of Dietary Supplements (Synbiotics and Probiotics in Combination with Plant Products and β-glucans) in Aquaculture. Aquac Nutr 22(1):4–24
Roos JD, Vuyst LD (2019) Microbial acidification, alcoholization, and aroma production during spontaneous lambic beer production. Sci Food Agric 99:25–38
Russell I, Stewart GG (2014) Whisky: technology, production and marketing, 2nd edn. Academic Press/Elsevier, Boston
Saithong P, Panthavee W, Boonyaratanakornkit M, Sikkhamondhol C (2010) Use of a starter culture of lactic acid bacteria in plaa-som, a Thai fermented fish. J Biosci Bioeng 110:553–557
Salampessy J, Kailasapathy K, Thapa N (2010) Fermented fish products. In: Tamang JP, Kailasapathy K (eds) Fermented foods and beverages of the world. CRC Press, Taylor and Francis Group, New York, pp 289–307

Saxelin M (2008) Probiotic formulations and applications, the current probiotics market, and changes in the marketplace: a European perspective. Clin Infect Dis 46:S76–S79

Schleifer KH (1986) Family I. Micrococcaceae Prevot 1961, 31. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 2. Williams & Wilkins, Baltimore, pp 1003–1008

Seenivasan C, Radhakrishnan S, Muralisankar T, Saravana Bhavan P (2016) Effects of probiotics on survival, growth and digestive enzymes activities in freshwater Prawn Macrobrachium Rosenbergii (De Man 1879). Proc Zool Soc 69(1):52–60

Sengün IY, Nielsen DS, Karapinar M, Jakobsen M (2009) Identification of lactic acid bacteria isolated from Tarhana, a traditional Turkish fermented food. Int J Food Microbiol 135:105–111

Shin MS, Han SK, Ryu JS, Kim KS, Lee WK (2008) Isolation and partial characterization of a bacteriocin produced by *Pediococcus pentosaceus* K23-2 isolated from kimchi. J Appl Microbiol 105:331–339

Soni SK, Sandhu DK, Vilkhu KS, Kamra N (1986) Microbiological studies on dosa fermentation. Food Microbiol 3:45–53

Steinkraus KH (1995) Classification of household fermentation techniques. In: Background Paper for WHO/FAO Workshop on Assessment of Fermentation as Household Technology for Improving Food Safety. Dec. 1115, Dept. of Health. Pretoria, South Africa

Steinkraus KH (1996) Handbook of indigenous fermented food, 2nd edn. Marcel Dekker, New York

Stevens HC, Nabors L (2009) Microbial food cultures: a regulatory update. Food Technol 63:36–41

Swain MR, Ray RC (2014) Fermented fruits and vegetables of asia: a potential source of probiotics. Biotechnol Res Int 2014:250424

Swain MR, Anandharaj M, Ray RC, Rani RP (2014) Fermented fruits and vegetables of Asia: a potential source of probiotics. Biotechnol Res Int. https://doi.org/10.1155/2014/250424

Tamang JP (2010) Diversity of fermented foods. In: Tamang JP, Kailasapathy K (eds) Fermented foods and beverages of the world. CRC Press, Taylor and Francis Group, New York, p 4184

Tamang B, Tamang JP (2009) Lactic acid bacteria isolated from indigenous fermented bamboo products of Arunachal Pradesh in India and their functionality. Food Biotechnol 23:133–147

Tamang JP, Watanabe K, Holzapfel WH (2016) Review: diversity of microorganisms in global fermented foods and beverages. Front Microbiol 7:377

Tamime AY, Robinson RK (2007) Yoghurt science and technology. Woodhead Publishing, Cambridge

Thapa S, Tamang JP (2006) Microbiological and physico-chemical changes during fermentation of kodo ko jaanr, a traditional alcoholic beverage of the Darjeeling hills and Sikkim. Indian J Microbiol 46:333–341

Toldra F (2007) Handbook of fermented meat and poultry. Blackwell Publishing, Oxford

Urushibata Y, Tokuyama S, Tahara Y (2002) Characterization of the *Bacillus subtilis* ywsC gene, involved in λ-polyglutamic acid production. J Bacteriol 184(2):337–343

Villar I, Garcia-Fontan MC, Prieto B, Tornadizo ME, Carballo J (2000) A survey on the microbiological changes during the manufacture of dry-cured lacon, a Spanish traditional meat product. J Appl Microbiol 89:1018–1026

Vogel RF, Hammes WP, Habermeyer M, Engel KH, Knorr D, Eisenbrand G (2011) Microbial food cultures-opinion of the senate commission on food safety (SKLM) of the German Research Foundation (DFG). Mol Nutr Food Res 55:654–662

Watanabe K, Fujimoto J, Tomii Y, Sasamoto M, Makino H, Kudo Y et al (2009) *Lactobacillus kisonensis sp. nov.*, *Lactobacillus otakiensis sp. nov.*, *Lactobacillus rapi sp. nov.* and *Lactobacillus sunkii sp. nov.*, hetero fermentative species isolated from sunki, a traditional Japanese pickle. Int J Syst Evol Microbiol 59:754–760

Wu YC, Kimura B, Fujii T (2000) Comparison of three culture methods for the identification of Micrococcus and Staphylococcus in fermented squid shiokara. Fish Sci 66:142–146

Yan PM, Xue WT, Tan SS, Zhang H, Chang XH (2008) Effect of inoculating lactic acid bacteria starter cultures on the nitrite concentration of fermenting Chinese paocai. Food Control 19:50–55

Chapter 4
Prebiotics for Probiotics

Ankita Vinayak, Gaurav Mudgal, Swati Sharma, and Gajendra B. Singh

Abstract Owing to their perceived therapeutic effects, functional foods have been a matter of extensive research in recent years. Prebiotics, probiotics and synbiotics are the important members of this family exhibiting property that improves health, mental well-being and particularly reflected as an outcome of optimized gastrointestinal health status. The most commonly employed probiotic strains are lactobacilli, bifidobacteria, *B. coagulans* and *L. johnsoni.* Prebiotics are the oligosaccharides, either extracted from plant, mammal's milk or synthesized industrially by enzymatic hydrolysis which are utilized by probiotic microorganisms in the intestine and impart health benefits such as stimulation of gut microflora, enhancing mineral absorption and host immunity, prevention of cardiovascular disease, cancer, diabetes and lowering cholesterol levels. Prebiotics are non-digestible oligosaccharides which are fermented in the intestine. The commonly known prebiotics are inulin, fructo-oligosaccharide, galacto-oligosaccharide, lactulose, lactosucrose, xylo-oligosaccharide and resistant starch. The prebiotic compounds when used in combination with probiotic microorganism are termed synbiotics which happen to be more effective and acceptable in terms of their beneficial effects. Presently, prebiotics and its synergistic therapy, i.e. synbiotic is gaining much importance because it manages human health at the same time providing nutrition. Till now, a number of foods like vegetables, fruits such as onion, tomato, garlic, carrots, banana, etc. are known to show good prebiotic effects. Various industrially formulated prebiotic products are also available in the market that resemble naturally found prebiotics and exhibit nutritional and therapeutic effects.

Keyword Prebiotics · Synbiotics · Functional foods · Oligosaccharide · Microflora · Synergistic · Gut

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4.1 Introduction

Crisis with food availability, its quality and affordability has a long history to talk about. Followed by green revolution and food fortification programs, many attempts have foreseen possibilities to minimize the deficits. On the other side of the story, globalization and technological advancements in various industrial sectors, which our twenty-first century modernization showcases, have not only transformed the lifestyles but also equally perturbed the food habits across most countries if not the whole globe. The ills of fluctuating eating schedules, upsurges in fast food joints and technical advancements at arranging 'ready to eat' versions of food backed by ever excelling packaging technologies all of which still are driving the so-called 'spoiled work culture' of this century have all contributed negatively to impact public health. To put as examples, a 'brunch' never existed before, people now do not dine together at home more often, and in the run for the phrase 'getting late to work', actually there were not much options before such as takeaways, online fast food booking, and if one would believe there never existed too many cookery shows over the media channels before. The point is that in the hassle of finding adjustments to make our lives easier (as we planned, but wrongly) we actually have succumbed to compromising our body for its natural routines and requirements. And the aftermath of this is seen in the rising number of patients in developed countries with health diseases, syndromes and disorders such as dementia, depression, cardiovascular problems, diabetes, joint pains, arthritis, skin problems and so on. This has led to discussions over the globe towards finding a quick fix to this rising concern.

From another view scope, the world is now witnessing a wave towards maintaining health and staying physically and mentally fit. This is exhibited in communities following practices like yoga, exercises, cosmetic and gastric sleeve surgeries, diet plans, etc. Besides other above ways, a strong positive correlation between diet, gut microbiota and health is widely understood supporting that a healthy gut is a sign of whole body fitness and endurance. Many studies have also reflected a consensus over the supportive role of gut microbiome at maintaining all round health of subject which includes effects on mood, anxieties, and at minimizing symptoms of some common gastrointestinal diseases, infections to life threatening situations like cancers. Not surprisingly, that these evidences do support the age-old sayings that go like, 'You become, what you eat', 'all health problems start and solve at gut' and so on. The idea in a nutshell foresees attempts towards maintaining a healthy gut microbial stature and translating it to life's homeostasis and increments in its expectancies (Falguera et al. 2012).

One may then ask, what would be those foods that keep the gut and its microbiota happy? Rising trends have proclaimed such food products frequently categorized into 'functional foods' or 'completely planned foods', which predominantly include food ingredients with potential health benefits in addition to delivering nutritional attributes. Typically, functional foods include products enriched with vitamins, minerals, omega fatty acids and supplemented products such as milk, yoghurt, baby foods and sport's drinks (Abuajah et al. 2015). More recently new versions

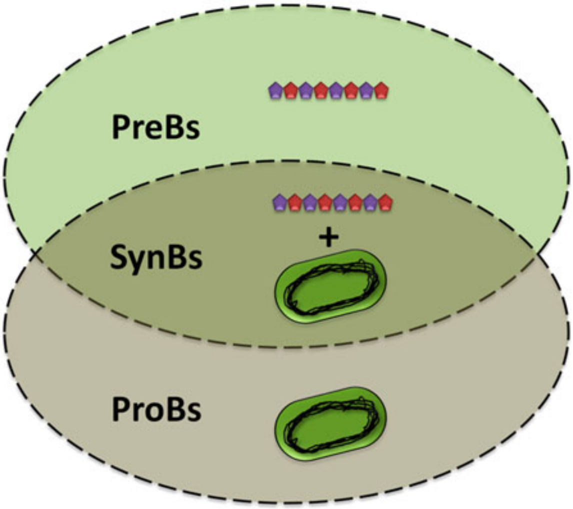

Fig. 4.1 Schematic defining emerging functional food versions. Shown here are PreBs: short chain sugars; ProBs: microbial entities native to gut microflora destined to maintain healthy gut functions; SynBs: a cocktail of PreBs and ProBs. See text for details

of functional foods have become a word of mouth which includes prebiotics (PreBs) and probiotics (ProBs). The former composes majorly significant fraction of either the fermented products alone, while the latter exclusively include microbes from the Generally Regarded As Safe (GRAS) category which naturally ferment foods in the gastric tract (Fig. 4.1). At the outset, both these versions of functional foods are increasingly being investigated under food biotechnology domain due to their nutritional and nutraceutical health benefits.

Yet another version of functional foods, called synbiotics (SynBs), has recently seen receiving more appreciation and actually is a combination of PreBs with ProBs (Fig. 4.1). The SynBs, however, were originally introduced in 1995 by Glenn R. Gibson and Roberfroid describing the use of the cocktail and its synergistic effects. Upon administration, SynBs are believed to stimulate microflora, especially probiotic microorganisms, native to the gut. SynBs exhibit a greater efficacy than PreBs or ProBs alone. The primary purpose of this synergy is to improve the survival and activity of ProBs (Mohanty et al. 2018).

The present chapter focuses on various PreBs, their sources, mode of action and their beneficial health effects. A brief account on SynBs and some of the commercially available PreBs' variants are also supplemented.

4.2 Prebiotics (PreBs)

These specifically include various short chain carbohydrates, indigestible by enzymes in the upper digestive tract. They are believed to deliver their beneficial effects in context to improving host health by stimulating metabolism of selected bacteria in the large intestine. Rather than being absorbed or hydrolyzed in the body, PreBs are fermented by bacteria, in turn altering the intestinal microflora and conferring positive effects (Al-Sheraji et al. 2013). Validation of potential ingredient to be PreBs considers three fundamental aspects: (i) resistance to the hydrolysis by digestive enzymes, (ii) fermentation by intestinal microflora and (iii) selective stimulatory effect of compound on growth and activity of gut microflora (Slavin 2013).

Among the intestinal microflora that get stimulated by the action of PreBs are the various strains of *Lactobacillus* and *Bifidobacteria* which are also added to fermented dairy products in the name of ProBs (Ahmad and Khalid 2018). The intestinal microorganisms have a natural tendency to utilize prebiotic sugars and rendering their enrichment for enhanced functional activity. Only a few non-digestible carbohydrates such as lactulose, inulin, fructo-oligosaccharide (FOS), lactulose, lactosucrose, resistant starch and galacto-oligosaccharide (GOS) are categorized into PreBs. They are resistant to hydrolysis and absorption in the mammalian digestive system (Khangwal and Shukla 2019). When administered, PreBs reach the lower gut in its undigested form by passing through small intestine where finally they are received by gut microflora for fermentation. Fermentation of the PreBs releases short chain fatty acids viz., butyric acid, acetic acid, lactic acid and propionic acid. The formation of short chain fatty acids is related to the enhancement of various physiological functions including mineral absorption, intestinal functions, regulation of glucose and lipid metabolism and reducing the risk of colon cancer (Sarao and Arora 2017). Also, the consumption of PreBs stimulates the growth of beneficial microflora and discourages the growth of pathogenic bacteria. Due to these health benefits, PreBs have been increasingly employed in food industries like in dairy products, infant feeds, bakery products and beverages (Ahmad and Khalid 2018). PreBS can be easily availed through extraction as bioactives from plants and microbes and also by biochemical means such as enzymatic hydrolysis of long chain oligo-saccharides. Many PreBs have seen commercial scale productions and are easily available as 'on counter packages' in pharmacies (Al-Sheraji et al. 2013).

4.3 How PreBs Qualify as Functional Foods?

PreBs are naturally found in plants and sometimes they are intentionally added to food products to improve nutritional values of products such as lactulose, inulin and galacto-oligosaccharide (Valcheva and Dieleman 2016). As discussed earlier, PreBs stimulate the growth and activity of probiotic microorganism in GI tract. When administered PreBs are not digested by gut microbiota, they reach unaltered into the colon. The non-digestible property of prebiotics is attributed to β-glycosidic linkage in sugar units as digestive enzymes in humans specifically cleave α-glycosidic bond (Sarao and Arora 2017). The possible mode for the action of PreBs on the probiotic organism may be direct or indirect mode (Mohanty et al. 2018). In direct mode, PreBs directly inhibit the pathogenic bacteria through the formation of antimicrobial

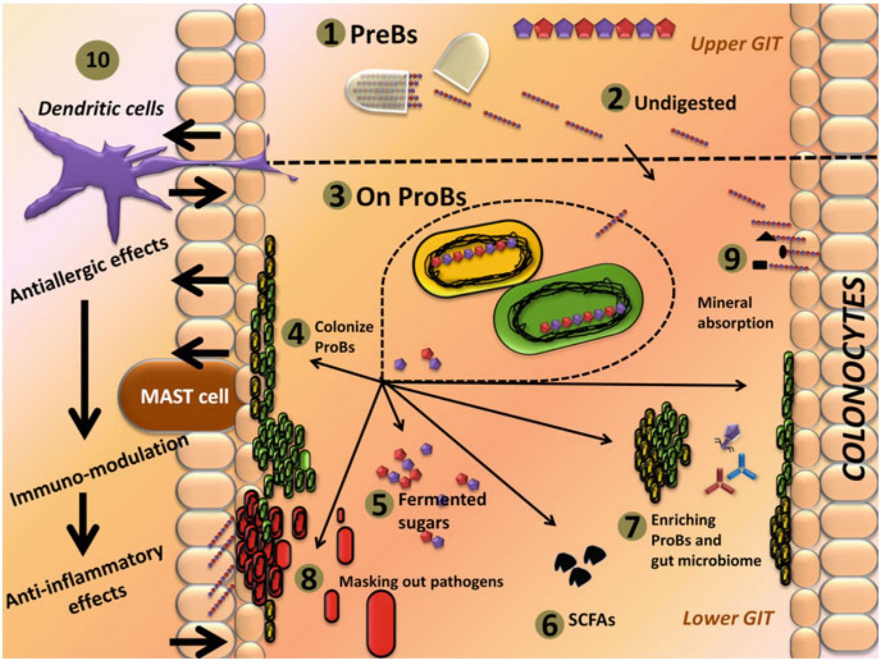

Fig. 4.2 Generalized cascade of events that suffice PreBs action and efficacy as a nutraceutical. Intake of short chain sugar PreBs (8 mer forms shown here capsulated and separately enlarged on the top right) formulation (in sequence 1) traverse through upper and lower gastrointestinal tract (GIT) (in 2 and 3). In 1 and 2 PreBs are unaffected from any enzymatic digestions in buccal cavity, stomach and the small intestine and remain structurally and functionally unaltered till they mark their entry into the lower GIT (in 3) wherein it readily is absorbed and assimilated by ProBs (microbes shown in green and yellow ovals) native to the gut. These three events are followed by a triggered cascade of reactions that variously stimulate enrichments of existing probiotics and the gut microflora as well (in 4–9). Ultimately prospective immunomodulatory changes (in 10) also effectuate in response to these changes. Ovals in red represent pathogenic microbes that are cleared by either the masking effect from increasing counts of the probiotics or otherwise favoured from the anticolonizing action of PreBs on them; shapes in 6 are the released small chain fatty acids that further contribute to de novo lipogenesis. Many PreBs do exclusively (other than following the indirect action mode involving interaction with ProBs) may directly cling onto GIT mucosa and may function as mineral absorbers, anticolonizing agents and/or favourably alter osmotic strength of the GIT fluid to positively let ProBs to flourish in lower GIT. For details see text

compounds and competing for the nutrient and adhesion to the epithelial wall. It also reduces the risk of cardiovascular diseases and gastrointestinal infections by forming the short chain fatty acids and modulating lipid and carbohydrate metabolism. Whereas in indirect mode, PreBs tend to enhance the absorption of essential minerals and nutrients like calcium and magnesium to nourish the gut microbiota for their enhanced growth (Slavin 2013). Being species specific in their mode of action, PreBs enhance the overall metabolism and microbial composition in intestine (Fig. 4.2).

4.4 Types and Sources of PreBs

PreBs comprises low molecular weight non-digestible oligosaccharide consisting of a variable number of carbohydrate units ranging from three to nine. Various non-digestible oligosaccharides such as lactulose, inulin type fructans, galacto-oligosaccharides are potential candidates for PreBs (Quigley 2019). Among all inulin types, galacto-oligosaccharide and fructo-oligosaccharide are most commonly used because they fulfil prebiotic fundaments (Huebner et al. 2007). The various types of PreBs and their natural sources are enlisted in Table 4.1.

4.4.1 Lactulose

Lactulose is a synthetic disaccharide synthesized by isomerization of lactose. It comprises galactose units linked to fructose via β-1,4 glycosidic bonds. It can be produced by enzymatic action of glucose isomerase and β-glycosidase on whey lactose. Lactulose is non-digestible for the human β-galactosidase, so it is not hydrolysed in the gut. It is fermented by intestinal microbiota whereby realizing its prebiotic effects like enhancing immunity and inhibiting pathogenic bacteria

Table 4.1 PreBs from natural sources

PreBs	Source of PreBs	References
Fructo-oligosaccharides	Asparagus, bananas, chicory root, garlic, leek, oats, onions, wheat, yacon, etc.	Sengun and Bingol (2018), Singh et al. (2016)
Galacto-oligosaccharide	Beans, chickpeas, cow/human milk, lentil, etc.	Belorkar and Gupta (2016), Cryan and Clarke (2016), Torres et al. (2010)
Inulin	Agave, artichoke, asparagus, bananas, barley, burdock camas, chicory root, coneflower, dandelion, elecampane, garlic, jicama, mugwort root, onions, rye, wheat, wild yam, yacon, etc.	Sengun and Bingol (2018), Kerry et al. (2018), Belorkar and Gupta (2016), Slavin (2013)
Isomalto-oligosaccharide	Honey, maltose, miso, sake, sauce, soy, sucrose	Anadón et al. (2016), Al-Sheraji et al. (2013), Patel and Goyal (2012)
Lactosucrose	Milk sugar	Kerry et al. (2018)
Lactulose	Milk and its products	Watson and Preedy (2015)
Resistant starch	Cereals, legumes, nuts, starchy fruits and vegetables	Anadón et al. (2016), Fuentes-Zaragoza et al. (2011), Kerry et al. (2018)
Soybean-oligosaccharide	Soybean	Espinosa-Martos and Rupérez (2006)
Xylo-oligosaccharide	Bamboo shoots, fruits, honey, milk, vegetables, wheat bran	Aachary and Prapulla (2011)

(Sitanggang et al. 2016). Very little amount of lactulose is present in natural food, so it is artificially added to enhance the nutritional values of foods like cake, yoghurt and chocolate (Nooshkam et al. 2018).

4.4.2 Lactosucrose

It is a synthetic oligosaccharide composed of glucose, fructose and galactose, synthesized from a mixture of sucrose and lactose with the aid of enzyme β-fructo-furanosidase (Silvério et al. 2015). The fermented products of lactulose beneficially alter the host intestine by reducing the pH, pathogenic bacteria and enhancing the mineral absorption. Various in vitro and in vivo studies were performed to evaluate prebiotic effect of lactosucrose. One reports that lactosucrose and its analogues enhance the growth and activity of bifidobacteria and lactobacilli (Li et al. 2015).

4.4.3 Inulin and Fructo-Oligosaccharide (FOS)

Inulin is a plant derived fructan polysaccharide stored as a nutrient in plants. It is commonly found in fruits, vegetables and edible grains such as wheat, garlic, chicory, onion, leek and bananas. It is synthesized during increasing sugar demand for photosynthesis. It is a linear chain oligosaccharide composed of fructose monomers with β-2,1 linkages exhibiting variable degrees of polymerization (Apolinário et al. 2014). It is used as a substitute for fat and sugar because of its low calorific value as it is rarely hydrolysed by digestive enzymes and thus is not used as an energy source. They are added to functional foods to increase the dietary fibre content and to exert beneficial effects in GI tract. Inulin when added to functional foods stimulates probiotic bacteria and biomass growth, simultaneously producing short chain fatty acids such as butyrate, acetate and propionate, which influences the carbohydrate and lipid metabolism in host (Walton et al. 2013).

Several reports evidence prebiotic effects of inulin in infants, adults and elder people by stimulating bifidogenic and lactobacillus in the gut. Additionally, in vivo and in vitro studies of faecal matter in models supplemented with inulin prebiotics suggest that inulin also discourages the growth of putrefactive and pathogenic bacteria in gut such as clostridium and thus aids in maintaining healthy microflora in digestive tract (Vandeputte et al. 2017).

4.4.4 Galacto-Oligosaccharide (GOS)

Galacto-oligosaccharide composed of galactose units is found naturally in the milk of mammals such as humans and cows. Industrially, they are synthesized by enzymatic action of β-galactosidase on lactose forming mixture of tri- and penta-saccharides having β-1,4, β-1,3 and β-1,6 bonds in galactose units (Tzortzis and Vulevic 2009). The fermented products of GOS augment the probiotic bacteria like bifidobacteria, enterobacteria and lactobacilli for the production of vitamins, minerals and provide immunity (Cardelle-Cobas et al. 2011).

Galacto-oligosaccharides administered to infants are reported to stimulate intestinal flora similar to bifidobacteria of breastfed infants. They also inhibit pathogenic bacteria in the intestine as they mimic the glycoconjugate cell surface receptor of pathogens. During a study it was observed that infant feeds prepared by incorporation of manufactured prebiotics like GOS could resemble breast milk especially in bifidogenic effects (Verkhnyatskaya et al. 2019). Based on this observation a mixture of prebiotics with varying concentrations of inulin and GOS has been prepared. On oral administration mixture exhibited prebiotic effect in terms of increasing gut and intestinal bacteria and faecal matter of these infants resembled those from breastfed infants (Mazzola et al. 2015). Various other studies on PreBs mixture have shown the same prebiotic and bifidogenic effect on infants and children.

4.4.5 Soybean-Oligosaccharide (SOS)

SOSs are the oligosaccharides found in soybean whey obtained during the soy protein production. They are composed of galactose and sucrose units joined by α-(1-6) linkage. When administered SOSs directly reach the colon and elicit its bifidogenic effect as digestive system in humans lacks enzyme α-galactosidase required for the digestion of SOS (Walton et al. 2013). SOS includes verbascose, raffinose and stachyose oligosaccharides, also termed as raffinose family oligosaccharides (Singh et al. 2017).

4.4.6 Xylo-Oligosaccharide (XOS)

It is another commercially synthesized disaccharide composed of pentose sugar xylose linked by β-(1-4) linkage. These are obtained by hydrolysis of xylan, constituent of hemicellulose—component of agricultural waste like husk, pulp, stalk and straw by the enzymatic action of endo 1,4-xylanse (Singh et al. 2015). XOS is fermented by bifidobacteria and lactobacilli, as it is a non-digestible dietary fibre because humans lack the β-xylosidase enzyme required for the digestion of xylan. After fermentation by intestinal bacteria, XOS is reported to show its bifidogenic effects in intestine. In vivo study that employed weaned pigs reported that XOS administration stimulated microbial communities, inflammatory status and gut barrier which is believed to aid in preventing gut dysfunction (Yin et al. 2019).

4.4.7 Isomalto-Oligosaccharide (IOS)

Isomalto-oligosaccharide is a starch derived oligosaccharide, a monomer of glucose units joined by α-(1-6) glycosidic linkages. It is synthesized industrially by enzymatic action of α-amylase, β-amylase and α-glycosidase on corn starch (Walton et al. 2013). IOS is resistant to digestion of the gut microbiota, enabling them to enter the colon and conferring bifidogenic effects. IMOs are low calorific value PreBs finding their use as sweeteners (Mitmesser and Combs 2017). They are added to commonly consumed foodstuff particularly to beverages and liquid foods due to their therapeutic values like enhancing gastrointestinal values and mineral absorption, lowering of blood glucose levels and cholesterol level in host (Sorndech et al. 2018).

Some of the other PreBs oligosaccharides like gentio-oligosaccharides, pectin-oligosaccharides, chitin-derived oligosaccharides, glycosylsucrose, arabino-oligosaccharide agaro-oligosaccharide and neo-agaro-oligosaccharide are also known to exhibit bifidogenic effects for human gut health (Al-Sheraji et al. 2013; Singh et al. 2017).

4.4.8 Resistant Starch

Resistant starch (RS) is composed of glucose monomers linked by α-1,4 and α-1,6 linkages resembling the chemical structure of starch. It is arranged in the form of polymeric granules, rendering it the benefit of both soluble and insoluble fibre. It is supplemented as low calorific value ingredient into food product, and it enhances their organoleptic and dietary fibre content (Sharma et al. 2008). The therapeutic effects of RS include regulation of cholesterol level, glycemic control, plasma glyceride level and mineral absorption. Studies to evaluate the prebiotic effect of lactosucrose have demonstrated that it tends to stimulate the probiotic bacteria in intestine such a lactobacilli and bifidogenic bacteria to enhance gastrointestinal functions (Keenan et al. 2015).

4.5 Therapeutic Effects of PreBs

PreBs are known to exhibit various clinical applications and play a major role in treating various diseases. Most of the PreBs and its derived products are selected based on their potential to stimulate growth and activity of probiotic microflora (De Paulo Farias et al. 2019). When ingested orally PreBs reach the gut and protect the epithelial walls of the digestive tract. Majority of the PreBs when administered tend to form short chain fatty acids which results in reducing pH, discouraging the growth of pathogenic microflora, modulating metabolism and enhancing mineral absorption (Pandey et al. 2015).

Based on the mechanism of action, PreBs elicit various preventive and curative effects against severe human diseases such as diarrhoea, bowel syndrome, cancer, bacterial infections, diabetes and obesity (Ahmad and Khalid 2018). In addition to above-mentioned effects it also improves immunity and mineral absorption in hosts. Possible health benefits of PreBs are demonstrated in Fig. 4.3.

4.5.1 Enhancing the Gut Health

PreBs supplementation in humans modulates gut microflora, i.e. stimulates protective bacteria such as bifidobacteria and lactobacilli and inhibits the pathogenic bacteria in the gut. It enhances immune response and absorptivity in the digestive tract, facilitating proliferation and normal functioning of microflora (Pandey et al. 2015). Various fortified prebiotic preparations have been evaluated for the treatment of gastrointestinal infections such as Crohn's disease, diarrhoea, constipation, irritable bowel syndrome, lactose intolerance, ulcerative colitis and traveller's diarrhoea (Azagra-Boronat et al. 2019). All of the above-mentioned infections are

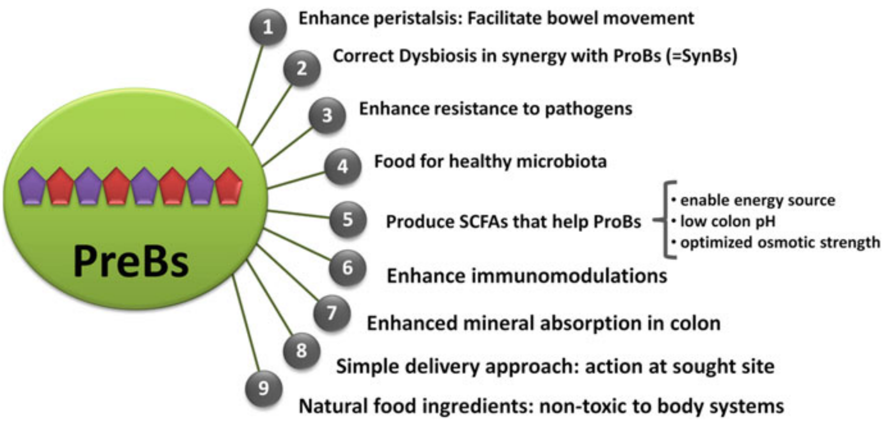

Fig. 4.3 Potential health benefits of PreBs

gastrointestinal infections having severe health effects. The conventional treatments such as consumption of antibiotics and spasmolytics cause various health implications, whereas use of PreBs for these GI infections is gaining attention (Wang et al. 2016). Various in vivo studies have reported that PreBs such as IOSs have the potential to regulate gut microbiota (Wu et al. 2017).

4.5.2 Immunological Effects

PreBs play a major role in stimulating the immune system and increasing the host immune response by modulating the expression of cytokines (Khangwal and Shukla 2019). The increase in the population of protective microorganism increases its competition with pathogenic bacteria for adherence and colonization, as well as through formation of antimicrobial compounds and short chain fatty acids which creates an unfavourable environment for pathogenic bacteria (Shokryazdan et al. 2017).

Several in vitro and in vivo studies have demonstrated the prebiotic effects on host immune system. For example, administration of mixture of inulin, GOS and FOS has shown enhancement for inflammatory response to food allergies and cell mediated immunity response (Bouchaud et al. 2016). Similarly, GOS in host has enhanced the level of protein, interleukins and tumour necrosis factor alpha (Yousefi et al. 2018).

4.5.3 Reducing the Risk of Colon Cancer

Cancer is termed as multiplication and growth of abnormal cells, known to be the leading cause of death in many countries. Colon rectal cancer ranks third among the most common malignancies, which leads to metastatic tumours. The existing chemo- and radiotherapy treatments in cancer worsen the situation more gravely as they confer immunocompromisation in patients. In contrast, administering a combination of live microorganisms (known as ProBs) with PreBs has shown cancer preventing abilities through the formation of metabolites such as butyrate (Thilakarathna et al. 2018; Ambalam et al. 2016). Galacto-oligosaccharide fermentation forms butyrate which reduces metastasis and induces apoptosis in colon cells. It is also known to stimulate the expression of enzymes required for inhibition of carcinogens (Fernández et al. 2018). The clinical studies have demonstrated the potential of synbiotic in reducing the rate of proliferation, inflammatory state and use of antibiotics to prevent incidences of cancer (Polakowski et al. 2019).

4.5.4 Bioavailability and Mineral Absorption

Micronutrients such as magnesium, potassium, calcium and iron are a requisite for the metabolism and proper functioning of the body systems. In recent years, prebiotic therapy for the absorption of minerals and nutrients is gaining much interest because of its enhanced effects. Various mechanisms are known to promote the bioavailability and absorption of nutrients in the body such as decrease in the colonic pH due to SCFAs which increase the nutrient solubility and exchange mechanism (Pandey et al. 2015). PreBs like GOS, inulin and FOS enhance magnesium and calcium uptakes for improving bone metabolism (Scholz-Ahrens 2016).

4.5.5 Lipid Metabolism and Cardiovascular Diseases

Elevated levels of low-density lipoproteins and blood cholesterol increase the risk of coronary heart diseases. There is increasing interest in the development of food products which could modulate the fats and lipids in the body such as cholesterol and triglycerides. Lactic acid bacteria in host could assimilate cholesterol and reduce its level. In addition, PreBs such as FOSs reduce the synthesis of triglycerides in the body (Prasad et al. 2017). Intake of prebiotic dietary fibres reduces the low-density lipoproteins and inflammatory elements, increases high density lipoproteins and ferulic acid concentrations and thereby imply lowering the risks with cardiovascular diseases and obesity (Mohanty et al. 2018).

4.5.6 PreBs and Skin

PreBs reduce the risk of skin allergies and infections by metabolizing aromatic amino acids. The product formed such as phenol and short chain fatty acids reduces the risk of allergic reactions such as atopic dermatitis (Davani-Davari et al. 2019). Galacto-oligosaccharides are reported to reduce skin pigmentation in experimental models and thus act as nutritional source vouching for skincare (Suh et al. 2019). A recent clinical trial demonstrates the potential of PreBs in skin infections reported that mixture of PreBs when supplemented to children improved atopic dermatitis (Ibáñez et al. 2018).

4.5.7 Reducing the Risk of Diabetes

PreBs are known to influence insulin and glucose level in blood. Formation of short chain fatty acids, delay in gastric emptying and reduction in intestine transit times reduce the glucose absorption into the bloodstream (Vyas et al. 2019). Prebiotic fermentation products such as propionates reduce gluconeogenesis, enhance glycolysis and stimulate glucose regulating hormones to optimize the insulin and glucose levels in the host (Gulzar et al. 2019).

4.6 Safety Concerns with PreBs

Currently, among all the functional foods PreBs are considered carrying huge potentials. PreBs exhibit a number of clinical applications such as improving gut health, immune system and cardiovascular system (Valcheva and Dieleman 2016). In recent years, various prebiotic products with their potential health benefits can be found catching wellness products markets. However, the primary concerns with PreBs are its efficacy, toxicity and safety. Inulin, FOS and GOS are extracted from edible parts of plants and also present in traditional food products, thus can be presumed non-toxic (Biswal et al. 2017). Nonetheless, safety issues still need to be addressed which are in dearth of much needed clinical trials and efficacy studies.

There are several safety aspects regarding the selection of a compound to be a safe prebiotic. Firstly, the active ingredient of prebiotic should be fully characterized in terms of structure, source, composition and concentration. Secondly, PreBs should be harmless to the host body and should not interfere with housekeeping metabolic activities (Malik et al. 2016). Promisingly, various animal models are used to assess target site and toxic effects of formulated prebiotic products. Studies, nonetheless, have reported that PreBs such as FOS are neither carcinogenic nor mutagenic. The optimum dosage of PreBs given will help in stimulation of gut microbiota but higher dosage of PreBs than normal may cause a gastrointestinal ailment. Several clinical trials have been demonstrated for dose dependent effects of PreBs. The gastrointestinal symptoms such as diarrhoea, abdominal bloating and gas production in host at dose higher than optimum level were observed (Davani-Davari et al. 2019).

4.7 Synbiotics (SynBs)

The term synbiotic was first introduced in 1995 by Glenn R. Gibson and Roberfroid with the idea that combination of probiotic living microorganism and prebiotic compounds confers an additional health benefit to host then individual therapy of probiotic or prebiotic. In combination therapy PreBs promote the growth and activity of probiotic microflora in the intestine (Quigley 2019). 'Synbiotic' term is defined as synergistic combination of probiotic, beneficial living organism and of prebiotic supplements (which modulates gut microflora) to aid implantation of ProBs in the digestive tract. SynBs were basically formulated to overcome drawbacks and problems encountered with probiotic survival as SynBs primarily affect the growth and functioning of probiotic organisms in the gut (Mohanty et al. 2018).

Table 4.2 Synbiotics and microbial sources

PreBs	ProBs	References
Inulin	Bifidobacteria, *Lactobacillus* genus bacteria	Markowiak and Śliżewska (2017), Astó et al. (2019)
FOS	*Bifidobacterium, Enterococcus faecium*, *Lactobacillus* genus bacteria	Markowiak and Śliżewska (2018)
XOS	*Bifidobacterium animalis* subsp. Lactis, *Bifidobacterium adolescentis*, *Lactobacillus plantarum*	Aachary and Prapulla (2011), Childs et al. (2014)
Isomalto-oligosaccharides	*Bifidobacterium*, *Bacteroides fragilis*	Kerry et al. (2018)
Lactosucrose	*Zymomonas mobilis*	Han et al. (2009)

FOS fructo-oligosaccharide, *XOS* xylo-oligosaccharide

The principal and significant approach used to develop synbiotic is complementarity and synergistic mode. In a complementary approach, probiotic is selected to specifically benefit the host and prebiotic is selected randomly just to enhance intestinal microbial loads. Whereas in synergistic approach probiotics are again chosen to stimulate host health but here prebiotic is selected which could specifically affect and improve functional properties of probiotic microbiota. Thus, both approaches indirectly and directly suffice the fundamental aspect of SynBs, improving host health. However, the synergistic approach of synbiotic is apparently more relevant and beneficial for the development of enhanced functional food products. Various in vivo and in vitro studies have been conducted to evaluate the synergistic effect of SynBs. It was reported that SynBs encourage growth, concentration and viability of intestinal microorganism than individual therapy that uses ProBs (Anzawa et al. 2019).

Various PreBs used in the development of SynBs are inulin, fructo-oligosaccharide, xylo-oligosaccharide and galacto-oligosaccharide (Table 4.2). The commonly used probiotic organisms in SynBs are *Bifidobacterium* and *Lactobacillus* species (Gourbeyre et al. 2011). Some of the considerable norms with the formulation of SynBs are: (i) prebiotic and probiotic selected should confer a beneficial effect on the host when given individually, (ii) stimulation of a probiotic organism by selected prebiotic should be standardized. Lastly, (iii) the chosen prebiotic should only improve the survival and functionality of beneficial microflora, having no or limited effect on other microorganism(s). The principal mechanism used by SynBs for their beneficial effect is the enhancement in the viability of probiotic microorganism which makes them tolerant to environmental conditions. It leads to the maintenance and modulation of intestinal functions and inhibition of intestinal pathogens through the formation of short chain fatty acids. The various health benefits of SynBs include enhanced immunomodulatory abilities, stimulation and maintenance of intestinal microflora, improved hepatic functions and prevention of bacterial and nosocomial infections (Sarao and Arora 2017).

4.8 Conclusions

There exist an increasing interest in assessing the prospects with PreBs, ProBs and SynBs at manipulating the gut microflora, given that many of the health benefits have been proclaimed variously (Fig. 4.3) (Markowiak and Śliżewska 2017). The chapter highlights these calls and shapes the positive vouch for the use of PreBs, ProBs and SynBs supported with obviousness as well as scientific evidences. Various commercially available products like fermented milk products act as delivery vehicles for these functional foods, imparting nutritional and health benefits, while at the same time influencing market and industrial growth (Mohanty et al. 2018). However, production and food processing treatments are susceptible to various challenges such as painstaking critical selection of abundant and cost-effective raw materials for PreBs productions, stability at high pH, process temperatures, storage and functional viability. New materials are variously being screened to meet these conditions. Mushrooms, for example, can be an invaluable source for cheap, non-dairy based raw material. Similarly, cereals and/or their husks can be a good source for the galactose and fructose oligosaccharides (Thatoi et al. 2018). Agricultural wastes could in addition be an alternatively rich source for vitamins, proteins, starch and phytochemicals to look for amenability in prebiotic production. Techniques such as microencapsulation, tableting, etc. amalgamated to cryoprotectants such as fruit juices may ensure extended functional viability and stability during storage of synthesized PreBs formulations (Mishra et al. 2018).

4.9 Opinions

At treating many health ailments ranging from lifestyle associated diseases, cardiovascular problems, immune system, and skin-related disorders, vector-borne diseases to many cancers, the orthodox medical systems have built over evidenced-based scientific studies. Perhaps, many of the treatment options are constrained principally in high cost, toxicity, and side-effects in the long run. Recent trends showcase a gradual shift in public acceptance for herbal, nutraceutical, and palliative care alternatives. This can be seen in developing countries as well in the form of a wave towards realizing the importance of sanitation, purity demands in food and confectionery commodities and changes in mindsets positively towards unorthodox systems of medicine such as Ayurveda, Chinese, Yunnanese, etc. Adding to the reasons above, people have also started believing in the importance of self-healing procedures like yoga, reiki, meditation, music therapy and many in the list which emphasize the treatment grassroots, the body and to keep it fit. Newer versions of foods have emerged in the markets such as planned food, nourishing shakes, energy drinks, and foods fortified with vitamins, minerals, and carbohydrates. Even the medical practitioners have started prescribing such solutions, a slight change in the sense of fortifying the orthodox solutions with yet not thoroughly justified and validated products. The commercial pharmaceutical market has sensed the profit potentials of the same and has also played a considerable role at making hype via various media platforms of the essentialities of such foods. This surely gives another reason for public to have strong confidence over their efficacies. However, only scientific trials remain the resort at unveiling if they are just the fads or do hold some water.

Other than the above races, but with equally relevant pace, exploring gut biosystems, microflora, its diversity and contribution of each and every good, bad and ugly forms of microbes (bacteria, fungi, protozoans, etc.) and even non-living biological entities (viz., bacteriophages, prions) have caught the recent attention of food biologists and biotechnologists, medical practitioners, pharmacologists and medical science communities around the world as well as the more fitness-savvy sections of society. It is estimated that compared to the human body's own cell count, the microbiota of gut accounts for some 100 fold higher numbers. In this context it may be debated what regulates the body and its life processes. Is it just the assembly of various tissue systems, the genetics, the immune system, the nervous system and its neural networks and so on of body's inherent units alone? Well, these are foods for thought and an out of the box queries the scientific communities are running about. To give a hint with evidences, many members in the gut microbiota can regulate mood, ageing, memory, perception, and from some studies are known to play an essential role at maintaining life and its propensities. These include various viruses even, like the BK viruses found in kidney, the bacteriophages that kill bacteria in the gut and so forth. Microbes and even viruses are used as therapeutics. Well, in this regard phage therapy and faecal microbiota transplant solutions can be read about by a little googling. So if these evidences are real, why then be foods left out to have these attributes.

We hope the readers must have framed a more holistic mindset to the theme of the present chapter with the above texts. It is imperative to see that extensive research is being pursued into ProBs and PreBs at investigating their health benefits in humans as well as other animal lives. PreBs should not be considered as magic bullets for the treatment of diseases because their effects are dose dependent and may exhibit side effects such as gastrointestinal symptoms. Currently, beyond market potentials and their tapping by commercial giants there exist scarce regulations at ascertaining and implementing safety parameters for the oral administration of PreBs. Safe and optimum dosage levels of individual PreBs might need thorough evaluations to eliminate side effects. In similar accord, formulated SynBs particularly containing recombinant strains as ProBs may also need to go through above acid tests for short-term and long-term safety profiles in various age groups. A plethora of research still awaits workbenches to be conducted to study molecular bases of interaction between PreBs with ProBs, human colonocytes, reactions with other by-products of ProBs, effect on pathogens and almost all domains that can be surmised from an outlined sequence of events in the generalized role-play of PreBs (Fig. 4.2). In line with this, genomics and metabolomics study will aid to understand and manipulate mechanisms exhibited by ProBs. All of the above-mentioned perspectives for the formulation of PreBs will aid in the prevention and management of prevalent human ailments like antibiotic resistance, obesity, allergy and cardiovascular diseases.

References

Aachary AA, Prapulla SG (2011) Xylooligosaccharides (XOS) as an emerging prebiotic: microbial synthesis, utilization, structural characterization, bioactive properties, and applications. Compr Rev Food Sci Food Saf 10(1):2–16

Abuajah CI, Ogbonna AC, Osuji CM (2015) Functional components and medicinal properties of food: a review. J Food Sci Technol 52(5):2522–2529

Ahmad A, Khalid S (2018) Therapeutic aspects of probiotics and prebiotics. In: Diet, microbiome and health. Elsevier, Amsterdam, pp 53–91

Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusof RM, Hassan FA (2013) Prebiotics as functional foods: A review. J Funct Foods 5(4):1542–1553

Ambalam P, Raman M, Purama RK, Doble M (2016) Probiotics, prebiotics and colorectal cancer prevention. Best Pract Res Clin Gastroenterol 30(1):119–131

Anadón A, Martínez-Larrañaga MR, Ares I, Martínez MA (2016) Probiotics: safety and toxicity considerations. In: Nutraceuticals. Elsevier, Amsterdam, pp 777–798

Anzawa D, Mawatari T, Tanaka Y, Yamsamoto M, Genda T, Takahashi S, Nishijima T, Kamasaka H, Suzuki S, Kuriki T (2019) Effects of synbiotics containing Bifidobacterium animalis subsp. lactis GCL2505 and inulin on intestinal bifidobacteria: a randomized, placebo-controlled, crossover study. Food Sci Nutr 7(5):1828–1837

Apolinário AC, De Lima Damasceno BPG, De Macêdo Beltrão NE, Pessoa A, Converti A, Da Silva JA (2014) Inulin-type fructans: a review on different aspects of biochemical and pharmaceutical technology. Carbohydr Polym 101:368–378

Astó E, Méndez I, Audivert S, Farran-Codina A, Espadaler J (2019) The efficacy of probiotics, prebiotic inulin-type fructans, and synbiotics in human ulcerative colitis: a systematic review and meta-analysis. Nutrients 11(2):293

Azagra-Boronat I, Rodríguez-Lagunas MJ, Castell M, Pérez-Cano FJ (2019) Prebiotics for gastrointestinal infections and acute diarrhea. In: Dietary interventions in gastrointestinal diseases. Elsevier, Amsterdam, pp 179–191

Belorkar SA, Gupta A (2016) Oligosaccharides: a boon from nature's desk. AMB Express 6(1):82

Biswal P, Pal A, Das AP (2017) Current trends and future prospective of prebiotics as therapeutic food. In: Microbial production of food ingredients and additives. Elsevier, Amsterdam, pp 57–88

Bouchaud G, Castan L, Chesné J, Braza F, Aubert P, Neunlist M, Magnan A, Bodinier M (2016) Maternal exposure to GOS/inulin mixture prevents food allergies and promotes tolerance in offspring in mice. Allergy 71(1):68–76

Cardelle-Cobas A, Corzo N, Olano A, Peláez C, Requena T, Ávila M (2011) Galactooligosaccharides derived from lactose and lactulose: influence of structure on Lactobacillus, Streptococcus and Bifidobacterium growth. Int J Food Microbiol 149(1):81–87

Childs CE, Röytiö H, Alhoniemi E, Fekete AA, Forssten SD, Hudjec N, Lim YN, Steger CJ, Yaqoob P, Tuohy KM (2014) Xylo-oligosaccharides alone or in synbiotic combination with Bifidobacterium animalis subsp. lactis induce bifidogenesis and modulate markers of immune function in healthy adults: a double-blind, placebo-controlled, randomised, factorial cross-over study. Br J Nutr 111(11):1945–1956

Cryan JF, Clarke G (2016) Gut microbiome and behavior, vol 131. Academic Press, Cambridge

Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, Berenjian A, Ghasemi Y (2019) Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods 8(3):92

De Paulo Farias D, De Araújo FF, Neri-Numa IA, Pastore GM (2019) Prebiotics: Trends in food, health and technological applications. Trends Food Sci Technol 93:23–35

Espinosa-Martos I, Rupérez P (2006) Soybean oligosaccharides. Potential as new ingredients in functional food. Nutr Hosp 21(1):92–96

Falguera V, Aliguer N, Falguera M (2012) An integrated approach to current trends in food consumption: Moving toward functional and organic products? Food Control 26(2):274–281

Fernández J, Moreno FJ, Olano A, Clemente A, Villar CJ, Lombó F (2018) Galacto-oligosaccharides derived from lactulose protects against colorectal cancer development in a animal model. Front Microbiol 9:2004

Fuentes-Zaragoza E, Sánchez-Zapata E, Sendra E, Sayas E, Navarro C, Fernández-López J, Pérez-Alvarez JA (2011) Resistant starch as prebiotic: A review. Starch-Stärke 63(7):406–415

Gourbeyre P, Denery S, Bodinier M (2011) Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. J Leukoc Biol 89(5):685–695

Gulzar N, Saleem IM, Rafiq S, Nadeem M (2019) Therapeutic potential of probiotics and prebiotics. In: Microbial Health-Role of Prebiotics and Probiotics. IntechOpen, New York

Han W-C, Byun S-H, Kim M-H, Sohn EH, Lim JD, Um BH, Kim CH, Kang SA, Jang K-H (2009) Production of lactosucrose from sucrose and lactose by a levansucrase from Zymomonas mobilis. J Microbiol Biotechnol 19(10):1153–1160

Huebner J, Wehling R, Hutkins RW (2007) Functional activity of commercial prebiotics. Int Dairy J 17(7):770–775

Ibáñez MD, Del Río PR, Alsina DG-S, Iglesias VV (2018) Effect of synbiotic supplementation on children with atopic dermatitis: an observational prospective study. Eur J Pediatr 177 (12):1851–1858

Keenan MJ, Zhou J, Hegsted M, Pelkman C, Durham HA, Coulon DB, Martin RJ (2015) Role of resistant starch in improving gut health, adiposity, and insulin resistance. Adv Nutr 6 (2):198–205

Kerry RG, Patra JK, Gouda S, Park Y, Shin H-S, Das G (2018) Benefaction of probiotics for human health: A review. J Food Drug Anal 26(3):927–939

Khangwal I, Shukla P (2019) Prospecting prebiotics, innovative evaluation methods, and their health applications: a review. 3 Biotech 9(5):187

Li W, Wang K, Sun Y, Ye H, Hu B, Zeng X (2015) Lactosucrose and its analogues derived from lactose and sucrose: Influence of structure on human intestinal microbiota in vitro. J Funct Foods 17:73–82

Malik JK, Ahmad AH, Kalpana S, Prakash A, Gupta RC (2016) Synbiotics: safety and toxicity considerations. In: Nutraceuticals. Elsevier, Amsterdam, pp 811–822

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

Markowiak P, Śliżewska K (2018) The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens 10(1):21

Mazzola G, Aloisio I, Biavati B, Di Gioia D (2015) Development of a synbiotic product for newborns and infants. LWT-Food Sci Technol 64(2):727–734

Mishra SS, Behera PK, Kar B, Ray RC (2018) Advances in probiotics, prebiotics and nutraceuticals. In: Innovations in technologies for fermented food and beverage industries. Springer, Cham, pp 121–141

Mitmesser S, Combs M (2017) Prebiotics: inulin and other oligosaccharides. In: The microbiota in gastrointestinal pathophysiology. Elsevier, Amsterdam, pp 201–208

Mohanty D, Misra S, Mohapatra S, Sahu PS (2018) Prebiotics and synbiotics: Recent concepts in nutrition. Food Biosci 26:152–160

Nooshkam M, Babazadeh A, Jooyandeh H (2018) Lactulose: Properties, techno-functional food applications, and food grade delivery system. Trends Food Sci Technol 80:23–34

Pandey KR, Naik SR, Vakil BV (2015) Probiotics, prebiotics and synbiotics-a review. J Food Sci Technol 52(12):7577–7587
Patel S, Goyal A (2012) The current trends and future perspectives of prebiotics research: a review. 3 Biotech 2(2):115–125
Polakowski CB, Kato M, Preti VB, Schieferdecker MEM, Campos ACL (2019) Impact of the preoperative use of synbiotics in colorectal cancer patients: a prospective, randomized, double-blind, placebo-controlled study. Nutrition 58:40–46
Prasad PD, Gurav A, Zhu H, Martin PM, Vijay-Kumar M, Singh N (2017) The relationship between probiotics and dietary fiber consumption and cardiovascular health. In: Dietary fiber for the prevention of cardiovascular disease. Elsevier, Amsterdam, pp 73–90
Quigley EM (2019) Prebiotics and probiotics in digestive health. Clin Gastroenterol Hepatol 17 (2):333–344
Sarao LK, Arora M (2017) Probiotics, prebiotics, and microencapsulation: A review. Crit Rev Food Sci Nutr 57(2):344–371
Scholz-Ahrens KE (2016) Prebiotics, probiotics, synbiotics and foods with regard to bone metabolism. In: Nutritional influences on bone health. Springer, Cham, pp 153–167
Sengun İY, Bingol MG (2018) Plants as prebiotic sources. Int J Environ Res Technol 1(2):11–14
Sharma A, Yadav BS, Ritika (2008) Resistant starch: physiological roles and food applications. Food Rev Int 24(2):193–234
Shokryazdan P, Jahromi MF, Navidshad B, Liang JB (2017) Effects of prebiotics on immune system and cytokine expression. Med Microbiol Immunol 206(1):1–9
Silvério SC, Macedo EA, Teixeira JA, Rodrigues LR (2015) Perspectives on the biotechnological production and potential applications of lactosucrose: a review. J Funct Foods 19:74–90
Singh RD, Banerjee J, Arora A (2015) Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides. Bioacti Carbohydr Diet Fibre 5(1):19–30
Singh RS, Singh RP, Kennedy JF (2016) Recent insights in enzymatic synthesis of fructooligosaccharides from inulin. Int J Biol Macromol 85:565–572
Singh SP, Jadaun JS, Narnoliya LK, Pandey A (2017) Prebiotic oligosaccharides: Special focus on fructooligosaccharides, its biosynthesis and bioactivity. Appl Biochem Biotechnol 183 (2):613–635
Sitanggang AB, Drews A, Kraume M (2016) Recent advances on prebiotic lactulose production. World J Microbiol Biotechnol 32(9):154
Slavin J (2013) Fiber and prebiotics: mechanisms and health benefits. Nutrients 5(4):1417–1435
Sorndech W, Nakorn KN, Tongta S, Blennow A (2018) Isomalto-oligosaccharides: recent insights in production technology and their use for food and medical applications. LWT 95:135–142
Suh MG, Hong YH, Jung EY, Suh HJ (2019) Inhibitory effect of galactooligosaccharide on skin pigmentation. Prev Nutr Food Sci 24(3):321
Thatoi H, Singdevsachan SK, Patra JK (2018) Prebiotics and their production from unconventional raw materials (mushrooms). In: Therapeutic, probiotic, and unconventional foods. Elsevier, Amsterdam, pp 79–99
Thilakarathna WW, Langille MG, Rupasinghe HV (2018) Polyphenol-based prebiotics and synbiotics: potential for cancer chemoprevention. Curr Opin Food Sci 20:51–57
Torres DP, Gonçalves MPF, Teixeira JA, Rodrigues LR (2010) Galacto-oligosaccharides: production, properties, applications, and significance as prebiotics. Compr Rev Food Sci Food Saf 9 (5):438–454
Tzortzis G, Vulevic J (2009) Galacto-oligosaccharide prebiotics. In: Prebiotics and probiotics science and technology. Springer, New York, pp 207–244
Valcheva R, Dieleman LA (2016) Prebiotics: definition and protective mechanisms. Best Pract Res Clin Gastroenterol 30(1):27–37
Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J (2017) Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut 66 (11):1968–1974

Verkhnyatskaya S, Ferrari M, de Vos P, Walvoort MT (2019) Shaping the infant microbiome with non-digestible carbohydrates. Front Microbiol 10:343

Vyas N, Nair S, Rao M, Miraj SS (2019) Childhood obesity and diabetes: role of probiotics and prebiotics. In: Global perspectives on childhood obesity. Elsevier, Amsterdam, pp 363–376

Walton GE, Swann JR, Gibson GR (2013) Prebiotics. The prokaryotes: human microbiology. Springer, Berlin, pp 25–43

Wang H, Geier MS, Howarth GS (2016) Prebiotics: a potential treatment strategy for the chemotherapy-damaged gut? Crit Rev Food Sci Nutr 56(6):946–956

Watson RR, Preedy VR (2015) Probiotics, prebiotics, and synbiotics: bioactive foods in health promotion. Academic Press, Cambridge

Wu Y, Pan L, Shang Q, Ma X, Long S, Xu Y, Piao X (2017) Effects of isomalto-oligosaccharides as potential prebiotics on performance, immune function and gut microbiota in weaned pigs. Anim Feed Sci Technol 230:126–135

Yin J, Li F, Kong X, Wen C, Guo Q, Zhang L, Wang W, Duan Y, Li T, Tan Z (2019) Dietary xylo-oligosaccharide improves intestinal functions in weaned piglets. Food Funct 10(5):2701–2709

Yousefi S, Hoseinifar SH, Paknejad H, Hajimoradloo A (2018) The effects of dietary supplement of galactooligosaccharide on innate immunity, immune related genes expression and growth performance in zebrafish (Danio rerio). Fish Shellfish Immunol 73:192–196

Chapter 5
Probiotics as Live Bio-therapeutics: Prospects and Perspectives

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Abstract Enhanced in-depth knowledge of the role of gut microbiota in development of several diseases and syndromes has increased interest in probiotics mediated novel health promoting strategies. A number of experiments have verified that these beneficial bacteria could be successfully administered for the prevention or treatment of a variety of pathological, metabolic and neural disorders. The beneficial effect of probiotics in prophylaxis and treatment of multifarious disorders has also been validated in animal and human clinical trials. The natural way of probiotics to restore a healthy human gut microbial ecosystem leads to the development of a new class of probiotics: bio-therapeutic probiotics. These are probiotics that deliver novel therapeutics effect efficiently and with site specificity. Nonetheless, probiotics were not widely accepted as therapeutic agents due to lack of well controlled and managed clinical trials on humans as well as lack of reliability of claims of their uses. High rate of inconsistency in the efficiency of probiotics has been reported. The reason behind this discrepancy is complex dynamics of the gastrointestinal microbial communities, individual specific genetic, epigenetic and nutritional factors. Thus, the establishment of probiotic as bio-therapeutics should depend on measurable markers and scientific evidence-based criteria by conducting multidisciplinary research to set up facts and mechanisms of action for selected probiotic strains. It is essential for the positioning of probiotic preparations as either a food, a food supplement or as pharmaceutical preparation.

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5.1 Introduction

Probiotics are the live microorganisms which when administered in adequate amount confer health benefits to the host. A wide variety of sources are being explored for isolation and identification of novel probiotic strains (Thakur et al. 2016). In the past decades, probiotic strains have gained high interest as supplement for both humans and animals due to their beneficial role in overall health improvement. Beneficial health effects of probiotics are well known for several gut and metabolic disorders including obesity, diabetes, gastroenteritis, etc. through their role in managing oxidative stress and inflammation (Achuthan et al. 2012; Chauhan et al. 2014; Sudhakaran et al. 2013), antagonistic activity against pathogens (Sharma et al. 2017; Singh et al. 2016) and secretion and expression of several gut hormones (Panwar et al. 2014, 2015, 2016).

Recently probiotics have attracted significant interest as bio-therapeutics for management of different metabolic and brain-based disorders. Beneficial gut microbiota produce several metabolites having direct and indirect impact on host pathophysiology. Modulating gut microbiota by probiotic intervention is emerging as a new frontier for possible management of multiple clinical scenarios. Probiotic administration balances the gut bacterial population and promotes their favourable metabolic actions. Recently gut-brain-axis has gained wide interest due to the emerging key role of gut microbiota in several disorders regulated through brain and central nervous system, expanding probiotic efficacy in chronic disorders apart from their established role in gut disorders. The book chapter summarizes and highlights the possible application of probiotic as bio-therapeutics in different clinical conditions.

5.2 Probiotics in Foodborne Diseases

Foodborne disease is becoming a global concern, as it is responsible for immense mortality and morbidity worldwide. Every year, approximately 48 million people suffered from foodborne diseases, 128,000 were hospitalized and cause 3000 mortality (Centers for Disease Control Prevention 2019). Foodborne infections are caused mainly by ingestion of foods that contains live harmful microorganisms, once reached in gut are multiplied, lysate and secrete toxins and/or invade intestinal wall. The severity of the foodborne diseases leads to development of various approaches to increase food safety and its preservation. Different physical (thermal treatment, hydrostatic pressure, pulsated electric field, fluctuating magnetic flux, high voltage and photo-dynamic), chemical (preservatives, antioxidants and essential oils) and biological (antibiotics) methods were used alone or in combination to reduce foodborne illness. However, these methods were effective in treating foodborne diseases but they are not 100% efficient and also have added side effects such as changes in organoleptic property of food, changes in appearance of food, chemical intolerance in body, antibiotic resistance in foodborne pathogens. Out of these, antibiotic resistance is of major concern; in the USA and across the globe, incidence of antibiotic resistant infections has increased immensely (Ventola 2015). Antibiotic resistant pathogen strains have immensely increased public health concerns, increased mortality, medical cost of treatment, increased length of medical care, etc. (Centers for Disease Control Prevention 2013). So, the alternative strategies should be required for surveillance and monitoring of foodborne diseases.

Probiotics can be an alternative approach in which "live microorganisms were administered in passable amounts which has health benefits for the consumer" (FAO/WHO 2002). Lactic acid bacteria, specifically species of *Lactobacillus* and *Bifidobacterium*, are most communal probiotic strains used in milk fermentation (Kirpich and McClain 2012). Probiotics gain immense popularity due to their health benefitting effects, ability to treat infections (Table 5.1) and various metabolic diseases (Sanders et al. 2013). Probiotics can prevent the infectious disease by major four proposed mechanism of action: (a) Competition, (b) Antimicrobial secretions, (c) Increased intestinal barrier function and (d) Immunomodulation (Wan et al. 2018).

a. Competition: bacterial–bacterial interactions were mediated by competitions for nutrient absorption and bacterial adhesion sites. The competition between the bacteria causes exclusion or reduced growth of one bacterial species. Certain probiotic bacterial species cause inhibition of establishment of pathogenic bacteria through release of antimicrobial substances (Schiffrin and Blum 2002), specific adhesion of protein interaction with surface protein and mucin (Servin 2004). Probiotic bacteria can impose steric hindrance to attachment of pathogenic bacteria at enterocyte pathogen receptors (Lee and Puong 2007). Administration of commercial probiotic of *Lactobacillus* spp. at 7.5×10^9 CFU/L reduced incidence of *Clostridium difficile* infections in pigs (Arruda et al. 2016). *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* competitively bind to Fe $(OH)_3$, rendering it unavailable for pathogens (Elli et al. 2000). *Lactobacillus* probiotic EcN releases siderophores that chelate and helps in competitive uptake of iron (Fe^{2+}/Fe^{3+}) via seven different uptake system (Große et al. 2006). Listeria infection in C2Bbe1 epithelial cell lines was observed to be inhibited by probiotic *Lactobacillus* due to combined production of acid (antimicrobial) and unidentified protein, whereas antimicrobial action of *Bifidobacterium* attributed to release of extracellular protein compound (Corr et al. 2007). A probiotic *Lactobacillus* bacterium secretes lectin (carbohydrate binding adhesins) which competitively eliminates enterobacteria (*S. typhimurium* KCCM 40253 and *E. coli* K88) because they share glycoreceptor adhesion sites in gut mucus and epithelium of host (Valeriano et al. 2016). Competition for same receptor,

Table 5.1 Probiotics intervention for management of foodborne pathogenic infections

Author	Animal Model	Pathogen	Probiotics	Findings
Lema et al. (2001)	Sheep	*E. coli* O157: H7	Consortium *of L. acidophilus*, *E. faecium*, *L. casei*, *L. fermentum* and *L. plantarum*	Administration of probiotic to sheep regularly for 7 weeks significantly decreased total number of pathogens in excreta and also improved production of meat
Ohya et al. (2000)	Calves	*E. coli* O157: H7	*Streptococcus bovis and L. gallinarum*	Administration of probiotic to infected animal inhibited shedding of *E. coli* O157:H7. There was significant increase in volatile fatty acid (acetic acid) which was correlated with attenuation of pathogen
Kim et al. (2013)	Rats	*Salmonella*	*L. acidophilus* heat killed	Heat killed *L. acidophilus* plays significant role in preventing Salmonella infection by increasing its excretion and lowering level of TNF-α and IL-1β as compared to control
Forkus et al. (2017)	Turkey	*Salmonella enteritidis*	Probiotic *E. coli* Nissle 1917	Administration of probiotic (*E. coli* secretes the antimicrobial peptide, Microcin J25) significantly decreased the pathogen load as compared to control after 2 weeks of *Salmonella* challenge
Filho-Lima et al. (2000)	Mice	*Shigella flexneri*	*L. acidophilus*, *Saccharomyces boulardii* and *E. coli*	Mixed probiotic administration in *Shigella flexneri* infected gnotobiotic mice resulted in antagonistic reaction (shown by *E. coli*) and protective mechanism such as competition for adhesion sites, regulation of toxin production and action, immunomodulation (shown by other two bacteria)
Puertollano et al. (2008)	Mice	*Listeria*	*L. plantarum*	In mice (challenged with *Listeria monocytogenes)*, administration of the probiotic significantly

(continued)

Table 5.1 (continued)

Author	Animal Model	Pathogen	Probiotics	Findings
				decreased the secretion of interleukin in blood serum
Dos Santos et al. (2011)	Mice	*Listeria*	*L. delbrueckii*	Probiotic bacteria elicited cytokine (TNF-α and IFN-c) production that bind to TLR of antigen presenting cell and cause activation of macrophages. Additionally they secrete more NO (nitric oxide) that eliminate intracellular bacteria
Arsi et al. (2015)	Chicks	*Campylobacter jejuni*	*Bacillus* and *Lactobacillus* spp	Probiotic administered chicks (on day of hatch) were challenged with mixed strains of *Campylobacter jejuni* on the 7th day. The cecal samples collected after two weeks showed significant reduction in campylobacter counts

degradation of carbohydrate receptor, receptor analogues, biofilm and biosurfactant production can inhibit the settlement of pathogens in host.

b. Antimicrobial secretions: Probiotic bacteria can secrete various metabolites like organic acids (acetic or lactic acid), H_2O_2 and bacteriocins that have ability to limit growth of pathogens. Lactic acid bacteria increased acidity of the surrounding medium by secreting lactic acid and acetic acid, making it unfavourable for growth of various pathogens (Makras et al. 2006). Organic acid enters inside pathogen and dissociates in cytoplasm, causes breakdown of proton motive force leading to inhibition of nutrient transport and lysis of cell. Probiotic bacteria can also produce some antibiotics, such as *Lactobacillus reuteri* strain ATCC55730 that secretes broad-spectrum antibiotic reuterin (3-hydroxypropionaldehyde), which are effective against Gram-positive, Gram-negative pathogen, viruses, fungi, yeast and protozoa (Cleusix et al. 2008). *Lactobacillus* bacteria can produce pH and heat stable ribosomally synthesized bacteriocins, non-cytotoxic, bactericidal and bacteriostatic peptides. Unlike antibiotics, bacteriocins were very target specific in their action. Bacteriocins are antimicrobial peptides that disrupt cell wall (by forming pore) and block synthesis of cell wall of target cell (Hassan et al. 2012). Combined application of nisin with citric acid and enterocin AS-48 with ethambutol were effective in inhibiting *Staphylococcus* *aureus*, *Listeria monocytogenes* (Zhao et al. 2017); and *Mycobacterium tuberculosis* (Aguilar-Pérez et al. 2018), respectively.

c. Increased intestinal barrier function: Intestinal epithelial cells serve as primary defence line (Dowarah et al. 2017) and are foremost for probiotic action (Lebeer et al. 2010). Probiotics were able to enhance the intestinal barrier function through stimulating (a) production of defensins peptides (Schlee et al. 2007); (b) improved immune response; (c) improved functionality of tight junctions; (d) inhibiting epithelial cell apoptosis and (e) generating cyto-protective molecules (Madsen 2012). Application of *Streptococcus thermophilus* and *Lactobacillus acidophilus* significantly reduced the entero-invasive *E. coli* adhesion, invasion and physiological dysfunction in in vitro experiment with HT-29 and Caco-2 cell lines. This occurs due to increased trans-epithelial resistance complemented with maintenance/enhancement of tight junctions, phosphorylation and cytoskeleton (Resta-Lenert and Barrett 2003). Additionally, VSL#3 increased expression of cell surface markers of various mucin protein (MUC2, MUC3 and MUC5AC) in human colon cancer cell line HT29 (Otte and Podolsky 2004). Probiotic strains stimulate release of small peptides known as defensins from epithelial cells, which can stimulate gut barrier function as well as inhibit growth of bacteria, fungi and viruses (Furrie et al. 2005).

d. Immunomodulation: Probiotic lactic acid bacteria are widely described to modulate innate as well as adaptive immunity, thus enhancing immune response and preventing pathogenic infections (Ashraf and Shah 2014). Pathogens have molecular patterns associated with their cell membrane which are recognized by pattern recognition receptors (PRR) of host. When host PRR comes in contact with pathogen associated molecular pattern, it leads to induction of inflammatory response and activation of innate immunity. Various immune cells (granulocytes, dendritic cells, macrophages, T- cells and B- cells) and cytokines (TNFα, IL-8, IL-6, IL-15, IL-1β interleukins) were involved in this response. Lactic acid bacteria can cause induction of cytokine production thus can activate innate as well as adaptive immune system (Kiczorowska et al. 2017). Administration of *L. acidophilus* at 1×10^8 CFU in feed increased anti-inflammatory properties in piglets infected with entero-toxigenic *E. coli*. The production of pro-inflammatory cytokine (IL-8 and TNF-α) was also found to be decreased in *in vivo* animal experiments (Li and Zhou 2016). *L delbrueckii* supplementation increased the anti-inflammatory cytokines in intestine (IL-10 and TGF-β) and decreased pro-inflammatory IL-8 cytokines in carp infected with *Aeromonas hydrophila* (Zhang et al. 2017). Administration of *L. plantarum* and *C. butyricum* at 10^7 CFU/kg and 10^6 CFU/kg of feed to broilers, respectively, enhanced the immune function by increasing IgG and IgA levels and broiler production (Han et al. 2018). Application of *L. plantarum* B2984 to Salmonella infected pigs encouraged production of IgM and IgA immunoglobulins (Naqid et al. 2015). So, probiotic application can directly re-establish the normal gut microflora; thereby eliminating pathogens, reducing the incidence of infection, and maintaining healthy well-being without causing any ill effects such as antibiotic resistance.

5.3 Probiotics and Genetic/Neurodevelopmental Disorders

Microbial population residing in the intestinal tract is widely linked with metabolic disorders and has been proposed as potential bio-therapeutics for management of several gut-related clinical scenarios as discussed in previous sections. It is now widely accepted that host genetics influences the gut microbiota composition. However, surge in information generating from metagenomics, metabolomics and bioinformatics analysis have emphasized role and significance of gut microbiota in shaping host genetics, immune and nervous system through gut-microbiota-brain-axis. Gut microbes produce several vital metabolites, *viz*. serotonin, dopamine, short chain fatty acids, etc., having role in brain functioning (Strati et al. 2017). Certain disorders arising from brain inflammation have recently been linked to the gut microbial composition (Petra et al. 2015). Any dysbiosis in gut healthy microbial community can lead to overexpression of Th1 and Th17 cells resulting in inflammation and disturbed central nervous system. The current section highlights the link between gut microbiota dysbiosis and genetic/ neurodevelopmental disorders and their possible management with probiotic therapy.

Several disorders, viz. intellectual disability, communication disorders, autism spectrum disorder, attention deficit/hyperactivity disorder (ADHD), conduct disorders, cerebral palsy, impairments in vision and hearing, schizophrenia, Parkinson's, etc., resulting from abnormal brain development or impaired central nervous system falls under category of neurodevelopmental or neurodegenerative disorders. The cumulative effect of genetic, biological, psychosocial and environmental factors disposes to the development of such neurodevelopmental disorders. Any disturbance in gut microbiota composition induced due to bacterial infection or antibiotic therapy has been connected as one of the probable contributors to autism (Li and Zhou 2016). Autistic patients frequently suffer from gastrointestinal disorders pointing out towards role of gut microbiota in gastrointestinal pathophysiology of neurodevelopmental disorders. One such recent study demonstrated altered bacterial and fungal community structure in cohort of autistic individuals. The study demonstrated increased Firmicutes/Bacteroidetes ratio with reduced Bacteroidetes concentration, and increased *Escherichia*, *Salmonella*, *Clostridium* and *Candida* spp. (Strati et al. 2017). Li and Zhou (2016) reviewed the potential management of autism spectrum disorder by targeting the microbiota-gut-brain axis through probiotic intervention. In a double-blind, placebo-controlled clinical study, supplementation of probiotic *Lactobacillus plantarum* WCFS1 improved the population of lactobacilli/enterococci and reduced *Clostridium* in the intestine of autistic subjects (Parracho et al. 2010). The change in gut microflora was also associated with healthier stool consistency and decreased the overall behaviour scores in the same study groups (Parracho et al. 2010). The possible mode of probiotic intervention in the management of autism could be mediated by microbiota-gut-brain axis. Precise mechanisms include the fine tuning of circulating neurotransmitters and neuroimmune related autism biomarkers within the axis, such as, myeloperoxidase (marker for inflammation and oxidation in autistic individuals), improved integrity of the intestinal barrier, and via altering serum metabolites etc. (Li and Zhou 2016).

Relationship between gut microbiome (entire genome of gut microorganisms), depression and anxiety has been evaluated in the past few years. Depression has been identified as one of the leading factors for mood and psychiatric disorders targeting almost every sector of the society in both developed and developing world. Stress induced neurological changes in brain have been strongly associated with depression and its associated complications. Gut microbiota through gut-brain-axis manipulate hypothalamic pituitary adrenal axis and affect mood through vagus nerve and endocrine system (Abdrabou et al. 2018). Few recent studies demonstrated beneficial effects on probiotic administration on depression and its related complications in animal and human subjects. *L. helveticus* NS8 administration to Sprague Dawley rats improved stress induced behaviour deficits and attenuated levels of corticosterone (Foster et al. 2017). In another study, *L. helveticus* R0052 and *Bifidobacterium longum* R0175 prevented stress-induced changes in neurogenesis, barrier integrity and stress reactivity in rat models (Ait-Belgnaoui et al. 2016). Kynurenine, a circulating metabolite, is known to promote depression. Mice treated with probiotic lactobacilli significantly decreased the levels of kynurenine in blood (Abdrabou et al. 2018). Anti-depression efficacy of probiotic strains and their metabolites has yet not been validated in human clinical studies, as there is only handful of published studies. Few recent reports proposed that altering gut microbiota composition by probiotic administration can be a viable adjuvant treatment option for subjects with major depressive disorder (Wallace and Milev 2017; Park et al. 2018). Recently Nikolova et al. (2019) reviewed the available literature on efficacy of probiotics for depression management in human subjects. Available literature indicated overall positive effects of probiotics on depression symptoms. Probiotics may be more effective against depression symptoms when administered alongside antidepressants.

Parkinson's and Alzheimer's diseases are among the neurodegenerative disorders that are commonly associated with aging. Reactive oxygen species mediated oxidative stress contributes to neuroinflammation. Brain areas affected in neurodegenerative disorders are sensitive to oxidative stress. Gut microflora, particularly probiotic strains, are known to contribute towards host by their strong antioxidant ability rendering protection to cells prone to oxidative stress. In general, aging is associated with a shift in gut microbial diversity. A significant variation in gut microbial diversity of elderly patients suffering with neurodegenerative disorders has been reported. The gastrointestinal tract also supports neuronal development and maintenance through gut-brain-axis. It has been proposed that the management of gut microflora can help in management of these neurodegenerative disorders (Westfall et al. 2017). Several microbial neurometabolites, biogenic amines, phenolic compounds, fatty acids, hormones, neuropeptides, etc. are known to contribute towards management of such disorders. In a recent randomized, double-blind, placebo-controlled trial, individuals with Parkinson's were administered probiotics for 12 weeks, following which significant changes in metabolic profile (reduced C-reactive protein and malondialdehyde; increased glutathione peroxidase) and

Parkinson's disease rating scale were recorded (Tamtaji et al. 2018). One of the possible routes through which gut microflora impact Parkinson's can be through reducing the expression of alpha-synuclein in gut and checking its spread to the central nervous system (Felice et al. 2016). Understanding the link between gut microbiota composition and genetic disorders particularly those linked to the central nervous system and identifying strains or consortium of probiotic strains having promising therapeutic effects can be a novel approach for prevention and/or management of these disorders.

5.4 Probiotics for Management of Cardiovascular Diseases

Cardiovascular diseases are the primary source of worldwide mortality. According to WHO approximately 31% of global death was caused by CVDs, out of these 85% were due to heart attack and stroke during the year 2016. It was also predicted that, till 2030, CVDs will affect around 23.6 million people over the globe (WHO 2006). CVD is a metabolic syndrome, which is associated with risks like increased ratio of low-density to high-density lipoprotein (LDL/HDL), increased triglyceride-rich lipoproteins (Vasquez et al. 2019), inflammation and autoimmune neural dysfunction. Genetic composition, high body mass index and medical history of an individual are contributing factors for cardiovascular diseases. This risk can be reduced by changing eating/food habits, sedentary life style and reducing consumption of tobacco and alcohol. Unhealthy life style and food habits have direct negative effect on diversity and activity of gut microbiota. Various reports verify that imbalance in gut microbiota is linked not only to pathogenic invasion in intestine but also to extraintestinal ailments, such as cardiovascular disease (CVD), diabetes and obesity. Therefore, gut microbiota can be a potent target to treat and/or prevent a metabolic disease. The articulation of gut microbiota using probiotics might offer new opportunities of prevention and/or disease treatment. Probiotics target various pathways that lead to development of cardiovascular diseases, as discussed below:

Probiotic in endothelial dysfunction and oxidative stress—Endothelium is a vascular layer of thin cells, serves as primary barrier between vascular muscles and bloodstream and tissue spaces, allows selective passage of solutes, fluids, inflammatory molecules (Malik et al. 2018), growth factors, etc. Endothelial cells have multiracial role in regulating vascular tone (through synthesis/release of vasodilator or vasoconstrictor), release of inflammatory molecules, reactive oxygen species, regulating immune response, along with this it has important participation in maintaining blood homeostasis, platelet aggregation, regulating antithrombotic/prothrombotic balance (Konukoglu and Uzun 2016). So, any adverse change in endothelial cells leads to disturbance in its functioning thus resulting into diseased conditions. Endothelial dysfunction is accepted as a major risk factor in the classic cardiovascular disease mechanisms. Endothelial dysfunction is characterized by reduced endothelium-mediated vasorelaxation, disturbance in hemodynamic regulation, reduced fibrinolytic ability, excessive release of growth factors and reactive oxygen species, enhanced expression of adhesive proteins and inflammatory genes, increased oxidative stress and increased cell layer permeability. Numerous studies recommended that consumption of probiotic can positively influence functionality of endothelial layer. Rashid et al. (2014) reported that application of probiotic formulation to rat having endothelial dysfunction of mesenteric artery ring with common bile duct ligation improved the pathological condition of the rat. Toral et al. (2014) showed that *Lactobacillus coryniformis* probiotic treatment improved the endothelial functioning and lipopolysaccharide (LPS) induced vascular oxidative stress in control mice. The oral administration of probiotic drink (Kefir) for 56 days could repair structure of vascular endothelium, decrease oxidative stress and increase nitrogen oxide bioavailability to rats. It also causes the recruitment of endothelial progenitor cells which diminishes endothelial dysfunctioning (Friques et al. 2015). Positive effects of probiotics were verified by Yap et al. (2016), they observed that lactic acid bacteria (LAB) were able to partially reverse the relaxation deficit of the aorta and increased the NO level in SHR. They observed that probiotic administration improved endothelial function by reducing vascular oxidation and inflammation. Gómez-Guzmán et al. (2015) reported that intake of probiotic consortium (*Lactobacillus fermentum*/*Lactobacillus coryniformis* with *Lactobacillus gasseri*) was able to establish normal gut microbiome and improve endothelial functioning, leading to reduced vascular pro-inflammation and pro-oxidation. Clinical trial on human and human cell lines had also shown the positive impact of probiotics on endothelial functioning. Cheng et al. (2013) reported that *Lactobacillus plantarum* or *Streptococcus thermophilus* fermented soy milk increased nitric oxide production and eNOS activity, signifying their impact in enhancing endothelial function. *Lactobacillus plantarum* administration (6 weeks) to stable coronary artery patient increased NO bioavailability along with reduced systemic inflammation, thus leading to improved endothelial function. Probiotic administration to obese post-menopausal women was recorded to improve normal functioning of endothelium, release of vascular endothelial growth factor, systolic blood pressure, IL-6, TNF-α and thrombomodulin (Szulińska et al. 2018a, b).

Probiotics in hypercholesterolaemia —Cholesterol is a vital constituent for body tissues but the elevated blood cholesterol can cause major threat to coronary heart problems (Aloglu and Öner 2006). Hypercholesterolaemia and dyslipidemia are most common cause of most of the cardiovascular diseases. Adhesion of probiotic bacterial cells to epithelial layer of intestine can lower cholesterol levels in body (Tomaro-Duchesneau et al. 2015). Three mechanisms of action have been proposed through which probiotics help in lowering cholesterol levels: (1) bile acid hydrolysis via microbial bile salt hydrolase (BSH) enzyme (Gilliland et al. 1985), (2) cholesterol incorporation in microbial cell (Noh et al. 1997) and (3) cholesterol conversion to easily assimilated metabolite (Gerard et al. 2007). Some researchers also suggested that probiotics were able to lower the cholesterol biosynthesis in the liver (Fukushima and Nakano 1996; Chiu et al. 2006). Various studies showed positive effects of probiotics on cholesterol levels in body. Lin and Chang (2000) observed attachment or assimilation of cholesterol on *L. acidophilus* surface, thus showing hypocholesterolaemic ability. Xiao et al. (2003) also observed a significant reduction in triglyceride, LDL and total cholesterol with administration of *Bifidobacterium* fermented milk. These effects were further confirmed by Abd El-Gawad et al. (2005) by feeding *B. lactis* or *B. longum* fermented yoghurt to the rats. Jones et al. (2011) reported an efficacious and safe application of encapsulated *Lactobacillus reuteri* NCIMB 30242 (having active BSH enzyme) fermented yoghurt formulation in decreasing low density lipid- cholesterol, total cholesterol, apoB-100 and non-high density lipid- cholesterol in hypercholesterolaemic organism .

Probiotics in hypertension—Hypertension leads to several pathological conditions such as heart failure, cardiac stroke, acute myocardial infarction, failure of renal system and main cause of premature death worldwide (Vasquez et al. 2019). Primary (or essential) hypertension depends upon various genetics, demographic and environmental conditions of individual (Freedman and Cohen 2016). Secondary hypertension is originated from behavioural conditions (drugs, smoking, etc.), endocrine disorders, cancer and excessive activation of the renin–angiotensin system (Rimoldi et al. 2014). Recent studies suggested that probiotics and their metabolites were able to improve total cholesterol level, LDL/HDL ratio, blood glycaemic levels, insulin resistance and functioning of renin–angiotensin system (Khalesi et al. 2014). Probiotics were able to secrete various effective bioactive metabolites having ACE inhibition activity that can be targeted for management of high BP. In RAS, renin causes inactivation of angiotensinogen via hydrolyses, resulting in inactive angiotensin-I, which enzymatically converted by ACE to angiotensin-II. Angiotensin-II can cause increased blood pressure and vasoconstriction; so, inhibition of ACE can serve as clinical target for management of high blood pressure (Thushara et al. 2016). Various probiotic fermented foods (milk, yogurt, cheese, ice-creams soymilk, etc.) were reported to have ACE inhibitory peptides (Fitzgerald and Murray 2006). Various clinical studies on humans also validate probiotics' ability to reducing high blood pressure levels. Naruszewicz et al. (2002) observed a significant reduction in blood pressure (systolic) with oral administration of *Lactobacillus plantarum*. Brantsaeter et al. (2011) reported that probiotic food intake can reduce the incidence of pre-clampsia, which is linked with hypertension and inflammation. Clinical trial based meta-analysis showed that probiotic consumption markedly reduced blood pressure in high BP patients. Khalesi et al. (2014) concluded that application of probiotics consortium can result in enhanced positive effects on hypertension condition.

Probiotic in autonomic control of cardiovascular function—Probiotic also benefits the autonomic neural control of cardiac pacemaker, baroreflex thereby controlling heart rhythm from brainstem integrative areas (Pimenta et al. 2018). Supplementation of kefir at least for 8 weeks reduced and incompletely reversed abnormal cardiac sympathetic predominance over the parasympathetic tone in SHR. Some studies showed that probiotics were significantly linked with reduced production of intravascular reactive oxygen species (ROS) and increased NO bioavailability. Reduction in cytokines and ROS production in hypothalamic paraventricular nucleus might be fundamental mechanism of probiotics on cardiac autonomic control that reduced hypertension and organ damage. Probiotic causes increased levels of anti-inflammatory molecules which diminish hypertension and organ

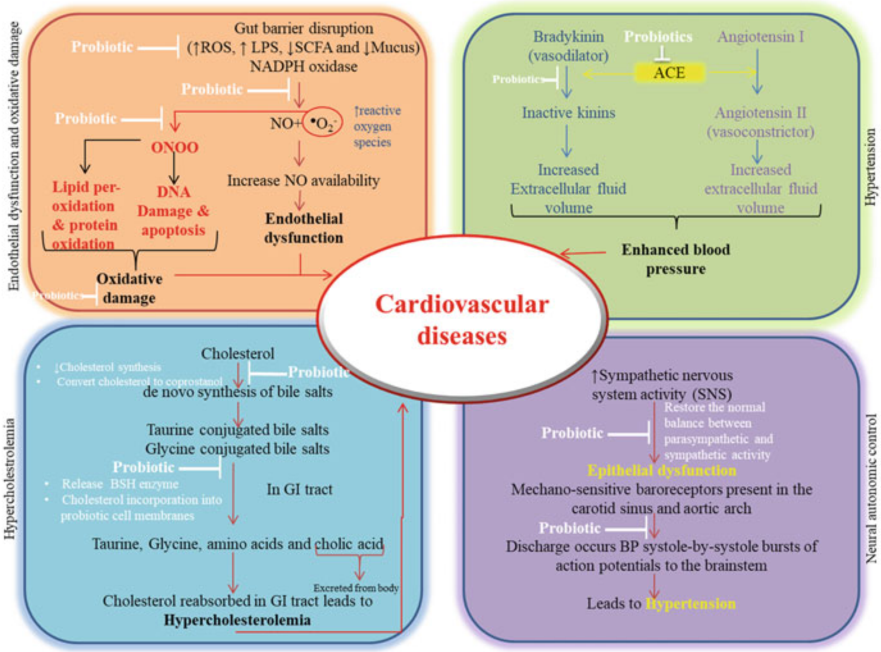

Fig. 5.1 Role of probiotic in management of cardiovascular diseases

damage thereby restoring regular functioning of parasympathetic/sympathetic system (Klippel et al. 2016). Probiotics directly or indirectly enhanced the normal functioning of various cardiovascular diseases (Fig. 5.1). Administration of probiotics (single or in consortium) has promising therapeutic effects on cardiovascular system, thus can serve as alternative approach to treat cardiovascular diseases.

5.5 Probiotics for Allergy and Autoimmune Disease

Estimates suggest that nearly 20% of the world population suffers from some form of allergic diseases (World Health Organization 2003). The increasing prevalence of allergic disease suggests prevalence of atopy in newer generations. Studies have shown that increased prevalence of atopy is nowadays connected with increase in sensitization to a variety of allergens rather than sensitization to one particular allergen (Jarvis and Burney 1998). The mechanism of allergy relies on the activity of different subtypes of T cells. Allergic person has dominance of Th2 cell mediated immune response. The type of immune response is controlled by Treg cells whose activity is crucial for immune tolerance and hyper reactivity. Activation of Th2 cells by Treg cells results in production of IL-4, IL-5, IL-13 and induction of inflammation by recruiting eosinophils and allergen specific IgE class switching in B cells

(Spacova et al. 2018). Moreover, atopic or allergic disorders develop under the influence of heterogeneous factors including genetic predisposition, environmental factors, tempering innate and acquired immune responses, etc.

Probiotic-induced protection against allergic diseases including atopic dermatitis, food allergies and airway allergy has been established in several animal studies and human clinical trials (Cuello-Garcia et al. 2015; Drago and Toscano 2015). Consumption of probiotics leads to modulation of immune system towards tolerance by activating maturation of Th1 cells and inhibition of Th2 cell response (Galdeano et al. 2019). Besides, the relation between composition of gut microflora and allergy has also been established by many researchers (Björkstén et al. 2001). Few other researchers have proposed that modification of gut colonization with the help of supplementation of probiotic strains during pregnancy and early infancy could down-regulate the onset of allergic diseases (Zuccotti et al. 2015). Recently a meta-analysis by Schmidt et al. (2019) explored the effect of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* subsp. *lactis* on the development of allergic diseases and sensitization in late infancy and early childhood. They observed that probiotics could effectively reduce incidence of eczema in probiotic consuming subjects. The mechanism behind this beneficial probiotic effect was explored by Forsberg et al. (2013) using pre- and postnatal consumption of *L. reuteri.* It was postulated from the study that supplementation of *L. reuteri* improved immunoregulatory capacity by decreasing Toll-like receptor-2 (TLR2) induced response during infancy. Even a direct effect of probiotic treatment in airway allergy has also been reported by Spacova et al. (2019). The study revealed that pre-treatment of *Lactobacillus rhamnosus* GG and *L. rhamnosus* GR-1 via intranasal route reduced all over airway hyperreactivity by decreasing eosinophil counts in bronchoalveolar lavage and IL-5 and IL-13 level in lungs in mouse model of allergic asthma. Similarly, Ma et al. (2019) reported that mucosal $CD103^+$ dendritic cells and differentiation of regulatory T cells were directly induced by probiotics during food allergy in murine model.

Probiotics also exert their beneficial effects on prevention of pathology of autoimmune diseases. For example, it has been deduced from number of studies that probiotics could improve the symptoms of rheumatoid arthritis. The disease develops by presence of autoantibodies called as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) causing inflammatory response at small joints such as hands, wrists, feet and progression of bone and join damage. The direct connection of the composition of gut and oral microbiota with pathogenesis of rheumatoid arthritis has been detected by many researchers (Bravo-Blas et al. 2016). However, the specific contribution of commensal microflora could not be recognized till date.

Probiotics are also reported to significantly modulate the markers in arthritis animal model (Achi et al. 2019). Precisely the modulation of cytokines involved lowering of pro-inflammatory cytokines (i.e., IL-1, IL-6, MCP-1 and TNF-α) and upregulation of the level of anti-inflammatory cytokines (i.e., IL-4, IL-10). Additionally, clinical benefits of probiotic supplements have also been reported in human subjects. Similarly, Rudbane et al. (2018) demonstrated that probiotics affected the

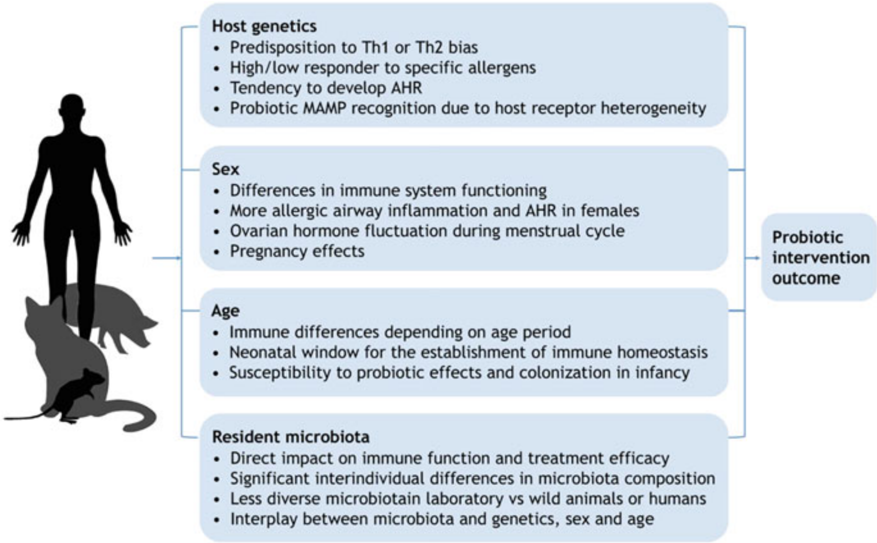

Fig. 5.2 Host factors affecting the impact of probiotic intervention in allergic diseases (figure adapted from Spacova et al. 2018)

level of inflammatory markers, i.e., erythrocyte sedimentation rate, TNF-α, IL-1β, IL-6, IL-10 and IL-12 as well as oxidative stress markers. This explains that the efficacy of probiotic treatment largely relies upon several host associated factors and varies from individual to individual.

In this line, a comprehensive article by Spacova et al. (2018) has explained that many factors like genetic build-up, age, sex and microbiological composition have significant influence on mechanism of immune system and how probiotics affect them (Fig. 5.2). The reason of conflicting results of probiotic interventions on allergic disease can be deciphered from the involvement of abovementioned variable factors which largely affect the results of clinical studies. An overview of currently present data demonstrates that supplementation of probiotics contributes to alleviation of allergy and autoimmune diseases by balancing between native microbiota, regulating inflammatory mediators and developing immune system. However, high levels of variability in related studies in the context of different formulations of probiotics for different types of patient populations and their respective outcomes have prevented from being observed any significant effect of probiotics on allergic diseases.

5.6 Probiotics for the Management of Obesity

Metabolism of dietary components is the best way to maintain homeostasis. Upon metabolic disbalance, complications like obesity, diabetes mellitus type 2, atherosclerosis, cardiovascular disorders, hypertension etc. may developed. The prevalence of these disorders is exponentially increasing, and predictions suggest, it will rise to 333 million by 2025 (Silveira et al. 2013). Among other things, obesity is a disorder that happens when the body accumulates excess fat. Influences such as hormonal, neural, hereditary and environmental are also involved in the development of obesity in addition to an unhealthy lifestyle. In order to manage high body weight, a healthy diet and physical exercise are always recommended. In addition, targeted appetite and fat absorption drugs are available, but their unnatural molecular structures are concerned with the safety of these synthetic drugs.

Gut microbiota composition has been closely associated with metabolic disorders. Scientific evidence indicates changes in gut microbiota contribute to differences in body weight index and body mass index (Cerdó et al. 2019). The diversity and abundance of few microbes can promote energy harvesting and metabolic pathways that lead to obesity. Consequently, modulation of intestinal microbiota may be a possible target for good health. In this regards, probiotic, the beneficial gut bacteria, has been found to encourage the abundance of intestinal microbiota, boost intestinal capacity and reinstate the microbiota changes that occurred during obesity (Table 5.2) (Mazloom et al. 2019). In addition, certain metabolic enzymes, pancreatic lipases and pancreatic phospholipase A2, which are responsible for digestion and dietary fat absorption, may be targeted for obesity control (Birari and Bhutani 2007; Wang et al. 2006; Shi and Burn 2004).

Although there are several unknown facts, a general concord states that gut microbiota are associated with obesity through dietary sugar fermentation, lipid metabolism and metabolic pathways leading to food intake control and energy balance regulation (Mazloom et al. 2019). Probiotics may enhance intestinal microbiota dysbiosis caused by high-calorie diets by improving the useful bacteria and dipping the pro-inflammatory bacteria (Kong et al. 2019). Recently, Ejtahed and co-researcher conducted a systematic review of obesity management through probiotics supplementation utilizing diverse databases like as PubMed, Cochrane, EMBASE, Web of Science and Scopus. The probiotic supplementation decreased weight increase or fat accretion in 72 animal studies in 61 trials utilizing specific strains of *Lactobacillus* and *Bifidobacterium*. In addition, three out of 15 clinical trials showed positive results as well (Ejtahed et al. 2019).

A study on overweight and obese individuals suggested that consumption of probiotic yogurt (including *L. acidophilus*, *L. casei*, *B. lactis* and *S. thermophiles*) along with low-calorie diet significantly increases the expression of genes such as toll-like receptor-2 and FOXP3, thus adding to the initiation of inflammatory responses and antigen-specific adaptive immunity (Yazdani et al. 2018). Moreover, an *L. reuteri* strain has also been reported to enhance indicators of obesity and pro-inflammatory factors, altering the gene expression of insulin and lipid metabolism in

Table 5.2 A list of probiotic strains shows anti-obesity effects in animal and human trails[a]

Strain	Anti-obesity effect	References
Lactobacillus rhamnosus	• Reduced weight gain, lipid accumulation and epididymal fat mass • Decreased adipose tissue mass and body weight	Lee et al. (2006), Kim et al. (2013), Ji et al. (2012)
Lactobacillus gasseri	• Prevented body weight gain and fat accumulation • Decreased mesenteric/retroperitoneal adipocyte size • Decreased abdominal adiposity and body weight	Kang et al. (2013), Sato et al. (2008) Kadooka et al. (2010)
Lactobacillus paracasei	• Attenuated mass increase and belly fat accumulation • Reduced body weight and visceral fat	Tanida et al. (2008), Thiennimitr et al. (2018)
Lactobacillus reuteri	• Decreased mass increase and belly fat pathology • Decreased dietary fat absorption	Poutahidis et al. (2013), Chung et al. (2016)
Lactobacillus plantarum	• Decreased body weight and adipose tissue mass • Reduced body mass, BMI and fat	Lee et al. (2007), Higashikawa et al. (2016)
Lactobacillus casei	• Decreased body weight, BMI, fat mass, leptin and glucose levels	Karimi et al. (2015)

[a]Adopted from Mazloom et al. (2019)

white adipose tissues as well (Chen et al. 2018). A soy fermented beverage with probiotic *Enterococcus faecium* and *Bifidobacterium longum* also reduced adipocytes and increased IL-6 and IL-10 levels in mice model (de Carvalho Marchesin et al. 2018).

A probiotic mix, including *L. Plantarum*, *L. Fermentum*, *B. Longum* with prebiotic Triphala, showed beneficial effects on physical obesity indicators including decrease in overall weight, triglyceride and glucose levels (Westfall et al. 2018). In a different study, adult mice nursed with *L. casei* fermented milk were observed with decreased body weight, increased number of immunoglobulin cells (A-positive) and macrophages in the small intestine (Núñez et al. 2014). Likewise, two *L. plantarum* and *L. curvatus* probiotic strains showed a decline in adipose tissue and liver fat accumulation (Yoo et al. 2013). Similarly, probiotic supplementation of *B. longum* reduced fat, size of adipocyte, expression of lipoprotein lipase, modulated leptin level and increased receptor-activated adiponectin and peroxisome proliferator-activated receptors-g expression in diet-induced obese rats (Karimi et al. 2017). Interestingly, a *B. breve* strain demonstrated the potential to prevent and improve obesity by decreasing weight gain, fat deposit on adipose tissue, size of adipocytes and macrophage (Roselli et al. 2018).

5.7 Probiotics as Bio-therapeutics for NAFLD

Non-alcoholic fatty liver disease (NAFLD) due to obesity has become a global health issue in both teenagers and adults. NAFLD is an increase in triglycerides build-up in liver cells (hepatocytes) which exceeds 5–10% of the total liver mass (Neuschwander-Tetri and Caldwell 2003). Till now, no medical therapy is approved for NAFLD treatment and the primary therapeutics is exercise and dietary management (life-style therapies) (El-Agroudy et al. 2019). Furthermore, NAFLD is a multifactorial disease and its pathogenesis is still unclear. The “two strike hypothesis” is one of the original hypotheses for NAFLD pathogenesis because steatosis is the first strike and oxidative pressure is the second hit (Day and James 1998; Byrne et al. 2009).

Gut microbiota, in other terms, the forgotten organ, consist of up to 2000 species, *Fusobacteria*, *Bifidobacteria*, *Eubacteria*, *Firmicutes*, *Pepto-streptococci* and *Bacteroides* (Salminen et al. 1998; Dethlefsen et al. 2006; Zihler 2010), in the intestine of human adults. Vespasiani-Gentilucci et al. (2018) recently identified a correlation between changes in gut microbiota and NAFLD pathogenesis. In NAFLD patients, stages of faecal *Bradyrhizobium*, *Propionibacterium acnes*, *Dorea*, *Peptoniphilus*, *Anaerococcus*, *Ruminococcus* and lower proportions of *Rikenellaceae* and *Oscillospira* increased (Del Chierico et al. 2017). In another study conducted by Zhu et al. (2013), they found that patients with obesity and NASH had different ratios of Firmicutes, Bacteroidetes, Actinobacteria and alcohol-producing bacteria in comparison to the vigorous patients and improved disease-causing bacterial strains to substitute them, like *Clostridium* sp.

The main metabolites of fermentation accumulated sugar like fructose in the intestine by various groups of gut microbiota are organic acids, e.g., lactic and succinic acids, fatty acids include ethanoate, propionic acid and butyric acid and ethanol (Salminen et al. 1998). Lactate, acetate plays a crucial function in the metabolic cross-feeding pathways of bacterial organisms such as *Anaerostipes* (*A.*) *caccae*/*Eubacterium hallii* (Belenguer et al. 2006) and *A. caccae* and the *Roseburia* sp. (Falony et al. 2006). Furthermore, SCFAs can attenuate accretion of triglycerides in hepatic cells (hepatocytes) via increasing the ratio of propionate to acetate which could inhibit lipogenesis (Daubioul et al. 2002).

Ethanol and acetaldehyde are essential sugar fermentation metabolites used by various gut microbiota, e.g., heterolactic intestinal bacteria. Ethanol can be absorbed into acetate and also acetaldehyde. Acetaldehyde may result in increased blood circulation leading to liver injury (Medina et al. 2004). Baker et al. (2010) stated that ethanol provided by intestinal microbiota in patients with NAFLD had contributed to NAFLD production. Zhu et al. (2013) also found that NASH patients also had improved alcohol-generating bacteria that increased serum levels of alcohol and increased oxidative stress. Elshaghabee et al. (2016) found that the non-mannitol generating intestinal hetero-fermentative lactobacilli (i.e., *Weissella confusa*) were observed to generate large quantities of ethanol from the fermentation of fructose in obese faecal slurries.

Furthermore, Lipopolysaccharide (LPS) endotoxin produced by Gram-negative (G^-) intestinal bacteria was shown to induce different pro-inflammatory cascades-associated NASH (Miele et al. 2009). On the other hand, gut microbiota fermentation of dietary fructans may stimulate glucagon hormone such as peptide-1 and also peptide YY (Uris-Silvas et al. 2008). They also enhance glucagon secretion such as peptide-2 which plays a vital part in enhancing the colonic integrity and tight junction and reducing blood circulation of LPS (Cani et al. 2007; Cani and Delzenne 2009). Results from the model of in vitro fermentation revealed that various gut microbiota could FOS (ferment short-chain), i.e., *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, *Lactobacillus and Roseburia* may ferment oligofructose, but few gut microbiota may use long-chain fructans (Ramirez-Farias et al. 2009; De Vuyst and Leroy 2011). Clinical experiments consuming breath hydrogen using a fermentation marker indicate that microbial fermentation is the individual mechanism for the production of hydrogen in the human body (Levitt 1969).

Probiotics represent one of the dietary strategies for reducing the risk of NAFLD. The definition of probiotics was evolved during the last years. FAO/WHO (2001) and Reid et al. (2003) well-defined probiotics as “creation of a product comprising feasible, specified microorganisms in adequate quantities that modify the microflora (by implantation or colonization) in the host section and thus have helpful health effects in the host”. Recently, Elshaghabee (2017) has defined probiotics as “Live bacterial strains with healthiness effect on the host when they ingest sufficient amounts daily (not less than 10^6–10^8 CFU/g) and are combined into gut microbiome”. The foremost genera of probiotics are *Lactobacillus* and *Bifidobacterium* and several fitness assistances have been investigated using in vitro models or different in vivo models (El-Deib et al. 2010; Hsieh et al. 2013; Wagnerberger et al. 2013), for example, modulation of the immune system, lessening of cholesterol, antioxidant and also anti-carcinogenic activity levels.

Several animal models were conducted to test the impact of various probiotic strains on NAFLD risk reduction as shown in Table 5.3. On the other hand, as shown in Table 5.4, few clinical trials had been directed to traverse the protective effect of probiotics against NAFLD. Further work is required on the mechanism through which probiotics could reduce NAFLD risk. Figure 5.3 indicates various potential pathways of probiotic health benefits for NAFLD.

One among the next generation probiotic, *Akkermansia muciniphila*, mainly isolated from the mammalian intestine can make use of mucin as a single source of carbon (Derrien et al. 2004), oval formed, gram-negative, immotile and strictly anaerobic, can make use mucin as a single source of carbon, release sulfur also the two main metabolites are acetate and propionate. A significant percentage (about 85%) of *A. muciniphila* may live in the gastrointestinal tract of vancomycin-treated mice (Hansen et al. 2012) and a multi-antibiotic patient (Dubourg et al. 2013). *Akkermansia muciniphila* regulates different metabolism pathways of the host either in the living or pasteurized form, whereas both forms could reduce the body and fat mass gain as well as reduce serum triglycerides and abstaining glucose, also enhance insulin sensitivity in mice (Everard et al. 2013). Besides, *Akkermansia muciniphila* could improve the gut barrier by enhancing the countenance of constricted connection of protein together with occludin, claudins and ZO-1, ZO-2 and ZO-3, by

Table 5.3 Different animal models used for evaluating the protective effect of probiotics against NAFLD

Author	Animal model	Diet induced NAFLD	Probiotics	Findings
Esposito et al. (2009)	Rat	High fat diet	VSL#3 probiotic culture contains a mixture of viable bifidobacteria, lactobacilli and *S. thermophilus*	Reduction of inflammatory signaling and limited oxidative liver damage
Wagnerberger et al. (2013)	Mice	High fructose solution	*L. casei* Shirota	Reduction of the TLR4 signaling cascade
Hsieh et al. (2013)	Rat	High fructose diet	*L. reuteri* GMNL 263	Decreased levels of inflammatory cytokines and normalizing the overexpression of lipogenic genes
Bhathena et al. (2013)	Hamster	Methionine deficient/ choline	*L. fermentum* ATCC 11976	Reduction of hepatic triglycerides and modulation of the gene expression of HMG-CoA reductase
Reichold et al. (2014)	Mice	Western diet	*B. adolescentis*	Modulation of expression of 88 mRNA and portal endotoxin levels. Activation of nuclear factor NF-κB and inhibited lipid peroxidation
Wang et al. (2015)	Mice	High fat diet	*L. paracasei* CNCM I-4270, *L. rhamnosus* I-3690 and *B. animalis* subsp. *lactis* I-2494	Modulation of the abundances of gut microbiota
Elshaghabee et al. (2016)	Rat	High fructose diet	Probiotic Karish cheese contains *L. acidophilus*, *B. longum* and *S. thermophiles*	Reduction of levels of faecal *Enterobacteriaceae*, and modulation of some biochemical parameters, e.g., cholesterol, glucose, IL-6 and IL-10
Al-Muzafar and Amin (2017)	Rat	High fat high sugar diet	Probiotic mixture containing *L. acidophilus*, *L. plantarum*, *B. bifidum* and *B. subtilis*	Reduction of levels of interleukin IL-6 and improve leptin as well as lipid profiles
Liu et al. (2020)	Mice	High fat high fructose diet + chronic intermittent hypoxia	*L. rhamnosus* GG culture supernatant	Enhancement of the metabolic function via fibroblast growth factor 21 adiponectin pathway

Table 5.4 Examples of different human studies used for evaluating probiotics as a complementary therapy for NAFLD

Author	N	Duration (week)	Probiotics	Main findings
Loguercio et al. (2005)	22	16	VSL#3	Probiotic strains could modulate liver biochemical parameters (ALT and AST) in volunteers. This effect is varied between different volunteers from significant to non-significant
Aller et al. (2011)	14	12	Yoghurt culture	
Wong et al. (2013)	10	24	*L. plantarum*, *L. bulgaricus* *L. acidophilus*, *L. rhamnosus* *B. bifidum*	
Alisi et al. (2014)	22	16	VSL#3	
Eslamparast et al. (2014)	52	28	*L. acidophilus*, *L. casei* *L. bulgaricus*, *L. rhamnosus* *B. longum*, *B. breve*, *St. thermophilus*	
Abdel Monem (2017)	15	68	*L. acidophilus*	
Famouri et al. (2017)	32	12	*L. acidophilus*, *L. rhamnosus* *B. lactis*, *B. bifidum*	
Mofidi et al. (2017)	25	28	*L. acidophilus*, *L. casei* *L. bulgaricus*, *L. rhamnosus* *B. longum*, *B. breve* *St. thermophilus*	
Kobyliak et al. (2018)	29	8	*Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Propionibacterium*, *Acetobacter*	

reducing the circulation of endotoxemia such as LPS stages and by inhibiting inflammatory reaction resulting in increased eGlucose and fat metabolism (Everard et al. 2013; Grander et al. 2018).

5.8 Probiotic Intervention for the Management of Celiac Disease

Celiac disease is a type of chronic enteropathy developed due to particular proteins of our diet which initiates immune response against self-cells in genetically predisposed humans. The causative proteins are known as gluten proteins,

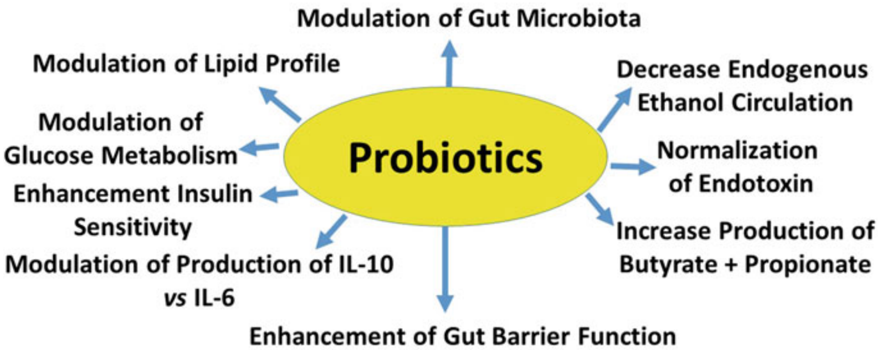

Fig. 5.3 Possible mechanisms of bio-therapeutic effects of probiotics against NAFLD

commonly available in cereals like wheat, Barley, oats and ray. Gluten is a glutamine- and proline-rich proteins and is partially hydrolysed in the gastrointestinal tract. The presence of an antigen on the surface of leukocyte, namely human leukocyte antigen HLA-DQ (human leukocyte antigen Class II with DQ2 and/or DQ8 molecules on antigen-presenting cells) system, is proved to be associated with genetic predisposition of celiac disease. These receptors help to recognize self and non-self molecules in the immune system. The indigested peptides enter the circulatory system and get in contact with the lymphocytes (Fig. 5.4). Recognition of peptides induces release of cytokines from lymphocytes and T-cells, i.e., interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) and matrix metalloproteinases (Kupfer and Jabri 2012). Activation of these components causes severe damage to the villi of small intestine. Clinical manifestation of celiac disease includes abdominal pain, weight loss, fatigue and some indirect consequences such as anaemia, osteoporosis, polyneuropathy, cerebellar ataxia, etc. (Lionetti and Catassi 2011). HLA-DQ genes are carried by 40% of the population but development of celiac disease is observed in only 1–2% population (Lionetti and Catassi 2011). This indicates involvement of additional environmental and host factors in progression of celiac disease pathogenesis.

The environmental factors such as, repeated gastrointestinal infections, and major change in the composition of gut microbiota has been seen to be connected with the advancement of celiac disease (Olivares et al. 2015; Nistal et al. 2016; Valitutti et al. 2019). In this line a case control study on newborns who had one or more first relative with the history of celiac disease was done by Olivares et al. (2018). They configured the components of gut microbiota using 16S rRNA gene sequencing and found that the infants at high risk of celiac disease had modifications in early composition of gut microbiota. The findings of study suggest that relative abundance of *Bifidobacterium breve* and *Enterococcus* spp. were associated with celiac disease development (Olivares et al. 2018). Recently, Bodkhe and team have studied the gluten degradation ability of faecal microbiota from healthy and celiac disease human subjects. They found that gut microbiota from celiac disease human had reduced gluten degradation ability in comparison to gut microbiota from healthy

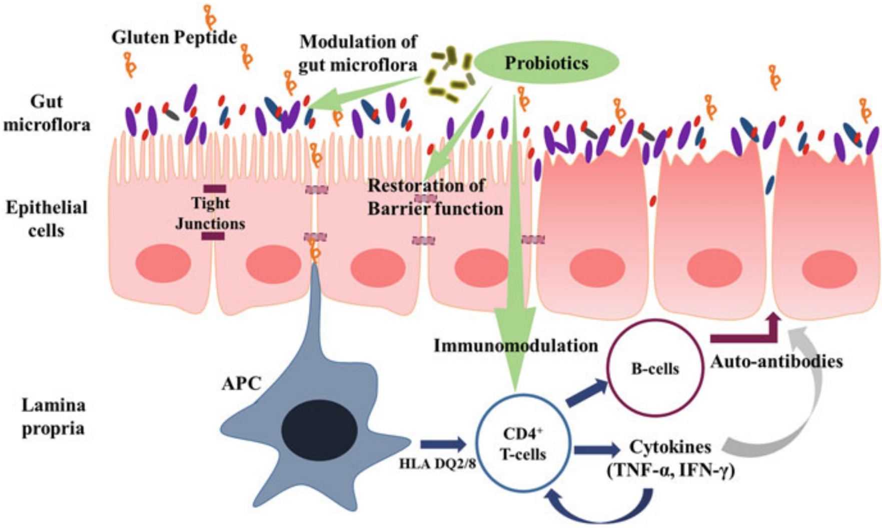

Fig. 5.4 Pathogenesis of celiac disease and probiotic interventions for prevention of the disease. Gluten peptides translocates to lamina propria and are presented to $CD4^{+}$T cells, which activates secretion of cytokines and autoantibodies. Altogether, the activity promotes degradation of matrix and causes deformation in villi. Probiotics improve integrity of intestinal barrier function by restabilising gut microbiota and by reducing inflammation. They also improvise the symptoms of disease by modulating immune cell response

control human. More precisely, celiac disease patients had higher abundance of genera *Megasphaera* and *Helicobacter* compared to the pre-disease condition subjects. However, both disease and pre-disease condition subjects also had reduced abundance of *Akkermansia* and *Dorea* (Bodkhe et al. 2019). The similar kind of observations recorded by several researchers indicates a direct correlation between composition of gut microbiota and development of clinical symptoms of celiac disease (Cenit et al. 2015; Garcia-Mazcorro et al. 2018; Rintala et al. 2018).

The knowledge of relation between the host genetic factors and individual's gut microbiota which perform an important role in development of celiac disease can be used for preventive measures by manipulating the gut environment. For this reason, probiotics are been thought to be used as an effective tool in the control of celiac disease using dietetic strategies. The management of celiac disease using probiotic intervention is also been proposed at the different levels of the disease. For example, in newly diagnosed celiac disease children, supplementation of probiotic *Bifidobacterium longum* CECT 7347 in conjunction with gluten-free diet improved health of the subjects by influencing the inflammation markers (Olivares et al. 2014). In later stage also, probiotic treatment has been reported to effectively improve the severity of symptoms of celiac disease patients. Another study by Francavilla et al. (2019) found apparent improvement in lactic acid bacteria, *Staphylococcus* and *Bifidobacterium*, in faecal microbiota of patients receiving probiotic treatment. The composition of gut microflora directly influences the stability of intestinal epithelium. Intestinal epithelium is the only shield between the receptors of intestinal immune cells and their ligands. The instability of epithelial barrier exposes the large number of antigens to the reactive immune cells and subsequently causes inflammation generated structural and functional damage to the interphase. Probiotics could restore the epithelial dysfunction by modulation of immune response at damage site (Krishnan et al. 2016; Zaylaa et al. 2018). The generation of pro-inflammatory cytokines in celiac disease patients is a critical step during autoimmune response. Probiotics are reported to decrease the production of these cytokines in such conditions. For example, the production of pro-inflammatory cytokine TNF-α was reduced in diseased children having gluten-free diet and two probiotic strains *Bifidobacterium breve* BR03 and *B. breve* B632 (Klemenak et al. 2015).

Moreover, probiotics can also modulate gluten antigen generated immune response. A recent study by Håkansson et al. (2019) explored the effect of oral consumption of *L. plantarum* HEAL9 and *L. paracasei* 8700:2 in children having genetic risk factors for the disease. The phenotyping of peripheral blood lymphocytes and IgA and IgG autoantibodies against tissue transglutaminase (tTG) was observed after probiotic treatment. The study concluded that in comparison to placebo group, probiotics temper the immune response of peripheral lymphocytes in children having celiac disease autoimmunity (Håkansson et al. 2019). Hence, after reviewing probiotic based studies at different levels, the probable mechanism behind the beneficial action of these organism can be identified (Fig. 5.4). At preliminary level, probiotics establish constancy of the gut microbiota, thus reduce the inflammation and enhance the intestinal barrier function integrity. On the other hand, probiotics could also act on modulation of immune cell response elicited by the presence of gluten peptide to reduce the deleterious effect of autoimmune response.

5.9 Probiotic Intervention for the Management of Diabetes Mellitus

Diabetes is a metabolic disorder characterized by impaired blood glucose levels. Currently, diabetes is prevalent among 10% adults worldwide besides the quantity of cases is predicted to be 592 million with 175 prediabetic patients till 2035, according to International Diabetes Federation (Zimmet et al. 2001; WHO 2006). The disease can be classified into two categories; type-I diabetes is an autoimmune disease triggered by the devastation of insulin-releasing β-cells in the pancreas, leading to absolute insulin deficiency, whereas type-II diabetes is a metabolic disorder characterized by a progressive loss of insulin secretion from pancreatic β-cells, low insulin release, insulin resistance or/and inefficiency of the body to utilize insulin (Cani et al. 2005). Type-II diabetes is a multifactorial disease, as decreased sensitivity or increased resistance towards insulin further leads to various metabolic variations like elevated free greasy acids, protein kinase C activation, oxidative stress, endothelial dysfunction and inflammation. Additionally, reduced incretin hormone secretion (glucagon-like peptide-1; GLP-1) besides increased cluster of extra counter-regulatory hormones (Di-peptidyl peptidase-4, α, β glucosidases, etc.) also contributes to diabetes (Cecilia et al. 2016).

The present-day lifestyle and high-fat diet habits alter gut microbiota, increase oxidation of fatty acids in the liver also adipocytes of adipose tissue. An unhealthy diet increases the mortality rate of Gram-negative bacteria, leading to the development of LPS in the intestine, also its transfer into intestinal capillaries as well as in general circulation. Reactive oxygen species (ROS) generated during the oxidation process reduces the development of mucus in the intestinal epithelium and causes injury to the epithelial cell membranes leading to increased permeability of intestinal tight junctions (Hall et al. 2001; Muccioli et al. 2010), while the compromised integrity of the intestinal barrier and LPS allows intestinal bacteria to be translocated to the lumen (Brownleea et al. 2007). In diabetes, improved Gram-negative to Gram-positive bacteria ratio contributes to gut symbiosis thus rises lipopolysaccharide (cell wall constituent of Gram-negative bacteria) levels in the gut which causes activation of toll-like receptors (TLR). Lipopolysaccharides binding to TLR-4 activate signaling cascade that exerts deleterious effects on pancreatic β-cell function (He et al. 2018). To counteract this situation, the establishment of normal gut microflora can serve as an alternative approach to improve gut health and anti-inflammatory effect (Zimmet et al. 2001; Everard et al. 2014; Wang et al. 2015).

The probiotic mechanisms are allied with positive properties such as various cell components, secreted proteins, carbon-based acids, bacteriocins also fatty acids (Yoo et al. 2013; O'Shea et al. 2012). Probiotic microbiota produce metabolites that could serve to increase the supply of nutrients used through intestinal epithelial cells (IEC), moreover, enhance the permeability of the intestine and prevent the passage of lipopolysaccharide (LPS) through systematic circulation thus decline metabolic endotoxemia. Probiotics microbiota can also express (MAMPs) microorganism-associated molecular patterns, which may bind to host pattern recognition receptors (PRRs) situated on the IEC cell surface also dendritic cells (Thomas et al. 2014). First, probiotics arouse dendritic cells that inhibit pro-inflammatory proliferation of CD4 + cells and trigger anti-inflammatory pathways and proliferation of plasma cells, resulting in the development of anti-inflammatory cytokines and IgA immunoglobulins (Moya-Perez et al. 2014). The anti-diabetic efficiency of probiotic strains investigated in vitro and in vivo conditions are briefly reviewed below.

The probiotics have been broadly studied for their great health benefits to improving immune system function and preventing diarrhoea (Hussein 2015), cancer (Ma et al. 2010), allergic (Felice et al. 2008; Lin et al. 2013) and HIV diseases (Villar-Garcia et al. 2015). Probiotics have also been shown to decrease blood glucose by enhancing inflammation and preventing β-cell death in animal models (Ushkalova 2014). Lactic acid bacteria strains (*Bifidobacterium bifidum*) isolated from vigorous newborn children (<9 months) showed that balancing incretin hormone emission also gene expression in the enteroendocrine cells can evoke GLP-1 and GIP emission (13 to 194-turn up) and balance gene expression. Incretin hormone secretion caused by LAB did not appear to include nutrient pathways nor was around a little sign of cytolysis. Instead, PCR array research included toll-like receptor system signaling agents including the significant changes observed in MyD88 and CD14 expression (Panwar et al. 2016). In another study, *L. plantarum*, *L. fermentum*, *L. casei* and *L. rhamnosus* showed broad-spectrum β glucosidase repressive actions as compared to its chemical positive control (acarbose*). L. rhamnosus* extract administered (1 g/1 kg) to glucose challenged mice reduced glucose excursions, hence had anti-diabetic potential (Panwar et al. 2014). Furthermore, approximately Lactobacillus strains (*L. plantarum* and *L. fermentum*), also, *Salmonella Typhimurium* and *E. coli*, potential sources of DPP-4 inhibitory activity were observed (10–32%; $p < 0.05$–0.001) in the enzymatic inactivation of incretin hormones. *L. plantarum* (12–25%) and *L. fermentum* strain had major inhibitory activity (14%) (Panwar et al. 2015).

Diverse animal models have been extensively explored for studying the role of probiotics in the management of diabetes. It had been found in a recent analysis that *L. Plantarum* improves the anti-diabetic activity of high fructose ingestion of insulin receptor substrate (IRS)-1, protein kinase B (AKT) and endothelial nitric oxide synthase (eNOS) reduced renal protein expression in rat and partial supplementations through *L. helveticus*. Dietary fructose-induced elevations of the tumour necrosis factor α (TNF-α), interleukin (IL)-1β, IL-6 and IL-10 levels in the renal tissue, besides, expression of IL-6 mRNA, were reduced, exclusively in *L. plantarum* treated rats. Treatment with *L plantarum* suppressed the increased renal expression of sodium-glucose cotransporter-2 (SGLT2), but not that of the glucose transporter type-5 (GLUT5) (Korkmaz et al. 2019). Probiotic Dahi comprising of *Lactobacillus acidophilus* and *Lactobacillus casei* significantly increased the adherence of lactobacilli to epithelial walls, thus decreasing the amount and adherence of coliforms in streptozotocin-induced diabetic rats. Oral intake of probiotic Dahi has also been found to minimize the oxidative stress marker thiobarbituric acid-reactive species in the intestinal tissue and haemoglobin glycosylation (Yadav et al. 2008). In additional research, the authors found that the anti-diabetic effects of specific lactic acid bacteria *Lactobacillus rhamnosus* GG by blocking glucose absorption from the intestine reduced postprandial blood glucose (Honda et al. 2012).

The probiotic and conventional yogurts containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* enriched with *Bifidobacterium animalis* subsp. *lactis* Bb12 (DSM 10140) and *Lactobacillus acidophilus* strain La5 were administered at 300 g/day for 8 weeks to type-2 diabetic patients. Patient serum analysis showed a substantial reduction in levels of HbA1c besides inflammatory marker TNF-α. The author also observed a non-significant reduction in IL-6 and hs-CRP levels in intervention set of serum relative to the control group (Mohamadshahi et al. 2014). Next, meta-analysis studies also showed that mixed probiotic administration to rats for a minimum of 8 weeks significantly reduced weighted mean difference and improved glucose metabolism (Zhang et al. 2016).

Besides, the outcome of probiotics on the regulation of hypertension and dyslipidemia on blood compression and the lipid contour of type-2 diabetic patients expressively decreased blood levels of TG and TC and LDL-C; despite, the impact of probiotics on increased HDL-C was not statistically significant besides rest on patient BMI and age (Hendijani et al. 2017). The outcome of probiotic fermented milk (kefir) on glucose also lipid outline was observed to diminish blood glucose and HbA1C levels in the fasting blood. The serum level of total cholesterol decreased compared with the two groups but this decrease was not statistically important. Such results indicate that probiotic fermented milk may be useful for controlling diabetic patients for medical nutrition (Ostadrahimi et al. 2015).

5.10 Conclusions

It is now well established that the benefits of probiotics include protection from vast varieties of illness including infectious diseases, immunological dysfunctions, neuro-degenerative disease and metabolic disorders. The efficacy of probiotics for human health promotion is backed with ample amount of scientific evidences and they are practiced by medical professionals as dietary, preventive or supplementary interventions for the treatment of several diseases. In this manner, we can say that probiotics hold a new ray of hope for betterment of human health.

References

Abd El-Gawad IA, El-Sayed EM, Hafez SA, El-Zeini HM, Saleh FA (2005) The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. Int Dairy J 15(1):37–44

Abdel Monem SM (2017) Probiotic therapy in patients with nonalcoholic steatohepatitis in Zagazig University Hospitals. Euroasian J Hepato-Gastroenterology 7(1):101–106. https://doi.org/10.5005/jp-journals-10018-1226

Abdrabou AM, Osman EY, Aboubakr OA (2018) Comparative therapeutic efficacy study of Lactobacilli probiotics and citalopram in treatment of acute stress-induced depression in lab murine models. Hum Microbiome J 10(2018):33–36

Achi SC, Talahalli RR, Halami PM (2019) Prophylactic effects of probiotic Bifidobacterium spp. in the resolution of inflammation in arthritic rats. Appl Microbiol Biotechnol 103:1–10

Achuthan AA, Duary RK, Madathil A, Panwar H, Kumar H, Batish VK, Grover S (2012) Antioxidative potential of lactobacilli isolated from the gut of Indian people. Mol Biol Rep 39:7887–7897

Aguilar-Pérez C, Gracia B, Rodrigues L, Vitoria A, Cebrián R, Deboosère N et al (2018) Synergy between circular bacteriocin AS-48 and ethambutol against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 62:e00359–e00318

Ait-Belgnaoui A et al (2016) Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology 37 (11):1885–1895

Alisi A, Bedogni G, Baviera G, Giorgio V, Porro E, Paris C, Giammaria P, Reali L, Anania F, Nobili V (2014) Randomised clinical trial: the beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. Aliment Pharmacol Ther 39(11):1276–1285. https://doi.org/10.1111/apt.12758

Aller R, De Luis DA, Izaola O, Conde R, Gonzalez SM, Primo D, De La Fuente B, Gonzalez J (2011) Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci 15:1090–1095

Al-Muzafar HM, Amin KA (2017) Probiotic mixture improves fatty liver disease by virtue of its action on lipid profiles, leptin, and inflammatory biomarkers. BMC Complement Altern Med 17 (1):43

Aloglu H, Öner Z (2006) Assimilation of cholesterol in broth, cream, and butter by probiotic bacteria. Eur J Lipid Sci Technol 108:709–713

Arruda PH, Madson DM, Ramirez A, Rowe EW, Songer JG (2016) Bacterial probiotics as an aid in the control of Clostridium difficile disease in neonatal pigs. Can Vet J 57:183–188

Arsi K, Donoghue A, Woo-Ming A, Blore P, Donoghue D (2015) The efficacy of selected probiotic and prebiotic combinations in reducing Campylobacter colonization in broiler chickens. J Appl Poult Res 24:327–334

Ashraf R, Shah NP (2014) Immune system stimulation by probiotic microorganisms. Crit Rev Food Sci Nutr 54:938–956. https://doi.org/10.1080/10408398.2011.619671

Baker SS, Baker RD, Liu W, Nowak NJ, Zhu L (2010) Role of alcohol metabolism in non-alcoholic steatohepatitis. PLoS One 5(3):e9570

Belenguer A, Duncan SH, Calder AG et al (2006) Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. Appl Environ Microbiol 72:3593–3599. https://doi.org/10.1128/AEM.72.5.3593-3599.2006

Bhathena J, Martoni C, Kulamarva A, Tomaro-Duchesneau C, Malhotra M, Paul A, Urbanska AM, Prakash S (2013) Oral probiotic microcapsule formulation ameliorates non-alcoholic fatty liver disease in bio F_1B golden Syrian hamsters. PLoS One 8(3):e58394

Birari RB, Bhutani KK (2007) Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today 12:879–889

Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M (2001) Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol 108(4):516–520

Bodkhe R, Shetty SA, Dhotre DP, Verma AK, Bhatia K, Mishra A, Kaur G, Pande P, Bangarusamy DK, Santosh BP, Ahuja V (2019) Comparison of small gut and whole gut microbiota of first-degree relatives with adult celiac disease patients and controls. Front Microbiol 10:164

Brantsaeter AL, Myhre R, Haugen M et al (2011) Intake of probiotic food and risk of preeclampsia in primiparous women: the Norwegian mother and child cohort study. Am J Epidemiol 174 (7):807–815

Bravo-Blas A, Wessel H, Milling S (2016) Microbiota and arthritis: correlations or cause? Curr Opin Rheumatol 28(2):161–167

Brownleea I, Knighta J, Dettmar PW, Pearson JP (2007) Action of reactive oxygen species on colonic mucus secretions. J Free Radic Biol Med 43:800–808

Byrne CD, Olufadi R, Bruce KD et al (2009) Metabolic disturbances in non-alcoholic fatty liver disease. Clin Sci 116:539–564. https://doi.org/10.1042/CS20080253

Cani PD, Delzenne NM (2009) The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des 15:1546–1558

Cani PD, Daubioul CA, Reusens B, Remacle C, Catillon G, Delzenne NM (2005) Involvement of endogenous glucagon-like peptide-1 amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. J Endocrinol 2005(185):457–465

Cani PD, Amar J, Iglesias MA et al (2007) Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56:1761–1772. https://doi.org/10.2337/db06-1491

Cecilia C, Low W, Connie NH, William RH, Goldfine AB (2016) Clinical update: cardiovascular disease in diabetes mellitus. Atherosclerotic cardiovascular disease and heart failure in type 2 diabetes mellitus – mechanisms, management, and clinical considerations. Circulation 133:2459–2502

Cenit MC, Olivares M, Codoñer-Franch P, Sanz Y (2015) Intestinal microbiota and celiac disease: cause, consequence or co-evolution? Nutrients 7(8):6900–6923

Centers for Disease Control Prevention (2013) National salmonella surveillance annual report, 2011. US Department of Health and Human Services, CDC, Atlanta

Centers for Disease Control Prevention (2019) US Department of Health and Human Services. CDC, Atlanta

Cerdó T, García-Santos JA, Bermúdez M, Campoy C (2019) The role of probiotics and prebiotics in the prevention and treatment of obesity. Nutrients 11(3):635

Chauhan R, Vasanthakumari AS, Panwar H, Mallapa RH, Duary RK, Batish VK, Grover S (2014) Amelioration of colitis in mouse model by exploring antioxidative potentials of an indigenous probiotic strain of *Lactobacillus fermentum* Lf1. BioMed Res Int 3:206732. https://doi.org/10.1155/2014/206732

Chen LH, Chen YH, Cheng KC, Chien TY, Chan CH, Tsao SP, Huang HY (2018) Antiobesity effect of *Lactobacillus reuteri* 263 associated with energy metabolism remodeling of white adipose tissue in high-energy-diet-fed rats. J Nutr Biochem 54:87–94

Cheng CP, Tsai SW, Chiu CP, Pan TM, Tsai TY (2013) The effect of probiotic-fermented soy milk on enhancing the NO-mediated vascular relaxation factors. J Sci Food Agric 93(5):1219–1225

Chiu CH, Lu TY, Tseng YY, Pan TM (2006) The effects of Lactobacillus-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. Appl Microbiol Biotechnol 71:238–245

Chung HJ, Jae GY, Lee IA, Liu MJ, Shen YF, Sharma SP, Jamal MA, Yoo JH, Kim HJ, Hong ST (2016) Intestinal removal of free fatty acids from hosts by Lactobacilli for the treatment of obesity. FEBS Open Bio 6(1):64–76

Cleusix V, Lacroix C, Vollenweider S, Le Blay G (2008) Glycerol induces reuterin production and decreases Escherichia coli population in an in vitro model of colonic fermentation with immobilized human feces. FEMS Microbiol Ecol 63(1):56–64

Corr SC, Gahan CGM, Hill C (2007) Impact of selected lactobacillus and bifidobacterium species on Listeria monocytogenes infection and the mucosal immune response. FEMS Immunol Med Microbiol 50:380–388

Cuello-Garcia CA, Brożek JL, Fiocchi A, Pawankar R, Yepes-Nuñez JJ, Terracciano L, Gandhi S, Agarwal A, Zhang Y, Schünemann HJ (2015) Probiotics for the prevention of allergy: a systematic review and meta-analysis of randomized controlled trials. J Allergy Clin Immunol 136(4):952–961

Daubioul C, Rousseau N, Demeure R et al (2002) Dietary fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker fa/fa rats. J Nutr 132:967–973

Day CP, James OF (1998) Steatohepatitis: a tale of two "hits"? Gastroenterology 114:842–845

de Carvalho Marchesin J, Celiberto LS, Orlando AB, de Medeiros AI, Pinto RA, Zuanon JA, Spolidorio LC, dos Santos A, Taranto MP, Cavallini DC (2018) A soy-based probiotic drink modulates the microbiota and reduces body weight gain in diet-induced obese mice. J Funct Foods 48:302–313

De Vuyst L, Leroy F (2011) Cross-feeding between bifidobacteria and butyrate-producing colon bacteria explains bifdobacterial competitiveness, butyrate production, and gas production. Int J Food Microbiol 149:73–80

Del Chierico F, Nobili V, Vernocchi P, Russo A, Stefanis C, Gnani D, Furlanello C, Zandona A, Paci P, Capuani G et al (2017) Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. Hepatology 65:451–464

Derrien M, Vaughan EE, Plugge CM, de Vos WM (2004) *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evolut Microbiol 54 (5):1469–1476

Dethlefsen L, Eckburg PB, Bik EM, Relman DA (2006) Assembly of the human intestinal microbiota. Trends Ecol Evol 21:517–523 dos Santos LM et al (2011) Mono association with probiotic Lactobacillus delbrueckii UFV-H2b20 stimulates the immune system and protects germfree mice against Listeria monocytogenes infection. Med Microbiol Immunol 200:29–38

Dowarah R, Verma AK, Agarwal N (2017) The use of *Lactobacillus* as an alternative of antibiotic growth promoters in pigs: a review. Anim Nutr 3:1–6. https://doi.org/10.1016/j.aninu.2016.11.002

Drago L, Toscano M (2015) Probiotics: immunomodulatory properties in allergy and eczema. In: Probiotics, prebiotics, and synbiotics: bioactive foods in health promotion. Elsevier, London, pp 831–837

Dubourg G, Lagier JC, Armougom F, Robert C, Audoly G, Papazian L, Raoult D (2013) High-level colonisation of the human gut by verrucomicrobia following broad-spectrum antibiotic treatment. Int J Antimicrob Agents 41(2):149–155. https://doi.org/10.1016/j.ijantimicag.2012.10.012

Ejtahed HS, Angoorani P, Soroush AR, Atlasi R, Hasani-Ranjbar S, Mortazavian AM, Larijani B (2019) Probiotics supplementation for the obesity management. A systematic review of animal studies and clinical trials. J Funct Foods 52:228–242

El-Agroudy NN, Kurzbach A, Rodionov RN, O'Sullivan J, Roden M, Birkenfeld AL, Pesta DH (2019) Are lifestyle therapies effective for NAFLD treatment? Trends Endocrinol Metab 30:701–709. https://doi.org/10.1016/j.tem.2019.07.013

El-Deib SM, Abd Rabo FHR, Badran SM, Abd El-Fattah AM, Elshaghabee FMR (2010) In vitro model for assessment of the health benefits of some microbial strains. Int J Probiot Prebiot 5:157

Elli M, Zink R, Rytz A, Reniero R, Morelli L (2000) Iron requirement of Lactobacillus spp. incompletely chemically defined growth media. J Appl Microbiol 88:695–703

Elshaghabee FMF (2017) Probiotics food supplement for NAFLD. J Nutr Health Food Eng 6 (4):00209. https://doi.org/10.15406/jnhfe.2017.06.00209

Elshaghabee FM, Bockelmann W, Meske D et al (2016) Ethanol production by selected intestinal microorganisms and lactic acid bacteria growing under different nutritional conditions. Front Microbiol 7:47

Eslamparast T, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hekmatdoost A (2014) Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. Am J Clin Nutr 99:535–542

Esposito E, Iacono A, Bianco G, Autore G, Cuzzocrea S, Vajro P, Canani RB, Calignano A, Raso GM, Meli R (2009) Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. J Nutr 139(5):905–911

Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM et al (2013) Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. PANS 110(22):9066–9071. https://doi.org/10.1073/pnas.1219451110

Everard A, Matamoros S, Geurts L, Delzenne NM, Cani PD (2014) Saccharomyces boulardii administration changes gut microbiota and reduces hepatic steatosis, low-grade inflammation, and fat mass in obese and type 2 diabetic db/db mice. MBio 5:e01011

Falony G, Vlachou A, Verbrugghe K, De Vuyst L (2006) Cross-feeding between *Bifidobacterium longum* BB536 and acetate converting, butyrate-producing colon bacteria during growth on oligofructose. Appl Environ Microbiol 72:7835–7841. https://doi.org/10.1128/AEM.01296-0610.1128/AEM.01296-06

Famouri F, Shariat Z, Hashemipour M, Keikha M, Kelishadi R (2017) Effects of probiotics on nonalcoholic fatty liver disease in obese children and adolescents. J Pediatr Gastroenterol Nutr 64:413–417

FAO/WHO (2001) Joint Expert Consultation on Evaluation of Health and Nutritional properties of probiotics in food including powder milk with live lactic acid bacteria

FAO/WHO (2002) Report on drafting guidelines for the evaluation of probiotics in food. London Ontario, Canada

Felice GD, Barletta B, Butteroni C (2008) Use of probiotic bacteria for prevention and therapy of allergic diseases: studies in mouse model of allergic sensitization. J Clin Gastroenterol 42:30–32

Felice VD, Quigley EM, Sullivan AM, O'Keeffe GW, O'Mahony SM (2016) Microbiota-gut-brain signalling in Parkinson's disease: implications for non-motor symptoms. Parkinsonism Relat Disord 2016:12. https://doi.org/10.1016/j.parkreldis.2016.03.012

Filho-Lima JVM, Vieira EC, Nicoli JR (2000) Antagonistic effect of *Lactobacillus acidophilus*, *Saccharomyces boulardii* and *Escherichia coli* combinations against experimental infections with *Shigella flexneri* and *Salmonella enteritidis* subsp. Typhimurium in gnotobiotic mice. J Appl Microbiol 88(3):365–370

Fitzgerald RJ, Murray BA (2006) Bioactive peptides and lactic fermentations. Int J Dairy Technol 59:118–125

Forkus B, Ritter S, Vlysidis M, Geldart K, Kaznessis YN (2017) Antimicrobial probiotics reduce salmonella enterica in Turkey gastrointestinal tracts. Sci Rep 7:40695

Forsberg A, Abrahamsson TR, Björkstén B, Jenmalm MC (2013) Pre-and post-natal Lactobacillus reuteri supplementation decreases allergen responsiveness in infancy. Clin Exp Allergy 43 (4):434–442

Foster JA, Linda R, John F, Cryan A (2017) Stress and the gut-brain axis: regulation by the microbiome. Neurobiol Stress 7:124–136

Francavilla R, Piccolo M, Francavilla A, Polimeno L, Semeraro F, Cristofori F, Castellaneta S, Barone M, Indrio F, Gobbetti M, De Angelis M (2019) Clinical and microbiological effect of a multispecies probiotic supplementation in celiac patients with persistent IBS-type symptoms: a randomized, double-blind, placebo-controlled, multicenter trial. J Clin Gastroenterol 53(3):e117

Freedman BI, Cohen AH (2016) Hypertension-attributed nephropathy: what's in a name? Nat Rev Nephrol 12(1):27–36

Friques AGF, Arpini CM, Kalil IC et al (2015) Chronic administration of the probiotic kefir improves the endothelial function in spontaneously hypertensive rat. J Transl Med 13(1):390

Fukushima M, Nakano M (1996) Effects of a mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat- and cholesterol-enriched diet. Braz J Nutr 76(6):857–867

Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'Neil DA, Macfarlane GT (2005) Synbiotic therapy (*Bifidobacterium longum*/synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut 54:242–249

Galdeano CM, Cazorla SI, Dumit JML, Vélez E, Perdigón G (2019) Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab 74(2):115–124

Garcia-Mazcorro J, Rivera-Gutierrez X, Cobos-Quevedo O, Grube-Pagola P, Meixueiro-Daza A, Hernandez-Flores K, Cabrera-Jorge F, Vivanco-Cid H, Dowd S, Remes-Troche J (2018) First insights into the gut microbiota of Mexican patients with celiac disease and non-celiac gluten sensitivity. Nutrients 10(11):1641

Gerard P, Lepercq P, Leclerc M, Gavini F, Raibaud P, Juste C (2007) *Bacteroides* sp. strain D8, the first cholesterol-reducing bacterium isolated from human feces. Appl Environ Microbiol 73 (18):5742–5749

Gilliland SE, Nelson CR, Maxwell C (1985) Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl Environ Microbiol 49(2):377–381

Gómez-Guzmán M, Toral M, Romero M et al (2015) Antihypertensive effects of probiotics Lactobacillus strains in spontaneously hypertensive rats. Mol Nutr Food Res 59(11):2326–2336

Grander C, Adolph TE, Wieser V, Lowe P, Wrzosek L, Gyongyosi B, Ward DW, Grabherr F, Gerner RR, Pfister A et al (2018) Recovery of ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. Gut 67(5):891–901. https://doi.org/10.1136/gutjnl-2016-313432

Große C, Scherer J, Koch D, Otto M, Taudte N, Grass G (2006) A new ferrous iron-uptake transporter, EfeuU (YcdN), from *Escherichia coli*. Mol Microbiol 62:120–131

Håkansson Å, Andrén AC, Brundin C, Oscarsson E, Molin G, Agardh D (2019) Effects of *Lactobacillus plantarum* and *Lactobacillus paracasei* on the peripheral immune response in children with celiac disease autoimmunity: a randomized, double-blind, placebo-controlled clinical trial. Nutrients 11(8):1925

Hall DM, Buettner GR, Oberley LW, Xu L, Matthes RD, Gisolfi CV (2001) Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. J Physiol Heart Circulat Physiol 280:H509–H521

Han J, Wang Y, Song D, Lu Z, Dong Z, Miao H et al (2018) Effects of *Clostridium butyricum* and *Lactobacillus plantarum* on growth performance, immune function and volatile fatty acid level of caecal digesta in broilers. Food Agric Immunol 29:797–807. https://doi.org/10.1080/09540105.2018.1457013

Hansen CHF, Krych L, Nielsen DL, Vogensen FK, Hansen LH, Sørensen SJ, Buschard K, Hansen AK (2012) Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. Diabetologia 55(8):2285–2294

Hassan M, Kjos M, Nes IF, Diep DB, Lotfipour F (2012) Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. J Appl Microbiol 113(4):723–736

He W, Yuan T, Choezom D, Hunkler H, Annamalai K, Lupse B, Maedler K (2018) Ageing potentiates diet-induced glucose intolerance, β-cell failure and tissue inflammation through TLR4. Sci Rep 8:2767. https://doi.org/10.1038/s41598-018-20909

Hendijani F, Pharm D, Akbari V, Pharm D (2017) Probiotic supplementation for management of cardiovascular risk factors in adults with type II diabetes: a systematic review and meta-analysis. Clin Nutr 37(2):532–541

Higashikawa F, Noda M, Awaya T, Danshiitsoodol N, Matoba Y, Kumagai T, Sugiyama M (2016) Antiobesity effect of *Pediococcus pentosaceus* LP28 on overweight subjects: a randomized, double-blind, placebo-controlled clinical trial. Eur J Clin Nutr 70(5):582–587

Honda K, Moto M, Uchida N, He F, Hashizume N (2012) Anti-diabetic effects of lactic acid bacteria in normal and type 2 diabetic mice. J Clin Biochem Nutr 51(2):96–101

Hsieh F-C, Lee CL, Chai CY, Chen WT, Lu YC, Wu CS (2013) Oral administration of *Lactobacillus reuteri* GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. Nutr Metab 10:1–14. https://doi.org/10.1186/1743-7075-10-35

Hussein HA (2015) Effect of probiotic yoghurt on management of antibiotic associated diarrhea among toddlers. J Nurs Sci 1(3):24–34

Jarvis D, Burney P (1998) The epidemiology of allergic disease. Br Med J 316(7131):607–610

Ji Y, Kim H, Park H, Lee J, Yeo S, Yang J, Park S, Yoon H, Cho G, Franz CM, Bomba A (2012) Modulation of the murine microbiome with a concomitant anti-obesity effect by *Lactobacillus rhamnosus* GG and *Lactobacillus sakei* NR28. Benefic Microbes 3(1):13–22

Jones ML, Martoni CJ, Parent M, Prakash (2011) Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active *Lactobacillus reuteri* NCIMB 30242 yoghurt formulation in hypercholesterolaemic adults. Br J Nutr 9:1–9

Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, Okano M, Kagoshima M, Tsuchida T (2010) Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr 64 (6):636–643

Kang JH, Yun SI, Park MH, Park JH, Jeong SY, Park HO (2013) Anti-obesity effect of *Lactobacillus gasseri* BNR17 in high-sucrose diet-induced obese mice. PLoS ONE 8:e54617

Karimi G, Sabran MR, Jamaluddin R, Parvaneh K, Mohtarrudin N, Ahmad Z, Khazaai H, Khodavandi A (2015) The anti-obesity effects of *Lactobacillus casei* strain Shirota versus Orlistat on high fat diet-induced obese rats. Food Nutr Res 59(1):29273

Karimi G, Jamaluddin R, Mohtarrudin N, Ahmad Z, Khazaai H, Parvaneh M (2017) Single-species versus dual-species probiotic supplementation as an emerging therapeutic strategy for obesity. Nutr Metab Cardiovasc Dis 27(10):910–918

Khalesi S, Sun J, Buys N, Jayasinghe R (2014) Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. Hypertension 64(4):897–903

Kiczorowska B, Samolinska W, Al-Yasiry ARM, Kiczorowski P, Winiarska-Mieczan A (2017) The natural feed additives as immunostimulants in monogastric animal nutrition – a review. Ann Anim Sci 17:605–625. https://doi.org/10.1515/aoas-2016-0076
Kim SW, Park KY, Kim B, Kim E, Hyun CK (2013) *Lactobacillus rhamnosus* gg improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production. Biochem Biophys Res Commun 431:258–263
Kirpich IA, McClain CJ (2012) Probiotics in the treatment of the liver diseases. J Am Coll Nutr 31 (1):14–23
Klemenak M, Dolinšek J, Langerholc T, Di Gioia D, Mičetić-Turk D (2015) Administration of *Bifidobacterium breve* decreases the production of TNF-α in children with Celiac disease. Dig Dis Sci 60(11):3386–3392
Klippel BF, Duemke LB, Leal MA et al (2016) Effects of kefir on the cardiac autonomic tones and baroreflex sensitivity in spontaneously hypertensive rats. Front Physiol 7:211
Kobyliak N, Abenavoli L, Mykhalchyshyn G, Kononenko L, Boccuto L, Kyriienko D, Dynnyk O (2018) A multi-strain probiotic reduces the fatty liver index, cytokines and aminotransferase levels in NAFLD patients: evidence from a randomized clinical trial. J Gastrointestin Liver Dis 27:41–49
Kong C, Gao R, Yan X, Huang L, Qin H (2019) Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. Nutrition 60:175–184
Konukoglu D, Uzun H (2016) Endothelial dysfunction and hypertension in hypertension: from basic research to clinical practice. In: Islam MS (ed) Advances in experimental medicine and biology, vol 956. Springer, Cham, pp 511–540
Korkmaz OA, Sumlu E, Koca BH, Pektas MB, Kocabas A, Sadi G, Akar F (2019) Effects of Lactobacillus plantarum and Lactobacillus helveticus on renal insulin signaling, inflammatory markers, and glucose transporters in high-fructose-fed rats. J Med 55(5):E207
Krishnan M, Penrose HM, Shah NN, Marchelletta RR, McCole DF (2016) VSL# 3 probiotic stimulates T-cell protein tyrosine phosphatase–mediated recovery of IFN-γ–induced intestinal epithelial barrier defects. Inflamm Bowel Dis 22(12):2811–2823
Kupfer SS, Jabri B (2012) Pathophysiology of celiac disease. Gastrointest Endosc Clin 22 (4):639–660
Lebeer S, Vanderleyden J, De Keersmaecker SCJ (2010) Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. Nat Rev Microbiol 8(3):171
Lee YK, Puong KY (2007) Competition for adhesion between probiotics and human gastrointestinal pathogens in the presence of carbohydrate. Br J Nutr 88:S101–S108
Lee HY, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y, Park JH (2006) Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. Biochim Biophys Acta 1761:736–744
Lee K, Paek K, Lee HY, Park JH, Lee Y (2007) Antiobesity effect of trans-10, cis-12-conjugated linoleic acid-producing *Lactobacillus plantarum* PL62 on diet-induced obese mice. J Appl Microbiol 103(4):1140–1146
Lema M, Williams L, Rao DR (2001) Reduction of faecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. Small Rumin Res 39:31–39
Levitt MD (1969) Production and excretion of hydrogen gas in man. N Engl J Med 281:122–127
Li Q, Zhou JM (2016) The microbiota-gut-brain axis and its potential therapeutic role in autism spectrum disorder. Neuroscience 324:131–139
Lin MY, Chang FJ (2000) Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. Dig Dis Sci 45:1617–1622
Lin TY, Chen CJ, Chen LK, Wen SH, Jan RH (2013) Effect of probiotics on allergic rhinitis in Df, D p or dust sensitive children: a randomized double blind controlled trial. Indian Pediatr 50 (2):209–213
Lionetti E, Catassi C (2011) New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. Int Rev Immunol 30(4):219–231

Liu Q, Liu Y, Li F, Gu Z, Liu M, Shao T, Zhang L, Zhou G, Pan C et al (2020) Probiotic culture supernatant improves metabolic function through FGF21-adiponectin pathway in mice. J Nutr Biochem 75:1–12. https://doi.org/10.1016/j.jnutbio.2019.108256

Loguercio C1, Federico A, Tuccillo C, Teraacciano F, D'Auria MV, De Simone C, Del Vecchio Blanco C (2005) Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. J Clin Gastroenterol 39(6):540–543

Ma LE, Choi J, Pothoulakis C, Rhee SH (2010) The anti-cancer effect of probiotic Bacillus poly fermenticus on human colon cancer cells is mediated through ErbB2 and ErbB3 inhibition. Int J Cancer 127(4):780–790

Ma J, Zhang J, Li Q, Shi Z, Wu H, Zhang H, Tang L, Yi R, Su H, Sun X (2019) Oral administration of a mixture of probiotics protects against food allergy via induction of CD103+ dendritic cells and modulates the intestinal microbiota. J Funct Foods 55:65–75

Madsen KL (2012) Enhancement of epithelial barrier function by probiotics. J Epithel Biol Pharmacol 5:55–59. https://doi.org/10.2174/1875044301205010055

Makras L, Triantafyllou V, Fayol-messaudi D, Adriany T, Zoumpopoulou G, Tsakalidou E, Servin A, De Vuyst L (2006) Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards Salmonella entericaserovar Typhimurium reveals a role for lactic acid and other inhibitory compounds. Res Microbiol 157(3):241–247

Malik M, Suboc TM, Tyagi S et al (2018) *Lactobacillus plantarum* 299v supplementation improves vascular endothelial function and reduces inflammatory biomarkers in men with stable coronary artery disease. Circ Res 123(9):1091–1102

Mazloom K, Siddiqi I, Covasa M (2019) Probiotics: how effective are they in the fight against obesity? Nutrients 11(2):258

Medina J, Fernández-Salazar LI, García-Buey L, Moreno-Otero R (2004) Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. Diabetes Care 27:2057–2066

Miele L, Valenza V, La Torre G et al (2009) Increased intestinal permeability and tight junction alterations in non-alcoholic fatty liver disease. Hepatology 49:1877–1887. https://doi.org/10.1002/hep.22848

Mofidi F, Poustchi H, Yari Z, Nourinayyer B, Merat S, Sharafkhah M, Malekzadeh R, Hekmatdoost A (2017) Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial. Brazilian J Nutr 117:662–668

Mohamadshahi M, Veissi M, Haidari F, Shahbazian H, Kaydani GA, Mohammadi F (2014) Effects of probiotic yogurt consumption on inflammatory biomarkers in patients with type 2 diabetes. J Biol Impacts 4(2):83–88

Moya-Perez A, Romo-Vaquero M, Tomas-Barberan F, Sanz Y, Garcia-Conesa MT (2014) Hepatic molecular responses to Bifidobacterium pseudocatenulatum CECT 7765 in a mouse model of diet-induced obesity. Nutr Metab Cardiovasc Dis 24:57–64

Muccioli GG, Naslain D, Bäckhed F, Reigstad CS, Lambert DM, Delzenne NM, Cani PD (2010) The endocannabinoid system links gut microbiota to adipogenesis. Mol Syst Biol 6:392–405

Naqid IA, Owen JP, Maddison BC, Gardner DS, Foster N, Tchórzewska MA et al (2015) Prebiotic and probiotic agents enhance antibody-based immune responses to *Salmonella Typhimurium* infection in pigs. Anim Feed Sci Technol 201:57–65. https://doi.org/10.1016/j.anifeedsci.2014.12.005

Naruszewicz M, Johansson ML, Zapolska-Downar D, Bukowska H (2002) Effect of *Lactobacillus plantarum* 299v on cardiovascular disease risk factors in smokers. Am J Clin Nutr 76 (6):1249–1255

Neuschwander-Tetri BA, Caldwell SH (2003) Nonalcoholic steatohepatitis summary of an AASLD single topic conference. Hepatology 37:1202–1219. https://doi.org/10.1053/jhep.2003.50193

Nikolova V, Zaidi SY, Young AH, Cleare AJ, Stone JM (2019) Gut feeling: randomized controlled trials of probiotics for the treatment of clinical depression: systematic review and meta-analysis. Therap Adv Psychopharmacol 9:1–7

Nistal E, Caminero A, Herran AR, Pérez-Andres J, Vivas S, Ruiz de Morales JM, Saenz de Miera LE, Casqueiro J (2016) Study of duodenal bacterial communities by 16S rRNA gene analysis in adults with active celiac disease vs non-celiac disease controls. J Appl Microbiol 120 (6):1691–1700

Noh DO, Kim SH, Gilliland SE (1997) Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. J Dairy Sci 80(12):3107–3113

Núñez IN, Galdeano CM, de LeBlanc AD, Perdigón G (2014) Evaluation of immune response, microbiota, and blood markers after probiotic bacteria administration in obese mice induced by a high-fat diet. Nutrition 30(11-12):1423–1432

O'Shea EF, Cotter PD, Stanton C, Ross RP, Hill C (2012) Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: bacteriocins and conjugated linoleic acid. Int J Food Microbiol 152:189–205

Ohya T, Marubashi T, Ito H (2000) Significance of fecal volatile fatty acids in shedding of Escherichia coli O157 from calves: experimental infection and preliminary use of a probiotic product. J Vet Med Sci 62:1151–1155

Olivares M, Castillejo G, Varea V, Sanz Y (2014) Double-blind, randomised, placebo-controlled intervention trial to evaluate the effects of *Bifidobacterium longum* CECT 7347 in children with newly diagnosed coeliac disease. Br J Nutr 112(1):30–40

Olivares M, Neef A, Castillejo G, De Palma G, Varea V, Capilla A, Palau F, Nova E, Marcos A, Polanco I, Ribes-Koninckx C (2015) The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. Gut 64(3):406–417

Olivares M, Walker AW, Capilla A, Benítez-Páez A, Palau F, Parkhill J, Castillejo G, Sanz Y (2018) Gut microbiota trajectory in early life may predict development of celiac disease. Microbiome *6*(1):36

Ostadrahimi A, Taghizadeh A, Mobasseri M, Rin NF, Payahoo LL, Gheshlaghi JB, Djabbari MV (2015) Effect of probiotic fermented milk (Kefir) on glycemic control and lipid profile in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. Iran J Public Health 44(2):228–237

Otte JM, Podolsky DK (2004) Functional modulation of enterocytes by Gram-positive and Gram-negative microorganisms. Am J Physiol Gastrointest Liver Physiol 286:G613–GG26

Panwar H, Calderwood D, Grant IR, Grover S, Green BD (2014) Lactobacillus strains isolated from infant faeces possess potent inhibitory activity against intestinal alpha- and beta-glucosidases suggesting anti-diabetic potential. Eur J Nutr 53(7):1465–1474. https://doi.org/10.1007/s00394-013-0649-9

Panwar H, Calderwood D, Grant IR, Grover S, Green BD (2015) Lactobacilli possess inhibitory activity against dipeptidyl peptidase-4 (DPP-4). Ann Microbiol 66:1. https://doi.org/10.1007/s13213-015-1129-7

Panwar H, Calderwood D, Gillespie AL, Wylie AR, Graham SF, Grant IR, Grover S, Green BD (2016) Identification of lactic acid bacteria strains modulating incretin hormone secretion and gene expression in enteroendocrine cells. J Funct Foods 2:23. https://doi.org/10.1016/j.jff.2016.02.040

Park C, Brietzke E, Rosenblat JD, Musial N, Zuckerman H, Ragguett RM, Pan Z, Rong C, Fus D, McIntyre RS (2018) Probiotics for the treatment of depressive symptoms: an anti-inflammatory mechanism? Brain Behav Immun 73:115–124

Parracho HMRT, Gibson GR, Knott F, Bosscher D, Kleerebezem M, McCartney AL (2010) A double-blind, placebo-controlled, crossover-designed probiotic feeding study in children diagnosed with autistic spectrum disorders. Int J Probiot Prebiot 5(2):69–74

Petra AI, Panagiotidou S, Hatziagelaki E, Stewart JM, Conti P, Theoharides TC (2015) Gut-microbiota-brain-axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. Clin Ther 37(5):984–995

Pimenta FS, Luaces-Regueira M, Ton AM et al (2018) Mechanisms of action of kefir in chronic cardiovascular and metabolic diseases. Cell Physiol Biochem 48(5):1901–1914

Poutahidis T, Kleinewietfeld M, Smillie C, Levkovich T, Perrotta A, Bhela S, Varian BJ, Ibrahim YM, Lakritz JR, Kearney SM, Chatzigiagkos A (2013) Microbial reprogramming inhibits Western diet-associated obesity. PLoS One 8(7):e68596

Puertollano E, Puertollano MA, Cruz-Chamorro L, de Cienfuegos GA, Ruiz-Bravo A, de Pablo MA (2008) Orally administered *Lactobacillus plantarum* reduces pro-inflammatory interleukin secretion in sera from *Listeria monocytogenes* infected mice. Br J Nutr 99:819–825

Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P (2009) Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and Faecalibacterium prausnitzii. Braz J Nutr 101:541–550

Rashid SK, Khodja NI, Auger C et al (2014) Probiotics (VSL#3) prevent endothelial dysfunction in rats with portal hypertension: role of the angiotensin system. PLoS One 9(5):e97458

Reichold A, Brenner SA, Spruss A, Förster-Fromme K, Bergheim I, Bischoff SC (2014) *Bifidobacterium adolescentis* protects from the development of nonalcoholic steatohepatitis in a mouse model. J Nutr Biochem 25(2):118–125

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

Resta-Lenert S, Barrett KE (2003) Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). Gut 52:988–997

Rimoldi SF, Scherrer U, Messerli FH (2014) Secondary arterial hypertension: when, who, and how to screen? Eur Heart J 35(19):1245–1254

Rintala A, Riikonen I, Toivonen A, Pietilä S, Munukka E, Pursiheimo JP, Elo LL, Arikoski P, Luopajärvi K, Schwab U, Uusitupa M (2018) Early fecal microbiota composition in children who later develop celiac disease and associated autoimmunity. Scand J Gastroenterol 53 (4):403–409

Roselli M, Finamore A, Brasili E, Rami R, Nobili F, Orsi C, Zambrini AV, Mengheri E (2018) Beneficial effects of a selected probiotic mixture administered to high fat-fed mice before and after the development of obesity. J Funct Foods 45:321–329

Rudbane SMA, Rahmdel S, Abdollahzadeh SM, Zare M, Bazrafshan A, Mazloomi SM (2018) The efficacy of probiotic supplementation in rheumatoid arthritis: a meta-analysis of randomized, controlled trials. Inflammopharmacology 26(1):67–76

Salminen S, Bouley C, Boutron-Ruault MC et al (1998) Functional food science and gastrointestinal physiology and function. Braz J Nutr 80:S147–S171. https://doi.org/10.1079/BNJ19980108

Sanders ME, Guarner F, Guerrant R, Holt PR, Quigley EM, Sartor RB, Sherman PM, Mayer EA (2013) An update on the use and investigation of probiotics in health and disease. Gut 62 (5):787–796

Sato M, Uzu K, Yoshida T, Hamad EM, Kawakami H, Matsuyama H, Abd El-Gawad IA, Imaizumi K (2008) Effects of milk fermented by *Lactobacillus gasseri* SBT2055 on adipocyte size in rats. Braz J Nutr 99:1013–1017

Schiffrin E, Blum S (2002) Interactions between the microbiota and the intestinal mucosa. Eur J Clin Nutr 56(S3):S60

Schlee M, Wehkamp J, Altenhoefer A, Oelschlaeger TA, Stange EF, Fellermann K (2007) Induction of human β-defensin 2 by the probiotic *Escherichia coli* Nissle 1917 is mediated through flagellin. Infect Immun 75:2399–2407

Schmidt RM, Pilmann LR, Bruun S, Larnkjaer A, Mølgaard C, Michaelsen KF, Høst A (2019) Probiotics in late infancy reduce the incidence of eczema: a randomized controlled trial. Pediatr Allergy Immunol 30(3):335–340

Servin AL (2004) Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol Rev 28:405–440

Sharma C, Singh BP, Thakur N, Gulati S, Gupta S, Mishra SK, Panwar H (2017) Antibacterial effects of *Lactobacillus* isolates of curd and human milk origin against food-borne and human pathogens. Biotech 7:31. https://doi.org/10.1007/s13205-016-0591-7

Shi Y, Burn P (2004) Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. Nat Rev Drug Discov 3:695
Silveira ST, Martínez-Maqueda D, Recio I, Hernández-Ledesma B (2013) Dipeptidyl peptidase-IV inhibitory peptides generated by tryptic hydrolysis of a whey protein concentrate rich in β-lactoglobulin. Food Chem 141:1072–1077
Singh N, Sharma C, Gulhane RD, Rokana N, Singh BP, Puniya AK, Attri S, Goel G, Panwar H (2016) Inhibitory effects of lactobacilli of goat's milk origin against growth and biofilm formation by pathogens: an in vitro study. Food Biosci 2:129–138. https://doi.org/10.1016/j.fbio.2018.02.001
Spacova I, Ceuppens JL, Seys SF, Petrova MI, Lebeer S (2018) Probiotics against airway allergy: host factors to consider. Dis Model Mech 11(7):034314
Spacova I, Petrova MI, Fremau A, Pollaris L, Vanoirbeek J, Ceuppens JL, Seys S, Lebeer S (2019) Intranasal administration of probiotic *Lactobacillus rhamnosus* GG prevents birch pollen-induced allergic asthma in a murine model. Allergy 74(1):100–110
Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, Jousson O, Leoncini S, Renzi D, Calabro A, De Filippo C (2017) New evidences on the altered gut microbiota in autism spectrum disorders. Microbiome 5(24):1–11
Sudhakaran AV, Panwar H, Chauhan R, Duary RK, Rathore RK, Batish VK, Grover S (2013) Modulation of anti-inflammatory response in lipopolysaccharide stimulated human THP-1 cell line and mouse model at gene expression level with indigenous putative probiotic lactobacilli. Genes Nutr 8(6):637–648. https://doi.org/10.1007/s12263-013-0347-5
Szulińska M, Łoniewski I, Skrypnik K et al (2018a) Multispecies probiotic supplementation favorably affects vascular function and reduces arterial stiffness in obese postmenopausal women-a 12-week placebo-controlled and randomized clinical study. Nutrients 10(11):1672
Szulińska M, Łoniewski I, Skrypnik K et al (2018b) Multispecies probiotic supplementation favorably affects vascular function and reduces arterial stiffness in obese postmenopausal women-a 12-week placebo-controlled and randomized clinical study. Nutrients 10(11):1672
Tamtaji OR, Taghizadeh M, Kakhaki RD, Kouchaki E, Bahmani F, Borzabadi S, Oryan S, Mafi A, Asemi Z (2018) Clinical and metabolic response to probiotic administration in people with Parkinson's disease: a randomized, double-blind, placebo-controlled trial. Clin Nutr 38:1031–1035. https://doi.org/10.1016/j.clnu.2018.05.018
Tanida M, Shen J, Maeda K, Horii Y, Yamano T, Fukushima Y, Nagai K (2008) High-fat diet-induced obesity is attenuated by probiotic strain *Lactobacillus paracasei* ST11 (NCC2461) in rats. Obes Res Clin Pract 2(3):159–169
Thakur N, Rokana N, Panwar H (2016) Probiotics: selection criteria, safety and role in health and disease. J Innov Biol 3(1):259–270
Thiennimitr P, Yasom S, Tunapong W, Chunchai T, Wanchai K, Pongchaidecha A, Lungkaphin A, Sirilun S, Chaiyasut C, Chattipakorn N, Chattipakorn SC (2018) *Lactobacillus paracasei* HII01, xylooligosaccharides, and synbiotics reduce gut disturbance in obese rats. Nutrition 54:40–47
Thomas LV, Ockhuizen T, Suzuki K (2014) Exploring the influence of the gut microbiota and probiotics on health: a symposium report. Br J Nutr 112(Suppl 1):S1–S18
Thushara RM, Gangadaran S, Solati Z, Moghadasian MH (2016) Cardiovascular benefits of probiotics: a review of experimental and clinical studies. Food Funct 7:632
Tomaro-Duchesneau C, Saha S, Malhotra M, Jones M, Rodes L, Prakash S (2015) *Lactobacillus fermentum* NCIMB 5221 and NCIMB 2797 as cholesterol-lowering probiotic biotherapeutics: in vitro analysis. Benefic Microbes 6(6):861–869
Toral M, Gómez-Guzmán M, Jiménez R et al (2014) The probiotic *Lactobacillus coryniformis* CECT5711 reduces the vascular pro-oxidant and pro-inflammatory status in obese mice. Clin Sci 127(1):33–45
Urıs-Silvas JE, Cani PD, Delmée E, Neyrinck A, López MG, Delzenne NM (2008) Physiological effects of dietary fructans extracted from Agave tequilana Gto. and Dasylirion spp. Brazilian J Nutr 99:254–261

Ushkalova EA (2014) Role of probiotics in the prevention and treatment of diarrhea of various genesis: focus on linex. Ter Arkh 86(3):100–105
Valeriano VD, Bagon BB, Balolong MP, Kang D-K (2016) Carbohydrate-binding specificities of potential probiotic lactobacillus strains in porcine jejunal (IPEC-J2) cells and porcine mucin. J Microbiol 54:510–519
Valitutti F, Cucchiara S, Fasano A (2019) Celiac disease and the microbiome. Nutrients 11 (10):2403
Vasquez EC, Pereira TMC, Veronica AP, Marcelo PB, Campos-Toimil M (2019). Probiotics as beneficial dietary supplements to prevent and treat cardiovascular diseases: uncovering their impact on oxidative stress. Oxidat Med Cell Longevity. https://doi.org/10.1155/2019/3086270
Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. J Formul Manage 40 (4):277–283
Vespasiani-Gentilucci U, Gallo P, Picardi A (2018) The role of intestinal microbiota in the pathogenesis of NAFLD: Starting points for intervention. Arch Med Sci 14:701–706
Villar-Garcia J, Hernandez JJ, Guerri-Fernandez R (2015) Effect of probiotics (Saccharomyces boulardii) on microbial translocation and inflammation in HIV-treated patients: a double-blind, randomized, placebo-controlled trial. J Acquir Immune Defic Syndr 68(3):256–263
Wagnerberger S, Spruss A, Kanuri G, Stahl C, Schröder M, Vetter W, Bischoff SC, Bergheim I (2013) *Lactobacillus casei* Shirota protects from fructose-induced liver steatosis: a mouse model. J Nutr Biochem 24:531–538. https://doi.org/10.1016/j.jnutbio.2012.01.014
Wallace CJK, Milev R (2017) The effects of probiotics on depressive symptoms in humans: a systematic review. Ann General Psychiatry 16:14. https://doi.org/10.1186/s12991-017-0138-2
Wan MLY, Forsythe SJ, El-Nezami H (2018) Probiotics interaction with foodborne pathogens: a potential alternative to antibiotics and future challenges. Crit Rev Food Sci Nutr 59 (20):3320–3333. https://doi.org/10.1080/10408398.2018.1490885
Wang S, Noh SK, Koo SI (2006) Green tea catechins inhibit pancreatic phospholipase A2 and intestinal absorption of lipids in ovariectomized rats. J Nutr Biochem 17:492–498
Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, van Hylckama Vlieg JE, Strissel K, Zhao L, Obin M, Shen J (2015) Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. ISME J 9:1–15
Westfall S, Lomis N, Kahouli I, Dia SY, Singh SP, Prakash S (2017) Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis. Cell Mol Life Sci 74:3769. https://doi.org/10.1007/s00018-017-2550-9
Westfall S, Lomis N, Prakash S (2018) A polyphenol-rich prebiotic in combination with a novel probiotic formulation alleviates markers of obesity and diabetes in Drosophila. J Funct Foods 48:374–386
WHO (2006) Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. World Health Organization, Geneva
Wong VW, Chan RS, Wong GL, Cheung BH, Chu WC, Yeung DK, Chim AM, Lai JW, Li LS, Sea MM, Chan FK, Sung JJ, Woo J, Chan HL (2013) Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial. J Hepatol 59 (3):536–542. https://doi.org/10.1016/j.jhep.2013.04.013
World Health Organization (2003) Prevention of allergy and allergic asthma (No. WHO/NMH/MNC/CRA/03.2). World Health Organization
Xiao JZ, Kondo S, Takahashi N et al (2003) Effects of milk products fermented by *Bifidobacterium longum* on blood lipids in rats and healthy adult male volunteers. J Dairy Sci 86(7):2452–2461
Yadav H, Jain S, Sinha PR (2008) The effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei on gastropathic consequences in diabetic rats. J Med Food 11(1):62–68
Yap WB et al (2016) *Lactobacillus casei* strain C1 attenuates vascular changes in spontaneously hypertensive rats. Korean J Physiol Pharmacol 20(6):621–628
Yazdani B, Shidfar F, Salehi E, Baghbani-arani F, Razmpoosh E, Asemi Z, Cheshmazar E, Zarrati M (2018) Probiotic plus low-calorie diet increase gene expression of toll-like receptor 2 and FOXP3 in overweight and obese participants. J Funct Foods 43:180–185

Yoo SR, Kim YJ, Park DY, Jung UJ, Jeon SM, Ahn YT, Huh CS, McGregor R, Choi MS (2013) Probiotics L. plantarum and L. curvatus in combination alter hepatic lipid metabolism and suppress diet-induced obesity. Obesity 21:2571–2578

Zaylaa M, Al KI, Alard J, Peucelle V, Boutillier D, Desramaut J, Dabboussi F, Pot B, Grangette C (2018) Probiotics in IBD: combining *in vitro* and *in vivo* models for selecting strains with both anti-inflammatory potential as well as a capacity to restore the gut epithelial barrier. J Funct Foods 47:304–315

Zhang Q, Wub Y, Fei X (2016) Effect of probiotics on glucose metabolism in patients with type 2 diabetes mellitus: a meta-analysis of randomized controlled trials. J Med 52(1):28–34

Zhang CN, Zhang JL, Guan WC, Zhang XF, Guan SH, Zeng QH et al (2017) Effects of *Lactobacillus delbrueckii* on immune response, disease resistance against *Aeromonas hydrophila*, antioxidant capability and growth performance of Cyprinus carpio Huanghe var. Fish shellfish. Immunology 68:84–91. https://doi.org/10.1016/j.fsi.2017.07.012

Zhao X, Zhen Z, Wang X, Guo N (2017) Synergy of a combination of nisin and citric acid against Staphylococcus aureus and Listeria monocytogenes. Food Addit Contam Part A 34:2058–2068. https://doi.org/10.1080/19440049.2017.1366076

Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR (2013) Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 57:601–609

Zihler A (2010) *In vitro* assessment of bacteriocinogenic probiotics for prevention and treatment of Salmonella in children using novel *in vitro* continuous colonic fermentation and cellular models. Dissertation ETH Zurich 19059. https://doi.org/10.3929/ethz-a-006354966

Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. J Nat 414:782–787

Zuccotti G, Meneghin F, Aceti A, Barone G, Callegari ML, Di Mauro A, Fantini MP, Gori D, Indrio F, Maggio L, Morelli L (2015) Probiotics for prevention of atopic diseases in infants: systematic review and meta-analysis. Allergy 70(11):356–1371

Chapter 6
Probiotics as Functional Foods

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Abstract It is recognized that lactic acid bacteria (LAB) provide a wide range of benefits primarily in host health and food, which is why they are considered to be probiotics; i.e., living microorganisms that administered in adequate amounts confer benefits on the host health. The most representative probiotic strains of LAB include members of the genus *Pediococcus*, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*. In terms of benefits, it has become evident that the consumption of probiotics has been shown to be useful in treating various clinical conditions ranging from childhood diarrhea, antibiotic-associated diarrhea, relapse of *Clostridium difficile* colitis, *Helicobacter pylori* infections, inflammatory bowel disease leading to cancer and urogenital infections. Other beneficial effects of probiotics include increasing nutrient utilization, lowering serum cholesterol, improving lactose intolerance, and decreasing antibiotic use. Its hypocholesterolemic, antimutagenic, antiosteoporotic, antihypertensive, and immunomodulatory effects have also been recognized. This chapter will describe the generalities of probiotics, the main metabolites by which they exert a beneficial effect, as well as the mechanisms by which they protect against various diseases.

Keywords Bacteriocins · Diabetes · Exopolysaccharides · Intestinal diseases · Lactic acid bacteria · Organic acids

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6.1 Introduction

The intestinal microbiota forms a relatively stable and highly diverse ecosystem that is increasingly being recognized for its impact on human health. Deviation from its normal structure is often related to systemic and localized diseases. Modulation of the intestinal microbiota could be helpful to improve health and could be achieved through different nutritional concepts, ranging from food-specific ingredients to complex diets or by ingestion of special living microorganisms such as probiotics.

Probiotics are defined as "living microorganisms which, when administered in appropriate dosage, provide health benefits to the host." In the last decades the contribution to the modulation of respiratory, gastrointestinal, and immunological functions that probiotics have has begun to be fully appreciated and scientifically evaluated. To this day, most commercially available probiotics are lactic acid bacteria (LAB), especially those of the genus *Lactobacillus*, but also bifidobacteria. A much smaller number of bacteria of the genus *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Enterococcus* are also used as probiotics.

It has been identified that the foods and beverages in which a fermentation process has been carried out contain various nutritional and therapeutic properties; in this sense, fermented products such as milk are benefited by LAB since they give them positive health effects. Such foods, beverages, and powders are highly acceptable to consumers due to their high nutritional values and flavors. Potential health benefits of fermented milk include anti-tumor and antimutagenic activity, reduction of serum cholesterol levels, and prevention of gastrointestinal diseases. Probiotics are commonly used for their gastrointestinal effects, of which the best demonstrated clinical benefits of probiotics are the treatment and prevention of antibiotic-related diarrhea; their use can also be extended to oral, skin, and vaginal health, as well as treatment for liver disorders, allergies, and metabolic diseases. Some examples of the health benefits of probiotics are shown in Table 6.1.

Normally, probiotics first colonize the intestinal tract and then strengthen host defense systems by inducing a generalized immune response from the mucosa, including dendritic cell modulation/interaction with natural killer cells, a balanced auxiliary T-lymphocyte response, polymeric immunoglobulin A (IgA) secretion, and self-limiting inflammatory response. Many studies have shown that LABs, mainly *Lactobacillus*, as well as *Bifidobacterium* and its fermentation products,

Table 6.1 Examples of beneficial effects provided by LAB used as probiotics (Mozzi 2016)

Health beneficial effect	Related LAB
Antidiarrheal	*Lactobacillus acidophilus*
Anti-cancer effects	*Lactobacillus paracasei*
Anti-tumor activity	*Lactobacillus reuteri*
Antimutagenic activity	*Lactobacillus casei*
Prevention of gastrointestinal infections	*Lactobacillus plantarum*
Decrease in serum cholesterol	*Lactobacillus rhamnosus*
Modulation of the immune response	*Lactobacillus delbrueckii* subspecies *lactis*

could improve innate and acquired immunity, alleviate allergies, prevent lesions in the gastric mucosa, and create a defense against intestinal infections. In addition, probiotic LABs are distinguished by being functional foods because they contain specific characteristics in order to potentially prevent or treat a variety of intestinal diseases including constipation, colon cancer, and inflammatory bowel disease (Mozzi 2016).

6.2 LABs as Probiotics

It is recognized that LABs provide a wide range of benefits primarily in host health and food, which is why they are considered probiotics. The most representative probiotic strains of LAB include members of the genus *Pediococcus*, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* (Buntin et al. 2008).

6.3 Generalities of LAB

Lactic acid bacteria (LAB) have been used since ancient times for the fermentation of various foods and is one of the oldest methods used to preserve food. The use of LAB dates back to 6000 B.C., with fermentation specifications in dairy products and 300 B.C. plants and 1500 B.C. meat. The conventional or typical LABs are positive Gram, immobile, negative catalase, non-spore formers, devoid of cytochromes, bacilli, or aerotolerant anaerobic coconuts, which are acid-resistant and generate lactic acid as the main final product of carbohydrate fermentation.

LABs can be located in any product, such as fermented foods and plants, and ecosystem and on the mucosal surfaces of the human body. Plants are preferred as a source for the isolation of LABs because of their metabolic activities and because of their specific flavor-forming properties. Each specific plant species provides a unique environment according to the competitiveness of microorganisms, natural plant antagonists, as well as the type, accessibility, and concentration of the substrate in various physical factors. These conditions allow the development and growth of a typical epiphytic microflora through which a population emerges and a series of fermentative processes begin after the plant material is prepared for fermentation. Strains derived from lactobacteria have demonstrated tolerance to high salt and pH concentrations, a high level of stress resistance compared to those from milk, and the ability to ferment various types of carbohydrates. Likewise, no really significant differences were observed in fermentation characteristics and enzyme profiles, such as peptidases, phosphatases, and lipases, needed to acquire various fermented dairy products from commercial lactobacteria plants and strains (Venugopalan and Dinesh 2010).

LABs have a general condition recognized as safe. They are very important within the dairy industry and are necessary for the production of several new and traditional dairy products. LABs (*Lactobacillus*, *Lactococcus*, *Leuconostoc, Enterococcus*, *Streptococcus*, and *Pediococcus*) are known to have probiotic activity, which beneficially influences diseases of the gastrointestinal tract (Benyacoub et al. 2005). In addition to probiotic activity, recent research has shown their ability to produce different biologically active metabolites (lactic acid, ɣ-aminobutyric acid, conjugated linoleic acid, bacteriocin and reutericycline, bioactive peptides, and exopolysaccharides). Food products supplemented with strains of LAB which contain probiotic properties (various biological activities) are functional foods with indisputable benefits for human health (Randazzo et al. 2016).

Phylogenetically, only bacteria called lactic acid bacteria (Holzapfel 2014) are within the order of Lactobacillales although some genera are more distant, they share similar physiological characteristics. Bifidobacteria and some Bacilli of the order Bacillales are examples of bacteria considered with similarities but these are not lactic acid bacteria. Bifidobacteria belonging to another phylum have frequently been considered as LAB.

However, the metabolism of LABs is different and unique and they do not share any genetic relationship. Similarly, some Bacilli, such as *Bacillus coagulans*, are used to generate lactic acid as the only fermentation product from pentoses and hexoses (Pleissner et al. 2016). However, in this case the metabolism is also different.

The strong adaptation of LABs to nutrient-rich niches, such as meat, fruit, or milk, leads to a truly significant reduction in their metabolic capacities. They are classified as nuisance microorganisms because they depend on external or exogenous sources for vitamins, precursor nucleic acids, and amino acids. This is an obvious disadvantage for its production in a chemical industry, since the necessary components are very expensive and in general, it is very complicated to carry out the purification of the products from the growing medium. These microorganisms have a quite efficient metabolism with only a few defined products and high flows. Moreover, this efficiency, which is thanks to adaptation, is reflected in their genomes.

6.4 Genome of Lactic Acid Bacteria: Low Redundancy

LAB have small genomes, ranging from 2.0 megabases (Mb) to 3.4 megabases (Holzapfel 2014). However, its genetic diversity is impressive despite the narrow functional definition (Sun et al. 2015). The historical definition of LAB is based on a comparable metabolism of carbohydrates (lactic acid production). In fact, the central genome of 213 Lactobacilli strains and closely associated species comprise 73 genes, none of which is related to carbohydrate metabolism (typically the LAB genome encompasses 1300–3000 genes and most of which have between 2000 and 2500) (Holzapfel 2014). On the other hand, pangenome has more than 44,000 gene families. 48 members of 133 known families of glycosyl hydrolases have been found, which at present have not been recognized for their biotechnological potential (Sun et al. 2015). The detailed analysis of the evolution of the central metabolism of carbon exposes an incredibly complex relationship between a large number of lactic acid bacteria (Salvetti et al. 2013), but it also exposes a great diversity based on the environment in which they are found. The process of reductive evolution was a consequence of the adaptation of LABs to nutrient-rich habitats, which led to the simplification of their metabolism through the accumulation of multiple auxotrophs (Makarova et al. 2006). Immediate adaptation to its environmental niche also requires less redundancy and less high level genetic control systems compared to other organisms, which makes it easier to have control of its metabolism. A correlation has been suggested between the reduced size of the genome and two components of the signal transduction system involved in stress signaling (Zúñiga et al. 2011).

6.5 Carbon Metabolism in LABs

The fermentation pathways of LAB are not favorable for obtaining energy (one or two ATP molecules per sugar molecule), which are mitigated by high conversion rates, therefore, the overall biomass gain is not high. In addition, the biosynthetic pathways and central carbon metabolism required for cell growth are highly decoupled in LABs because they adapt to nutrient-rich niches. These niches make it easier for bacteria to occupy several building cell structures, rather than synthesizing them from carbon sources. As a consequence of the aforementioned, energy is obtained from carbon sources but is inefficient. This is why, huge amounts of sugar are fermented and not much of the sugar is lost for biomass generation. This combination results in optimal yields during the fermentation process, making them interesting for the industrial area, and some applications have already been suggested.

It should be noted that LABAs are classified into three groups: obligate homolactic fermenters (lactic acid as the sole fermentation product); obligate heterolactic fermentation organisms; and obligate heterolactic fermenters (which generate lactic acid, CO_2, and ethanol/acetate) (Gänzle 2015). In general, ATP is earned when sugar is oxidized to pyruvate. To reduce pyruvate, it is necessary to close the balance through the removal of electrons. The direct reduction of pyruvate produces lactic acid, which is a product of industrial importance and there are various processes for its formation, based on the diversity of LAB (Hofvendahl, and Hahn-Hägerdal 2000).

Another important aspect is the ability of many LABs to make use of various pentoses and hexoses, which makes it the perfect starting condition to take advantage of lignocellulosic sugars. Such sugars are the most abundant renewable resources on our planet and have been researched as the oil of the future (Abdel-rahman et al. 2011; Boguta et al. 2014).

A particularly interesting feature of LAB's carbon metabolism is the flexibility of electron acceptors to carry out metabolism. Commonly, electron acceptors incorporate a significant variety to the configuration of metabolism. One of the fundamental electron acceptors is oxygen, but sometimes there is no presence of oxygen and replaces it with pyruvate. If oxygen is present, it is reduced to water without any energy conservation. Glycerol or fructose are compounds that accept other molecules that benefit the chemical industry: many heterofermentative LABs have the ability to grow in the presence of fructose, and that on the one hand fructose itself is used as a carbon source and is reduced to mannitol on the other hand. For the production of biomass and energy gain, growth in the presence of glucose is used, with fructose as an electron acceptor (Tyler et al. 2016). But, when both carbon sources are present, microorganisms use glycerol as an electron acceptor, which significantly increases the efficiency of sugar energy production, and this transforms glycerol to 1,3-propanediol with high efficiency (Pflügl et al. 2014). The first step in the use of glycerol as an electron sink is the redox-neutral action of glycerol dehydrate, generating 3-hydroxypropionaldehyde by dehydration. The second step is the reduction of aldehyde. This is the actual reaction for electron dissipation.

It is important to mention that this and similar reactions (such as the conversion of 1,2-propanediol to propionaldehyde or 2,3-butanediol to 2-butanone (Ghiaci et al. 2014)) are not linked to either energy or redox metabolism. In nature, these ketone or aldehyde intermediates never accumulate in cells that are growing due to their high toxicity (Doleyres et al. 2005).

6.6 Search for New Probiotics

Among the characteristics that a microorganism must present for it to be considered a probiotic is the ability to resist passage through the different parts of the gastrointestinal system since these microorganisms will interact with substances such as bile acids and acid pH, as well as the ability to adhere to the intestinal mucosa as this increases the benefits that these microorganisms provide to the body (Lee et al. 2008).

Probiotic microorganisms and LABs are generally associated with dairy foods; however, some foods are developed every day and marketed as functional foods, which contain probiotic microorganisms, which are generally added or supplemented to the food to obtain the benefits of this type of microorganisms. After dairy products, fruit or vegetable juices are the most technologically developed foods in relation to probiotic microorganisms, mainly this interest is associated with the potential to provide probiotic strains to people intolerant to lactose with reduced cholesterol content, which makes it of interest to many regular consumers. As mentioned above, fruits and vegetables are generally considered potential matrices for containing probiotics because they are rich in nutrients such as vitamins, dietary fiber, sugars, minerals, and polyphenolic compounds (antioxidants). Microorganisms such as *Lactobacillus rhamnosus*, *L. fermentum*, *L. reuteri*, and *L. plantarum* have been found in commercial fruit products containing a pH of 4.2 and stored 80 days at 4 °C; these strains showed resistance to storage, while the *L. acidophilum* strain showed low resistance in the juice matrix. This shows that the acid pH of some fruit juices represents a technological change for the formulation of foods containing LAB. Similarly, high levels of viable probiotic microorganism cells have been found in fruit juices at levels between 10^9 and 10^{10} CFU/mL after 28–50 days of storage at 4 °C in carrot, peach, and orange juices, demonstrating that fruit juices are a good matrix for delivering an adequate concentration of these microorganisms to consumers without the use of dairy additives (Freitas et al. 2015).

During the process of deterioration of fresh meat a wide variety of LAB can also be obtained; the main genera are *Leuconostoc*, *Carnobacterium*, *Lactococcus*, *Weissella*, *Enterococcus*, and *Lactobacillus*. Species derived from this genus (*L. curvatus*, *L. algidus*, *L. fuchuensis*, *L. oligofermentans*) are associated with severe acidification, emission of unpleasant odors, among others (Pothakos et al. 2015). The main producer of organic acids, such as acetic acid, is the genus *Leuconostoc* and is found mainly in all types of meat and meat packaging conditions (Pothakos et al. 2015). They have also been obtained from the study of fermented milk, where it has been found that they provide health benefits due to the metabolic products generated by the biological activities of lactic acid bacteria (Domínguez Gonzáleza et al. 2014).

LABs that perform the yogurt fermentation process can be produced in different environments, especially in plant materials (de Almeida Júnior et al. 2015). LAB strains to generate prebiotic foods, which are inert substances that stimulate the growth of probiotic organisms (Quigley 2012), are isolated from humans, since they have a greater capacity to adhere to the intestinal wall and colonize it; but on the other hand, also strains of animal origin can be used since they have positive effects on the human organism (Ruiz et al. 2017). One of the reasons for the increased consumption of fermented dairy products is that they contain probiotics and are readily available on the market (Savadogo et al. 2006). In yogurt, the main species are *Streptococcus*, such as *S. lactis*, *S. cremoris* (cheese, yogurt, butter) and *S. thermophilus* in yogurt and cheese (Ramírez et al. 2011). Finally, some LAB genera such as *Lactobacillus*, *Bifidobacterium,* and *Pediococcus* are commonly found in the intestinal microbiota of mammals (Porto et al. 2017). They have also been isolated from microbiota from freshwater fish, ocean or saltwater fish, as well as from minimally processed aquatic products (Ghanbari et al. 2013). Benavides et al. (2016) also isolated them from mature and immature subtropical fruits, such as guava, berries, and other inflorescences.

6.7 Resistance of Probiotics to Food Processing: The Case of High Hydrostatic Pressures

High hydrostatic pressures (HHP) technology has been used in food products that contain lactic acid bacteria or probiotics, such as dairy products. However, one of the main functions of high pressure as food treatment is the reduction of microbial load in order to increase the shelf life of products, so there is no selectivity between the destruction of pathogens and probiotic bacteria. In this sense, some techniques have been proposed that help these microorganisms to be used in foods treated with HHP. For example, methods of attenuation of lactic acid bacteria have been proposed for use as adjuvants in cheese making. Such is the case of *Lactobacillus lactis* spp. *lactis* treated at 300 MPa and *Lactobacillus casei* spp. *casei* treated at 350 MPa, which can be added to cheese during processing, which does not allow microorganisms to produce acid during processing, but provide an extra contribution of enzymes that accelerate the maturation of cheeses; likewise, the attenuation of *Lactobacillus lactis* ssp. *cremoris*, treated at 200 MPa for 20 min at 20 °C, can be used in combination with primary strains in the production of cheddar cheese, producing high levels of free amino acids and accelerating secondary proteolysis in cheese (Juan et al. 2016).

The effect of HHP on products containing conventional probiotic microorganisms has been widely studied. However, few studies have focused on the effects on the microflora of final products in this type of dairy foods (i.e., yogurt). In the processing of this type of products with HPP, it was found that 400 MPa was the minimum pressure necessary to inactivate *Lactobacillus delbrueckii* sp. *bulgaricus*; however, at these same conditions, *Streptococcus thermophilus* was resistant to pressure, and during storage of yogurt after pressurization a decrease in the concentration of the microorganism was observed, likewise, no acidification of the product was observed during cold storage (Juan et al. 2016).

Within the probiotic microorganisms, some endospore-producing bacteria are known (these forms of cell maintenance are very resistant to different environmental conditions), which gives it a high survival capacity under different environmental conditions. Thus, some bacteria belonging to the genus *Bacillus* sp. have been classified as probiotic microorganisms with high potential for use in the food industry.

Unlike lactic acid bacteria (LAB) used in dairy products such as those belonging to the genera *Lactobacillus* sp., *Streptococcus* sp., among others, the genera *Bacillus* sp. present a greater resistance to HHP processes. Bacterial spores require higher pressures (>1200 MPa) to be inactivated; for this process of inactivation, or inhibition of the germination of bacterial spores to be carried out, a combination with moderate heat pre-treatments or other treatments such as ultrasound is required. For example, for the inactivation of *Clostridium botulinum* spore-forming bacteria, a thermal process of 90–121 °C is required in conjunction with a pressure of 500–800 MPa (Rastogi and Shukla 2013).

Foods with high acidity content (low pH) are the best candidates for HHP technology; however, their stability may be compromised because the treatment does not destroy bacterial spores, so it will be necessary to establish coupled processes to meet the requirement to eliminate spore-producing pathogens (Rastogi and Shukla 2013). However, this same behavior can be used as a benefit to obtain food containing spore-forming bacteria (SFB) and considered as probiotics. By not destroying SFB with conventional high hydrostatic pressure (600 MPa), it is possible to take advantage of this characteristic of microbial resistance and inoculate food with a certain number of microorganisms, and in this way, it will be possible to destroy the detrimental microorganisms of food and pathogens and maintain a minimum acceptable level of the content of spore-producing probiotics. Thus, food containing probiotics can be obtained without compromising the safety and security of the food.

An advantage of acidic foods treated with HHP is that the acid pH (<4.5) is that, at this level of acidity, bacterial pathogen spores are not able to initiate growth. For this reason, this food group represents the majority of the products on the market.

6.8 Probiotics as a Functional Food

Probiotics exert their effects by mechanisms still unclear; however, there are hypotheses that explain how these microorganisms provide these benefits to the host (O'Toole and Cooney 2008). The competition between probiotics and pathogenic bacteria to colonize the gastrointestinal tract is one of the mechanisms of action that probiotics exert to prevent harmful microorganisms from causing some pathology adhering to the intestinal mucosa. This mechanism was tested with bacteria of the genus *Lactobacillus* (*plantarum* and *rhamnosus*) as they showed the ability to inhibit the adhesion of *E. coli* to the epithelium of the intestine (Mack et al. 1999).

Another possible mechanism is the secretion of antimicrobial substances that keep the intestinal microbiota in balance (Rolfe 2000). One of the substances secreted by these microorganisms are bacteriocins, in addition to other substances such as hydrogen peroxide, diacetyl and short-chain acids burn to be secreted by probiotics, modifying the intestinal microbiota for a proper balance. Probiotics also improve the immune response by stimulating the secretion of a greater amount of immunoglobulins, especially the IgA type (Link-Anster et al. 1994), natural killer cells, and the phagocytic action of macrophages, since through this increase in cells defending the immune system can inhibit the gastrointestinal tract colonization by pathogenic bacteria, thus benefiting the composition of the microbiota (Fuller and Gibson 1997). Probiotics also compete with pathogenic microorganisms for nutrients (Vandenplas and Benninga 2009), as in the case of *Clostridium difficile* which needs carbohydrates for its growth and colonization; this is where probiotics act using the largest amount of monosaccharides leaving this and many pathogens without nutrients (Wilson and Perini 1988) thus avoiding the incidence of diseases caused by the spread and colonization of pathogens.

6.9 Metabolites Produced by Probiotics

Probiotics are commonly used in foods and also act as preservatives and enhancers of texture, flavor, and odor. These properties result from the ability of these bacteria to produce different types of sugars and metabolites such as ethanol, organic acids (lactic, acetic), diacetyl, acetone, exopolysaccharide, specific proteases, and bacteriocins (Porto et al. 2017).

6.9.1 Polysaccharides

Some probiotics have the ability to synthesize glycosidic polymers. Usually, such molecules are secreted and can be kept covalently attached to the cell surface, typically in a capsule form; released into the environment; or remain slightly attached to the surface (Kumar et al. 2007; Viana de Souza and Silva Dias 2017). Probiotics belonging to the genera *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Weissella*, and *Streptococcus* have been identified as producers of exopolysaccharides (EPS) (Amari et al. 2013).

The EPS obtained by these bacteria can be different in: (1) composition, since they have different monosaccharides attached by different types of bonds; (2) molecular weight; (3) structure, since they contain several degrees and types of branching; and (4) overall structural conformation.

The role of EPS in food is determined by the characteristics mentioned above, as well as the concentration of polymeric compounds and their way of interacting with food matrices. Taking into account its composition, the EPS are classified into: (1) homopolysaccharides (HoPS), consisting of monosaccharides of a single type or (2) heteropolysaccharides (HePS), consisting of monosaccharides of two or more types. Based on the different links that it contains and the carbon involved in the link, HoPS are separated into four groups: α-d-glucans, β-D-glucans, β-D-fructans, and others (i.e., polygalactans) (Pérez-Ramos et al. 2015).

The interest in EPS has increased over the years because of the diversity generated by probiotics. The isolated forms of EPS are not yet used industrially because the prices of polysaccharides of bacteria are high compared to polysaccharides isolated from plants, animal sources (pectin, alginate, galactomannan, starch, among others), and algae (Zannini et al. 2016). However, EPS produced by LABs are industrially used as stabilizers, thickeners, emulsifiers, viscous agents, gelling agents, and water-binding agents.

Despite the above, in many food industries, the production of EPS by LABs with probiotic attributes is carried out by in situ fermentation and this is decisive in the organoleptic characteristics that it presents as they influence the consistency and rheology of fermented products (Notararigo et al. 2013), so they are used as viscosifiers, stabilizers, emulsifiers, or gelling agents (Viana de Souza and Silva Dias 2017). EPS also contribute to water retention in certain products such as cheese, which benefits the product in texture and calorie reduction (da Silva Ferrari et al. 2016). In addition, some EPS have anticholesterolemic and anti-tumor activity. They have the capacity to decrease the formation of biofilms by pathogens and the produced β-glucans have immunomodulatory, antitumorigenic, antiviral, and antiulcerous activities (Notararigo et al. 2014; Nwodo et al. 2012). In addition, they can also act as prebiotics by increasing the growth of beneficial microorganisms (de Moreno de LeBlanc et al. 2018).

It is evident that EPS synthesized by LABs have greater potential for the production of cereal and dairy products (Badel et al. 2011). Table 6.2 shows a list of LABs, the EPSs they produce and the foods in which they are found.

Table 6.2 Exopolysaccharides produced by probiotics in foods (Zarour et al. 2017)

Food	Microorganisms	Exopolysaccharide
Cheese	*Lactobacillus casei, Lactobacillus helveticus, L. delbrueckii* subspecies *bulgaricus, L. citreum, L. johnsonii*, *L. reuteri*, *Streptococcus mutans*	Heteropolysaccharides Inulin
Yogurt	*Lactobacillus delbrueckii* subspecies *bulgaricus, L. mucosae, L. johnsonii*, *L. reuteri, Leuconostoc citreum, Streptococcus thermophiles*, *S. mutans*	Heteropolysaccharides Inulin
Kefir	*Lactobacillus kefiranofaciens*, *Lactococcus lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis*, *Leuconostoc mesenteroides*, *S. thermophilus*	Kefiran
Bakery products	*Lactobacillus reuteri, Lactobacillus buchneri, L. citreum, L. mesenteroides*, *S. mutans*, *Weissella cibaria*, *W. confusa*	Reuteran Dextran Heteropolysaccharide

6.9.2 Aromatic Compounds

Some probiotics enhance the organoleptic characteristics of fermented foods by producing various compounds that are involved in the formation of other aromas and flavors. They can influence cheese maturation by transforming milk components into aromatic and flavored compounds through the metabolism of carbohydrates, proteins, citrate, and milk lipids.

The aroma formed in fermented foods is due to the action of lysed and intact LABs. Cytoplasmic enzymes are released during cell lysis, which continue their activity even outside the cell, producing metabolites in the fermented food matrix (Lortal and Chapot-Chartier 2005). Different amino acids are presented in food matrices or can be obtained in proteolysis, their catabolism also results in the synthesis of aromatic compounds. Isoleucine, leucine, and valine (branched-chain amino acids) give sweet and fruity flavors; phenylalanine, tryptophan, and tyrosine (aromatic amino acids) through catabolism generate floral flavors; aspartate is catabolized into butter flavors; and cysteine and methionine (sulfur amino acids) are metabolized into compounds flavored with meat, garlic, and boiled cabbage (Ardö 2006). Through citrate metabolism, LABs also generate aromatic compounds.

6.9.3 Aromatic Compounds with Four Carbons

Few probiotics generate aromatic four-carbon compounds from citrate such as *L. lactis* subspecies *lactis* biovar. *diacetylactis (L. diacetylactis*) (Hugenholtz 1993), some from *Leuconostoc* (Hemme and Foucaud-Scheunemann 2004), *Lactobacillus plantarum* (Minervini et al. 2010), a few of *Enterococcus* (Martino et al. 2016), *Oenococcus oeni* (Bartowsky and Henschke 2004), and *Weissella paramesenteroides* (García-Quintáns et al. 2008).

Citric acid is an organic acid present in fermentable food products, such as vegetables, fruits, fruit juice, cheese, and milk. Under acidic and anaerobic conditions, bacteria have the ability to metabolize citrate through the pyruvate pathway to produce aromatic four-carbon compounds such as acetoin, diacetyl, ethanol, and 2,3-butanediol (Smid and Kleerebezem 2014). The *Leuconostoc* and *L. diacetylactis* species are the main citrate fermenting bacteria found in milk starters (Drider et al. 2004).

The efficiency of the synthetic routes of diacetyl will depend on the activity of the enzymes responsible for transforming citrate to pyruvate, and the concentration gradient is influenced by the rate of citrate disappearance in an intracellular medium (Laëtitia et al. 2014). Most of the pyruvate generated is converted to lactate by the action of the enzyme lactate dehydrogenase (LDH) (Ko et al. 2016).

6.9.4 Antimicrobial Compounds

LABs have been shown to produce antimicrobial compounds as a form of defense against pathogens, thus protecting food from spoilage. LABs generate a variety of compounds, such as organic acids (acetic acid and lactic acid), hydrogen peroxide, diacetyl, bacteriocins, and bactericidal proteins, all of which have bacteriostatic or antibacterial properties. These metabolic products have the function of extending the shelf life and inhibiting the growth of pathogenic microorganisms in the food, additionally also positively impacting the color, taste, smell, and texture of the food.

There is some concern about manufacturing processes involving heat treatment (sterilization, heating, and pasteurization), dehydration, acidification, and the addition of preservatives (nitrite, antibiotics, and sulfur dioxide) and organic compounds such as sorbate, benzoate, acetate, lactate, and propionate. Although the above procedures are generally effective, there is growing public demand for naturally occurring and microbiologically safe products that give consumers positive health effects (Zacharof and Lovitt 2012). Consequently, bacteriocins are becoming increasingly interesting as they can be found in natural sources and contribute to food safety (Yang et al. 2014).

6.9.5 Bacteriocins

Bacteriocins are defined as ribosomally synthesized antimicrobial peptides and are commonly used as preservatives in foods (Chikindas et al. 2018) because they inhibit or annihilate other microorganisms that are closely related to the producer, without harming the harmless microbiota (Cotter et al. 2013). There is a greater amount and structural variability of bacteriocins produced by Gram-positive bacteria compared to those obtained from Gram-negative bacteria. LABs are a group of gram-positive bacteria identified with a high potential to generate bacteriocins. Most

LAB bacteriocins are non-toxic to eukaryotic cells and are activated in the nanomolar range (Messaoudi et al. 2013).

Bacteriocins cause cell death in bacteria at much lower concentrations compared to antimicrobial peptides in prokaryotic cells, and this is probably because bacteriocins interact with specific receptors in target cells. Therefore, interest in its potential use in food products has increased. Bacteriocins are inactivated by the action of digestive proteases, which causes them to have a minimal effect on the intestinal microbiota, a property that supports their use as food preservatives. Some of its characteristics are that they are heat and pH tolerant, have not been related to antibiotic resistance, and are usually encoded by genes that are located in a plasmid, which facilitates genetic manipulation (Gálvez et al. 2007). Bacteriocins may act as bacteriostatic or bactericidal. Their action will depend on various factors such as, for example, the dose of bacteriocin and the degree of purification and experimental conditions such as pH, temperature, or the existence of agents that modify the integrity of the cell wall (Juodeikiene et al. 2002).

The incorporation of bacteriocins for food preservation could have several advantages: (1) to minimize the risk of diseases caused by pathogens present in foods such as food poisoning; (2) to provide extra protection during excessive temperature conditions and to decrease the use of chemical preservatives; (3) to extend the shelf life of food products; (4) to allow lower intensity physical treatments, thereby preserving nutrients, organoleptic properties of foods, and vitamins, as well as lowering processing costs; (5) to reduce waste due to food spoilage; and (6) to provide alternative preservation barriers to "novel" foods in the hope of meeting consumer demands for safe, ready-to-eat, fresh-tasting, minimally processed foods (Messaoudi et al. 2013).

Most LAB produce bacteriocins, such as *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium*, *Aerococcus*, *Oenococcus*, *Tetragenococcus*, *Vagoccus,* and *Weissella* (Ananou et al. 2007; Gálvez et al. 2007; Gálvez et al. 2008; Leistner and Gorris 1995; Oliveira et al. 2008); however, only bacteriocin obtained from *Lactococcus lactis*, named nisin, which has been approved by the FDA (Sobrino-López and Martín-Belloso 2008; Zacharof and Lovitt 2014), is commercially permitted, so research on new bacteriocins is of paramount importance, given the benefits they bring.

6.9.6 Hydrogen Peroxide (H_2O_2)

Some LABs contain flavoprotein oxidases, which gives them the ability to produce and accumulate H_2O_2 in the presence of molecular oxygen. This compound has bactericidal and sporicidal action, due to its strong oxidizing effect, which is generated by the tendency of H_2O_2 to form highly reactive metabolites, such as hydroxyl and superoxide radicals, to which damage to membrane lipids, DNA, and other essential cellular compounds is attributed (Holzapfel et al. 2003).

A food product in which H_2O_2 has great importance is in raw milk, since H_2O_2 exerts its characteristic bactericidal and sporicidal action, which benefits this product by extending its shelf life. These antagonistic actions are easier to perform in raw milk because it has the enzyme lactoperoxidase that has the function of catalyzing the oxidation of thiocyanate inherent in the presence of H_2O_2, obtaining from this reaction a compound capable of producing the desired effect. Therefore, in places without optimal refrigeration equipment, milk or cheese is stored by adding H_2O_2 (Martin et al. 2014). However, the addition of H_2O_2 is not allowed in the USA and several places around the world, with the exception of certain applications in cheese, mainly because this compound can cause gastrointestinal problems, the degradation of vitamins (folic acid) and certain essential amino acids (methionine) (Antelmann and Helmann 2011; Rutala and Weber 2008).

H_2O_2 has the advantage of having a broad spectrum of inhibition since it acts against viruses, bacteria, yeasts, and bacterial spores, having antagonistic effect on Gram-positive and Gram-negative bacteria (Viana de Souza and Silva Dias 2017), which is used in the food industry using this compound during bottling, transport and packaging (Imlay 2003).

6.9.7 Diacetyl

Diacetyl is obtained through citrate metabolism in which LABs such as *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc* spp., *Lactobacillus paracasei,* and *Lactobacillus rhamnosus* are involved. Its obtained form is non-enzymatic, by means of the oxidative reaction of the acetate, which originated from pyruvate (Díaz-Muñiz et al. 2006; Hugenholtz 1993; Tamang et al. 2015).

In its pure form, diacetyl is a yellow liquid with a strong butter aroma (Starek-Swiechowicz and Starek 2014). It is used in products such as wine, dairy products, roasted coffee, and beer (Shibamoto 2014). Because of its characteristic aroma and flavor, this compound is used in a variety of applications, such as microwave popcorn (Brass and Palmer 2017). It is also used as an antagonist agent, by inhibiting pathogenic microorganisms, generating this effect by having the ability to penetrate bacterial membranes and interfere with the functions of essential metabolites. It is known that the antagonistic effect caused by diacetyl is through the blocking of the catalytic site of the enzymes responsible for the utilization of arginine, causing cells to be unable to synthesize this essential protein (Hor and Liong 2014).

Microorganisms that inhibit diacetyl and have been reported are: *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Escherichia coli,* and *Salmonella anatum* (Naidu 2000). According to Ferrari (2016), LABs isolated from goat's milk have a strong potential to produce this antimicrobial compound.

6.9.8 Organic Acids

During the fermentation process, a wide variety of organic acids are produced: lactic acid, acetic acid, succinic acid, propionic acid, formic acid, and butyric acid (Haller et al. 2001; Özcelik et al. 2016; Ross et al. 2002). The use of these acids is highly regarded by the food industry for their ability to improve food quality and safety (Hwanhlem et al. 2011). The antimicrobial effect that characterizes these acids is due to their ability to reduce pH below the optimal growth range of various microorganisms and to metabolic inhibition by undissociated organic acid molecules (Crowley and Mahony 2013). The decrease in pH caused by organic acids also has the objective of giving them greater liposolubility, allowing this to interfere with the conservation of the potential of the cell membrane. As a result, the active transport is inhibited, the cell membrane is crossed, and the cytoplasm is reached, thus causing the reduction of intracellular pH and the inhibition of several metabolic functions in the pathogenic microorganism (Haller et al. 2001; Özcelik et al. 2016; Ross et al. 2002).

6.9.9 Lactic Acid

Lactic acid is mainly used in the pharmaceutical, chemical, cosmetic, and food industries (Li et al. 2015). It can be obtained through fermentation or chemical synthesis. However, 90% of this product is preferably produced worldwide by bacterial fermentation (Vijayakumar et al. 2008). The identified LABs involved in this process are those belonging to the genera *Lactobacillus*, *Sporolactobacillus*, *Enterococcus*, *Lactococcus*, *Bacillus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacteria* (Naidu 2000).

About 70% of the lactic acid produced is used in the food sector (Martinez et al. 2013). It is found naturally or as a fermentation product in: olives and pickled vegetables, yogurt, butter and other fermented milk, fermented dough bread, and other fermented products. It is also used as an acidulant, condiment, pH buffer of the decomposition generated by bacteria of various processed products such as sweets, bread and bakery products, non-alcoholic beverages, soups, sorbets, dairy products, beer, jams and jellies, mayonnaise, processed eggs, among others (Datta and Henry 2006).

The mechanism used by lactic acid against pathogenic microorganisms focuses on its ability to cross its cell membrane, resulting in intracellular pH reduction and the failure of the transmembrane proton motive force. During the process, the acid dissociation of the molecules is carried out, as well as the incorporation of a set of factors that benefit in the antimicrobial capacity of the lactic acid like the accumulation of toxic anions, the stress provoked in the cellular homeostasis, the inhibition of the metabolic reactions and the inhibitory effect on the division that comes to cause the death of the microorganism (Smith et al. 1993; Pisoschi and Negulescu 2011).

LAB such as *Lactobacillus* and *Bidifobacterium* come to generate lactic acid and has been observed the inhibition of this against *Escherichia coli* O157:H7 (Reis et al. 2012). It also has this effect against *Salmonella typhimurium*, Enterobacteriaceae, *Salmonella bareilly,* and *Listeria monocytogenes* (Mani-López et al. 2012; Naidu 2000), in addition to *Mycobacterium tuberculosis*, *Bacillus coagulans*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Clostridium botulinum*, Enterobacteriaceae, Lactobacillaceae, *Aspergillus* sp., *Pseudomonas fragi*, *Clostridium sporogenes*, *Vibrio vulnificus*, *Helicobacter pylori,* and *Pseudomonas* sp. (Naidu 2000).

Applications of lactic acid in non-food are in the production of plastics, dyes, pesticides, synthetic polymers, glue (acetic acid), and even in the washing of corpses (Reis et al. 2012).

6.9.10 Acetic Acid

Acetic acid is an another product derived from the fermentation of some substrates by LAB. It is used in almost all countries as vinegar (Panda et al. 2016). It is used in the food industry as an additive to control pH levels and improve taste (Supharoek et al. 2017). As well as for the preservation of various foods, as this product has the effect of inhibiting growth and reducing the viability of Gram-positive and Gram-negative bacteria, yeasts, and fungi. It presents bacteriostatic activity at 0.2% and bactericidal activity at 0.3% (Ray 2004), so it is said that acetic acid has a more lethal effect against pathogenic microorganisms than lactic acid. Due to its pungent odor and taste, the use of this product in food is limited (Erginkaya et al. 2014); for example, in Canada it is only permissible to have a concentration of 4.1–12.3% acetic acid in vinegar (Panda et al. 2016). Excessive consumption or application of this organic acid is also not recommended, because its corrosive properties can cause stomach diseases (Supharoek et al. 2017).

6.9.11 Proteolytic Capacity

LABs are commonly used as a source of carbon the sugars and citrate and as a source of nitrogen the caseins. They are unable to synthesize several amino acids and have to be able to degrade peptides and proteins in order to meet their amino acid requirements.

One of the essential biochemical processes for obtaining fermented dairy products is the proteolysis that is involved in the organoleptic characteristics of the final product (Chaves-López et al. 2011). The proteolytic systems of the LABs are made up of: (1) proteinases that hydrolyze caseins to originate peptides; (2) transport systems to translocate peptides to the cytoplasm; and (3) peptidases that transform peptides into amino acids. For example, the taste of cheese during ripening is formed by the action of *L. lactis*, this is mainly caused by the proteolysis of casein-generating free amino acids, which behave as substrates for catabolic reactions generating aromatic compounds (Smid and Kleerebezem 2014).

It is also known that proteolysis can activate bioactive peptides (Gobbetti et al. 2002), metabolites of great importance since they show a wide range of desirable effects such as antimicrobial activity, antithrombotic antihypertensive, immunomodulatory, etc. (de Moreno de LeBlanc et al. 2018). According to Pescuma et al. (2009) this biochemical event has come to prevent and reduce frequent allergies in children under 3 years because of poor digestion of milk proteins.

6.10 Beneficial Effects of Probiotics

6.10.1 Immunology

Probiotics can modulate the immune response in animals and humans not only at the level of the intestinal mucosa, but also at the systemic level (Manzano et al. 2012). The probiotic lactic acid bacteria (LAB) to modulate their actions through the intestinal mucosal immune system must also interact with the normal intestinal flora and the food that is ingested. The interaction of probiotics with intestinal epithelial cells (enterocytes) generates cytokines and chemokines that initiate immunomodulatory events. Several studies have shown that numerous lactobacilli can alert the intestinal immune system and secondarily favor the rejection of potentially harmful infectious microorganisms; this can be done through the production of specific type A immunoglobulins (Kaila et al. 1992) or the activation of K cells ("natural killer") (Gill et al. 2001). Other immunomodulatory effects of these probiotics derive from their ability to increase the phagocytic activity of intestinal leukocytes, promote increased proliferation of B lymphocytes along with increased secretion of immunoglubulins A and G, as well as stimulate cytosines such as interleukin IL-2, IL-6, or tumor necrosis factor (TGB). The probiotics *Lactobacillus casei*, *L. rhamnosus*, *Bifidobacterium breve*, *Lactobacillus gasseri* are some of the examples studied to demonstrate this action, as well as the general activation of B lymphocytes.

Given their immunomodulatory properties, the usefulness of probiotics in the preventive or therapeutic management of inflammatory diseases is currently being evaluated. The consumption of probiotics could have a positive effect on human health in some situations that may alter the balance of the intestinal microbiota and influence the immune response of the individual, such as feeding with infant formulas, treatment with antibiotics, physiological changes related to aging, gastrointestinal diseases, and stress.

In summary, the different studies show the viability of using probiotics to modulate the immune system, prevent infections, and control the inflammatory process, but the results are diverse, so it is necessary to conduct research to reduce the gap between the differences found with factors such as: strain or species used, dose of probiotic, supplementation time, and characteristics of the subjects studied. This will allow comparisons to be made and conclusions to be drawn that will benefit the therapeutic and preventive use of probiotics on the immune system.

Recently, the function of probiotics as modulators of the immune system has been identified where mucosal immunity is of primary importance (Donkor et al. 2010; Koninkx et al. 2010). The effects of probiotics on the immune system can be classified into two major categories, involving the activation of cells of the innate immune system, such as phagocytes and natural killer cells, and the inhibition of abnormal immune responses. The former is expected to have an inhibitory effect against infections and cancer, and the latter may have an inhibitory effect against inflammatory bowel diseases, allergies, and autoimmune diseases (Chiba et al. 2010; Rook and Brunet 2005).

6.10.2 Respiratory Infections

In many occasions, probiotics are used for the prevention of certain infections, such is the case of respiratory infections, where a study carried out by Di Pierro et al. (2014) demonstrated the beneficial effects they produce. The study included 61 children diagnosed with recurrent oral streptococcal disorders, who were given the *S. salivarius* K12 strain through a tablet. Only 30 children completed the test, consuming the probiotic for 90 days in order to prevent tonsillitis. The results showed a significant reduction in episodes of streptococcal pharyngeal infection compared to the previous year's infection rates. It should also be noted that the product supplied was well tolerated and had no side effects.

Reported strains with the potential to reduce the load of pathogens in the respiratory system are *Lactobacillu*s and *Bifidobacterium*. Their mechanism is linked to the action of immune cells such as natural killer and macrophages (Hardy et al. 2013).

6.10.3 Diabetes

In diabetes mellitus, satisfactory results from the use of probiotics have been perceived, since it has been observed, in experimentation with rats, that strains such as *Lactobacillus reuteri* GMN-32 reduce blood glucose levels from 4480 to 3620 mg/L when applied at a concentration of 10^7 CFU/d. In addition, the changes in the heart that cause this disease were also reduced (Lin et al. 2014).

Most LABs, especially *Lactobacillus* species, have great antidiabetic potential. A higher content of *Lactobacillus acidophilus* and *Lactobacillus casei* has been found in fermented milk products such as Dahi, which improves hyperglycemia, hyperinsulinemia, dyslipidemia and reduces oxidative stress in diabetic rats

(Yadav et al. 2008). *Lactobacillus casei* has an important preventive effect against diabetes, being tested in non-obese diabetic mice that elevates plasma glucose and reduces plasma insulin levels due to the destruction of pancreatic cells β.

6.10.4 Cancer

The use of probiotic therapy for treatment and prevention of cancer through probiotics has increased the interest of clinical nutritionists, scientists, and industry (Soa et al. 2017; Vafaeie 2016), as it has been recognized the beneficial effect they come to generate against different types of cancer: colon and rectum, breast, blood, cervical, prostate and bladder, skin, esophagus, liver, gallbladder, head, and neck (Dasari et al. 2016). The advantage of probiotics over other cancer treatments is their absence or minimal presence of side effects (Soa et al. 2017; Vafaeie 2016). Isolated strains of fermented milk with antitumor activity are *Bifidobacterium infantis, B. bifidum*, *B. animalis*, *L. acidophilus*, and *L. paracasei*. When studied on the growth of a breast cell line it was demonstrated that the most effective species were *B. infantis* and *L. acidophilu*s (Biffi et al. 1997).

However, only the anti-cancer effect of LABs has been proven to be in vitro studies (George Kerry et al. 2018), so it is necessary to apply the research carried out to confirm this activity in humans.

6.11 The Role of Probiotics in Intestinal Diseases

6.11.1 Diseases Associated with Imbalances of the Intestinal Microbiota

The intestine is formed by a complex microbial ecosystem that has specific and protective metabolic functions (Parra 2012). The intestinal microbiota is the community of living microorganisms residing in the digestive tract (Icaza-Chávez 2013). It is composed of microorganisms that are classified as pathogenic, neutral, or beneficial to the host. This last group of bacteria is commonly included mainly in dairy derivatives (Parra 2012). That is why it is essential for proper body growth, the development of immunity and nutrition as it exercises nutritional, metabolic, and protective functions that make it indispensable for the host while it delivers nutrients and adequate conditions for growth (Morales and Brignardello 2010). The intestinal microbiota participates mainly in multiple functions such as the metabolism of some carbohydrates, specialization and activation of the immune system, regulation of intestinal cell growth, and synthesis of certain vitamins (K and B). Some bacteria in the intestinal microbiota have enzymes that are capable of digesting certain carbohydrates that cannot otherwise be processed. More or less complex polysaccharides, such as those that make up insoluble dietary fiber, undergo fermentation processes that give rise to products such as short-chain fatty acids, which have beneficial effects on the metabolism of carbohydrates and cholesterol. This fermentation also produces gases and flatulences with odors characteristic of feces.

Imbalances in their composition or a lack of microbial richness have been found to be risk factors for certain pathologies such as obesity, diabetes, asthma, or some types of cancer and may play an important role in others such as Parkinson's or autism (Sáez 2015). Several acute diarrheal diseases are due to pathogens that proliferate and have invasive characteristics or produce toxins. Diarrhea associated with antibiotics is due to an imbalance in the composition of the intestinal flora with the proliferation of pathogenic species, such as some strains of *Clostridium difficile* that produce toxins that cause pseudomembranous colitis (Guarner 2007).

6.11.2 The Role of Probiotics in Colon Cancer

The colon is the first part of the large intestine that absorbs water and nutrients from food and serves as a storage place for solid waste. Colon-rectal cancer develops in the digestive system, which is the primary mechanism for food processing, energy production, and solid waste disposal (Parra 2012). Currently experimental models have shown that intestinal bacteria can play a role in the initiation of colon cancer through the formation of carcinogenic products (Guarner 2007). The high number and diversity of the human gut microflora are reflected in its wide and varied metabolic capacity, especially in relation to the biotransformation of xenobiotics and the synthesis and activation of carcinogens (Burns and Rowland 2003).

The molecular genetic defects that appear in human colorectal cancer are well known, and appear to be a consequence of the genotoxicity of products generated in light from the intestine. Epidemiological data suggest that environmental factors such as diet play an important role in the development of colon cancer. Consumption of animal fat and red meat, particularly processed meat, is associated with higher risk (Guarner 2007). On the other hand, some probiotics are able, by mechanisms not completely clarified, to degrade mutagenic substances present in the intestine decreasing their genotoxicity, to bind to them (as to heterocyclic amines), to decrease levels of enzymes (such as azoreductase, nitroreductase, ß-glucuronidase, ß-glucosidase, and 7-a-dehydrolase) that can regenerate toxic substances that had been detoxified in the liver and excreted for disposal (Wollowski et al. 2018).

Evidence from a wide range of sources supports the view that colonic microflora intervenes in the etiology of cancer. It follows that modification of the intestinal microflora would interfere with the carcinogenesis process, and this opens up the possibility of dietary modifications to reduce the risk of colon cancer (Burns and Rowland 2003).

This type of cancer is a major cause of death in the Western world. Approximately 70% of people with this type of cancer are associated with environmental factors, probably mostly diet. Most colon cancers arise from polyps that begin to grow in the inner lining of the colon or rectum. In this regard, studies have shown that fermented dairy products prevent rectal colon cancer (Parra 2012). Probiotic lactic-acid bacteria have been shown to deactivate carcinogenic genotoxic substances. while in in vitro model systems they can prevent mutations. While in in vivo colon tissues they can prevent DNA damage and stimulate protective systems. These bacteria have potential as chemoprotective agents against genotoxic chemicals and further research is needed to clarify and quantify their beneficial effect on the prevention of colon cancer in humans (Wollowski et al. 2018). Other studies indicate that probiotics would be useful in preventing colon cancer, through the production of short-chain fatty acids, which acidify the environment (Parra 2012), exert anti-inflammatory and apoptotic effects of cells that could become carcinogenic associated with lower cancer risk.

6.12 Conclusion

The main application of these bacteria occurs in the fermentation process of food. Due to the multiple metabolites they produce, probiotics have been a very effective way for natural preservation. One of its applications are food additives that provide flavors, odors, textures, and even nutritional value. They have long been used in industrial applications mainly as starters for food fermentation, biocontrol agents, or as probiotics. They are widely used by the chemical industry, due to the production potential of polylactides as biodegradable plastic polymers and biocompatible derivatives of petrochemical products. Several LAB strains have proven probiotic properties, and their biomass can be considered a high value product (Mazzoli et al. 2014).

Since LABs are also found within the gastrointestinal microbiota, they have the ability to produce different substances to avoid some adverse effects produced by some microorganisms, only by modifying their metabolism without destroying it and thus their population is diminished. As mentioned before, LABs have a great fermentative capacity of which organic acids are produced from simple carbohydrates, which determines an increase in intestinal acidity that limits the growth of bacteria, especially Gram-negative bacteria. The function of LABs is very useful in the food industry: the preservation of food by bacteriocins produced by *Lactobacillus* has obtained successful results in meat foods, to control pathogenic microorganisms that can cause alterations in food, such as *Salmonella* spp. and *E. coli* (Ruiz et al. 2017).

Probiotics have a highly developed defense against stress factors; they can survive in environments with a large number of changes or conditions. It is necessary to know the response of these bacteria to stress conditions for a good selection of them (D’Angelo et al. 2017) because their different applications depend on what conditions they can be found, and through research, can be found more fields in which can be used this large group of bacteria.

References

Abdel-rahman MA, Tashiro Y, Sonomoto K (2011) Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: overview and limits. J Biotechnol 156(4):286–301. https://doi.org/10.1016/j.jbiotec.2011.06.017

Amari M, Fernanda L, Arango G, Gabriel V, Robert H, Morel S et al (2013) Characterization of a novel dextransucrase from Weissella confusa isolated from sourdough. Appl Microbiol Biotechnol 97:5413–5422. https://doi.org/10.1007/s00253-012-4447-8

Ananou S, Maqueda M, Martínez-Bueno M, Valdivia E (2007) Biopreservation, an ecological approach to improve the safety and shelf-life of foods. In: Communicating current research and educational topics and trends in applied microbiology. FORMATEX, Badajoz, pp 475–486

Antelmann H, Helmann JD (2011) Thiol-based redox switches and gene regulation. Antioxid Redox Signal 14(6):1049–1063. https://doi.org/10.1089/ars.2010.3400

Ardö Y (2006) Flavour formation by amino acid catabolism. Biotechnol Adv 24(2):238–242. https://doi.org/10.1016/j.biotechadv.2005.11.005

Badel S, Bernardi T, Michaud P (2011) New perspectives for Lactobacilli exopolysaccharides. Biotechnol Adv 29(1):54–66. https://doi.org/10.1016/j.biotechadv.2010.08.011

Bartowsky EJ, Henschke PA (2004) The 'buttery' attribute of wine—diacetyl—desirability, spoilage and beyond. Int J Food Microbiol 96(3):235–252. https://doi.org/10.1016/j.ijfoodmicro.2004.05.013

Benavides AB, Ulcuango M, Yépez L, Tenea GN (2016) Evaluación de las propiedades bioactivas in vitro de bacterias ácido lácticas aisladas de nichos ecológicos nativos del Ecuador. Rev Argent Microbiol 48(3):236–244. https://doi.org/10.1016/j.ram.2016.05.003

Benyacoub J, Pérez PF, Rochat F, Saudan KY, Reuteler G, Antille N et al (2005) Enterococcus faecium SF68 enhances the immune response to giardia intestinalis in mice 1. J Nutr 135:1171–1176

Biffi A, Coradini D, Larsen R, Riva L, Di Fronzo G (1997) Antiproliferative effect of fermented milk on the growth of a human breast cancer cell line. Nutr Cancer 28(1):93–99. https://doi.org/10.1080/01635589709514558

Boguta AM, Bringel F, Martinussen J, Jensen PR (2014) Screening of lactic acid bacteria for their potential as microbial cell factories for bioconversion of lignocellulosic feedstocks. Microb Cell Factories 13:97. https://doi.org/10.1186/s12934-014-0097-0

Brass DM, Palmer SM (2017) Models of toxicity of diacetyl and alternative diones. Toxicology 388:15–20. https://doi.org/10.1016/j.tox.2017.02.011

Buntin N, Chanthachum S, Hongpattarakere T (2008) Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. Songklanakarin J Sci Technol 30:141–148

Burns AJ, Rowland IR (2003) Prebióticos y probióticos en la prevención del cáncer de colon. Gastroenterol Hepatol 26(Supl 1):73–84

Chaves-López C, Serio A, Martuscelli M, Paparella A, Osorio-cadavid E, Suzzi G (2011) Microbiological characteristics of kumis, a traditional fermented Colombian milk, with particular emphasis on enterococci population. Food Microbiol 28(5):1041–1047. https://doi.org/10.1016/j.fm.2011.02.006

Chiba Y, Shida K, Nagata S, Wada M, Bian L, Wang C et al (2010) Well-controlled proinflammatory cytokine responses of Peyer's patch cells to probiotic Lactobacillus casei. Immunology 130(3):352–362. https://doi.org/10.1111/j.1365-2567.2009.03204.x

Chikindas ML, Weeks R, Drider D, Chistyakov VA, Dicks LM (2018) Functions and emerging applications of bacteriocins. Curr Opin Biotechnol 49:23–28. https://doi.org/10.1016/j.copbio.2017.07.011

Cotter PD, Ross RP, Hill C (2013) Bacteriocins—a viable alternative to antibiotics? Nat Rev Microbiol 11(2):95–105. https://doi.org/10.1038/nrmicro2937

Crowley S, Mahony J (2013) Current perspectives on antifungal lactic acid bacteria as natural. Trends Food Sci Technol 33(2):93–109. https://doi.org/10.1016/j.tifs.2013.07.004

D'Angelo LD, Cicotello J, Zago M, Guglielmotti D, Suárez V (2017) Leuconostoc strains isolated from dairy products: response against food stress conditions. Food Microbiol 66:28. https://doi.org/10.1016/j.fm.2017.04.001

da Silva Ferrari I, de Souza JV, Ramos CL, da Costa MM, Schwan RF, Dias FS (2016) Selection of autochthonous lactic acid bacteria from goat dairies and their addition to evaluate the inhibition of Salmonella typhi in artisanal cheese. Food Microbiol 60:29–38. https://doi.org/10.1016/j.fm.2016.06.014

Dasari S, Kathera C, Janardhan A, Praveen Kumar A, Viswanath B (2016) Surfacing role of probiotics in cancer prophylaxis and therapy: a systematic review. Clin Nutr 36:1465. https://doi.org/10.1016/j.clnu.2016.11.017

Datta R, Henry M (2006) Lactic acid: recent advances in products, processes and technologies - a review. J Chem Technol Biotechnol 82:1115–1121. https://doi.org/10.1002/jctb

de Almeida Júnior WLG, da Silva Ferrari Í, de Souza JV, da Silva CDA, da Costa MM, Dias FS (2015) Characterization and evaluation of lactic acid bacteria isolated from goat milk. Food Control 53:96–103. https://doi.org/10.1016/j.foodcont.2015.01.013

de Moreno de LeBlanc A, Luerce TD, Miyoshi A, Azevedo V, LeBlanc JG (2018) Functional food biotechnology. In: Omics technologies and bio-engineering. Academic Press, London, pp 105–128. https://doi.org/10.1016/B978-0-12-815870-8.00007-3

Di Pierro F, Colombo M, Zanvit A, Risso P, Rottoli AS (2014) Use of Streptococcus salivarius K12 in the prevention of streptococcal and viral pharyngotonsillitis in children. Drug Healthc Patient Saf 6:15–20

Díaz-Muñiz I, Banavara DS, Budinich MF, Rankin SA, Dudley EG, Steele JL (2006) Lactobacillus casei metabolic potential to utilize citrate as an energy source in ripening cheese: a bioinformatics approach. J Appl Microbiol 101(4):872–882. https://doi.org/10.1111/j.1365-2672.2006.02965.x

Doleyres Y, Beck P, Vollenweider S, Lacroix C (2005) Production of 3-hydroxypropionaldehyde using a two-step process with Lactobacillus reuteri. Appl Microbiol Biotechnol 68:467–474. https://doi.org/10.1007/s00253-005-1895-4

Domínguez Gonzáleza KN, Cruz Guerrero AE, Márquez HG, Gómez Ruiz LC, García-Garibay M, Rodríguez Serrano GM (2014) El efecto antihipertensivo de las leches fermentadas. Rev Argent Microbiol 46(1):58–65. https://doi.org/10.1016/S0325-7541(14)70050-1

Donkor ON, Shah NP, Apostolopoulos V, Vasiljevic T (2010) Development of allergic responses related to microorganisms exposure in early life. Int Dairy J 20(6):373–385. https://doi.org/10.1016/j.idairyj.2009.12.017

Drider D, Bekal S, Prévost H (2004) Genetic organization and expression of citrate permease in lactic acid bacteria. Genet Mol Res 3(2):273–281

Erginkaya Z, Ünal E, Kalkan S (2014) Food processing: strategies for quality assessment. Springer, New York. https://doi.org/10.1007/978-1-4939-1378-7

Freitas DR, Campos JMS, Marcondes MI, Valadares SC, Franco MO, Martins EC et al (2015) Levedura seca integral na alimentação de vacas lactantes. Arq Bras Med Vet Zootec 67:211–220

Fuller R, Gibson GR (1997) Modification of the intestinal microflora using probiotics and prebiotics. Scand J Gastroenterol 32(sup222):28–31. https://doi.org/10.1080/00365521.1997.11720714

Gálvez A, Abriouel H, López RL, Omar NB (2007) Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol 120(1–2):51–70. https://doi.org/10.1016/j.ijfoodmicro.2007.06.001

Gálvez A, López RL, Abriouel H, Valdivia E, Omar NB (2008) Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. Crit Rev Biotechnol 28(2):125–152. https://doi.org/10.1080/07388550802107202

Gänzle MG (2015) Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr Opin Food Sci 2:106–117. https://doi.org/10.1016/j.cofs.2015.03.001

García-Quintáns N, Blancato V, Repizo G, Magni C, López P (2008) Citrate metabolism and aroma compound production in lactic acid bacteria. In: Mayo B, López P, Pérez-Martín G (eds) Molecular aspects of lactic acid Bacteria for traditional and new applications. Kerala, Research Signpost, pp 64–88

George Kerry R, Patra JK, Gouda S, Park Y, Shin H-S, Das G (2018) Benefaction of probiotics for human health: a review. J Food Drug Anal 26:927–939. https://doi.org/10.1016/j.jfda.2018.01.002

Ghanbari M, Jami M, Domig KJ, Kneifel W (2013) Seafood biopreservation by lactic acid bacteria - a review. LWT Food Sci Technol 54(2):315–324. https://doi.org/10.1016/j.lwt.2013.05.039

Ghiaci P, Lameiras F, Norbeck J, Larsson C (2014) Production of 2-butanol through *meso* -2,3-butanediol consumption in lactic acid bacteria. FEMS Microbiol Lett 360(1):70–75. https://doi.org/10.1111/1574-6968.12590

Gill HS, Rutherfurd KJ, Cross ML (2001) Dietary probiotic supplementation enhances natural killer cell activity in the elderly: an investigation of age-related immunological changes. J Clin Immunol 21(4):264–271

Gobbetti M, Stepaniak L, De Angelis M, Corsetti A, Di Cagno R, Fitzgerald D (2002) Latent bioactive peptides in milk proteins: proteolytic activation and significance in dairy processing. Crit Rev Food Sci Nutr 42(3):223–239. https://doi.org/10.1080/10408690290825538

Guarner F (2007) Papel de la flora intestinal en la salud y en la enfermedad. Nutr Hosp 22:14–19

Haller D, Colbus H, Ganzle M, Scherenbacher P, Bode C, Hammes WP (2001) Metabolic and functional properties of lactic acid bacteria in the gastro-intestinal ecosystem: a comparative in vitro study. Syst Appl Microbiol 226(24):218–226. https://doi.org/10.1128/AEM.02063-08

Hardy H, Harris J, Lyon E, Beal J, Foey AD (2013) Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Nutrients 5:1869. https://doi.org/10.3390/nu5061869

Hemme D, Foucaud-Scheunemann C (2004) Leuconostoc, characteristics, use in dairy technology and prospects in functional foods. Int Dairy J 14(6):467–494. https://doi.org/10.1016/J.IDAIRYJ.2003.10.005

Hofvendahl K, Hahn-Hägerdal B (2000) Factors affecting the fermentative lactic acid production from renewable resources1. Enzym Microb Technol 26(2–4):87–107. https://doi.org/10.1016/S0141-0229(99)00155-6

Holzapfel WH (2014) Lactic acid bacteria: biodiversity and taxonomy. Wiley, Chichester

Holzapfel WH, Schillinger U, Gelsen R, Lücke F (2003) Starter and protective cultures. In: Russell NJ, Gould GW (eds) Food preservatives. Springer, Boston, MA, p 291

Hor YY, Liong MT (2014) Use of extracellular extracts of lactic acid bacteria and bifidobacteria for the inhibition of dermatological pathogen Staphylococcus aureus. Dermatol Sin 32(3):141–147. https://doi.org/10.1016/j.dsi.2014.03.001

Hugenholtz J (1993) Citrate metabolism in lactic acid bacteria. FEMS Microbiol Rev 12 (1–3):165–178. https://doi.org/10.1016/0168-6445(93)90062-E

Hwanhlem N, Buradaleng S, Wattanachant S, Benjakul S, Tani A, Maneerat S (2011) Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (Plasom) and production of Plasom from selected strains. Food Control 22(3–4):401–407. https://doi.org/10.1016/j.foodcont.2010.09.010

Icaza-Chávez ME (2013) Microbiota intestinal en la salud y la enfermedad. Rev Gastroenterol Mex 78(4):240–248. https://doi.org/10.1016/j.rgmx.2013.04.004

Imlay JA (2003) Pathways of oxidative damage. Annu Rev Microbiol 57(1):395–418. https://doi.org/10.1146/annurev.micro.57.030502.090938

Juan B, Zamora A, Quevedo JM, Trujillo AJ (2016) Proteolysis of cheese made from goat milk treated by ultra high pressure homogenisation. LWT - Food Sci Technol 69:17–23. https://doi.org/10.1016/j.lwt.2015.12.013

Juodeikiene G, Bartkiene E, Viskelis P, Urbonaviciene D, Eidukonyte D, Bobinas C (2002) Fermentation processes using lactic acid bacteria producing bacteriocins for preservation and

improving functional properties of food products. In: Advances in applied biotechnology. IntechOpen, London
Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H (1992) Enhancement of the circulating antibody secreting cell response in human diarrhea by a human lactobacillus strain. Pediatr Res 32(2):141
Ko J, Nam S, Park J, Wee Y, Kim D, Lee WS et al (2016) Synthesis and characterization of glucosyl stevioside using Leuconostoc dextransucrase. Food Chem 211:577. https://doi.org/10.1016/j.foodchem.2016.05.046
Koninkx JFJG, Tooten PCJ, Malago JJ (2010) Probiotic bacteria induced improvement of the mucosal integrity of enterocyte-like Caco-2 cells after exposure to Salmonella enteritidis 857. J Funct Foods 2(3):225–234. https://doi.org/10.1016/j.jff.2010.06.001
Kumar AS, Mody K, Jha B (2007) Bacterial exopolysaccharides—a perception. J Basic Microbiol 47:103–117. https://doi.org/10.1002/jobm.200610203
Laëtitia G, Pascal D, Yann D (2014) The citrate metabolism in homo- and heterofermentative LAB: a selective means of becoming dominant over other microorganisms in complex ecosystems. Food Nutr Sci 05(10):953–969. https://doi.org/10.4236/fns.2014.510106
Lee K, Lee H-G, Pi K, Choi Y-J (2008) The effect of low pH on protein expression by the probiotic bacterium Lactobacillus reuteri. Proteomics 8(8):1624–1630. https://doi.org/10.1002/pmic.200700663
Leistner L, Gorris LGM (1995) Food preservation by hurdle technology. Trends Food Sci Technol 6(2):41–46. https://doi.org/10.1016/S0924-2244(00)88941-4
Li X, Chen Y, Zhao S, Chen H, Zheng X, Luo J, Liu Y (2015) Efficient production of optically pure l-lactic acid from food waste at ambient temperature by regulating key enzyme activity. Water Res 70:148–157. https://doi.org/10.1016/j.watres.2014.11.049
Lin C-H, Lin C-C, Shibu MA, Liu C-S, Kuo C-H, Tsai F-J et al (2014) Oral Lactobacillus reuteri GMN-32 treatment reduces blood glucose concentrations and promotes cardiac function in rats with streptozotocin-induced diabetes mellitus. Br J Nutr 111(04):598–605. https://doi.org/10.1017/S0007114513002791
Link-Anster H, Rochat F, Saudan KY, Mignot O, Aeschlimann JM (1994) Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Med Microbiol 10:55–63
Lortal S, Chapot-Chartier M (2005) Role, mechanisms and control of lactic acid bacteria lysis in cheese. Int Dairy J 15:857–871. https://doi.org/10.1016/j.idairyj.2004.08.024
Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA (1999) Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. Am J Physiol Gastrointest Liver Physiol 276(4):G941–G950. https://doi.org/10.1152/ajpgi.1999.276.4.G941
Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E et al (2006) Comparative genomics of the lactic acid bacteria. Proc Natl Acad Sci 103:15611
Mani-López E, García HS, López-Malo A (2012) Organic acids as antimicrobials to control Salmonella in meat and poultry products. Food Res Int 45(2):713–721. https://doi.org/10.1016/j.foodres.2011.04.043
Manzano C, Estupiñán D, Poveda E (2012) Clinical efects of probiotics: what does the evidence says. Rev Chil Nutr 39:98–110
Martin N, Friedlander A, Mok A, Kenet D, Martin W, Boor K (2014) Peroxide test strips detect added hydrogen peroxide in raw milk at levels affecting bacterial load. J Food Prot 76(2):333–337. https://doi.org/10.4315/0362-028X
Martinez FA, Balciunas EM, Salgado JM, González JM, Converti A, de Souza Oliveira RP (2013) Lactic acid properties, applications and production: a review. Trends Food Sci Technol 30:70–83. https://doi.org/10.1016/j.tifs.2012.11.007
Martino G, Quintana IM, Espariz M, Blancato VS, Magni C (2016) Aroma compounds generation in citrate metabolism of Enterococcus faecium: genetic characterization of type I citrate gene cluster. Int J Food Microbiol 218:27–37. https://doi.org/10.1016/j.ijfoodmicro.2015.11.004

Mazzoli R, Bosco F, Mizrahi I, Bayer EA, Pessione E (2014) Towards lactic acid bacteria-based biorefineries. Biotechnol Adv 32(7):1216–1236. https://doi.org/10.1016/j.biotechadv.2014.07.005

Messaoudi S, Manai M, Kergourlay G, Prévost H, Connil N, Chobert J, Dousset X (2013) Lactobacillus salivarius: bacteriocin and probiotic activity. Food Microbiol 36:296. https://doi.org/10.1016/j.fm.2013.05.010

Minervini F, De Angelis M, Di Cagno R, Pinto D, Siragusa S, Rizzello CG, Gobbetti M (2010) Robustness of Lactobacillus plantarum starters during daily propagation of wheat flour sourdough type I. Food Microbiol 27(7):897–908. https://doi.org/10.1016/j.fm.2010.05.021

Morales P, Brignardello J (2010) La microbiota intestinal: un nuevo actor en el desarrollo de la obesidad. Rev Med Chil 138:1020–1027

Mozzi F (2016) Lactic acid bacteria. In: Encyclopedia of food and health. Academic Press, Amsterdam, pp 501–508. https://doi.org/10.1016/B978-0-12-384947-2.00414-1

Naidu AS (2000) Natural food antimicrobial systems. CRC Press, London

Notararigo S, Nácher-Vázquez M, Ibarburu I, Werning M, De Palencia PF, Dueñas MT et al (2013) Comparative analysis of production and purification of homo- and hetero-polysaccharides produced by lactic acid bacteria. Carbohydr Polym 93(1):57–64. https://doi.org/10.1016/j.carbpol.2012.05.016

Notararigo S, de las Casas-Engel M, de Palencia PF, Corbí AL, López P (2014) Immunomodulation of human macrophages and myeloid cells by 2-substituted (1–3)-β-d-glucan from P. parvulus 2.6. Carbohydr Polym 112:109–113. https://doi.org/10.1016/j.carbpol.2014.05.073

Nwodo UU, Green E, Okoh AI (2012) Bacterial exopolysaccharides: functionality and prospects. Int J Mol Sci 13(11): 14002–14015. https://doi.org/10.3390/ijms131114002

O'Toole PW, Cooney JC (2008) Probiotic bacteria influence the composition and function of the intestinal microbiota. Interdiscip Perspect Infect Dis 2008:1. https://doi.org/10.1155/2008/175285

Oliveira RBP, Oliveira ADL, Glória MBA (2008) Screening of lactic acid bacteria from vacuum packaged beef for antimicrobial activity. Braz J Microbiol 39(2):368–374. https://doi.org/10.1590/S1517-83822008000200031

Özcelik S, Kuley E, Özogul F (2016) Formation of lactic, acetic, succinic, propionic, formic and butyric acid by lactic acid bacteria. LWT Food Sci Technol 73:536–542. https://doi.org/10.1016/j.lwt.2016.06.066

Panda SK, Mishra SS, Kayitesi E, Ray RC (2016) Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids: biotechnology and scopes. Environ Res 146:161–172. https://doi.org/10.1016/j.envres.2015.12.035

Parra R (2012) Yogur en la salud humana. Rev Lasallista Invest 9(2):162–177

Pérez-Ramos A, Nácher-Vázquez M, Notararigo S, López P, Mohedano ML (2015) Current and future applications of bacterial extracellular polysaccharides. In: Watson RR, Preedy VR (eds) Probiotics, prebiotics, and synbiotics. Elsevier, Oxford, pp 329–344

Pescuma M, Hébert EM, Dalgalarrondo M, Haertlé T, Mozzi F, Chobert JM, De Valdez GF (2009) Effect of exopolysaccharides on the hydrolysis of ß-lactoglobulin by lactobacillus acidophilus CRL 636 in an in vitro gastric/pancreatic system. J Agric Food Chem 57(12):5571–5577. https://doi.org/10.1021/jf9006505

Pflügl S, Marx H, Mattanovich D, Sauer M (2014) Heading for an economic industrial upgrading of crude glycerol from biodiesel production to 1, 3-propanediol by Lactobacillus diolivorans. Bioresour Technol 152:499–504. https://doi.org/10.1016/j.biortech.2013.11.041

Pisoschi AM, Negulescu GP (2011) Methods for total antioxidant activity determination: a review. Biochem Anal Biochem 1(1):1–10. https://doi.org/10.4172/2161-1009.1000106

Pleissner D, Neu A-K, Mehlmann K, Schneider R, Puerta-Quintero GI, Venus J (2016) Fermentative lactic acid production from coffee pulp hydrolysate using Bacillus coagulans at laboratory and pilot scales. Bioresour Technol 218:167–173. https://doi.org/10.1016/j.biortech.2016.06.078

Porto MCW, Kuniyoshi TM, Azevedo POS, Vitolo M, Oliveira RPS (2017) Pediococcus spp.: an important genus of lactic acid bacteria and pediocin producers. Biotechnol Adv 35(3):361–374. https://doi.org/10.1016/j.biotechadv.2017.03.004

Pothakos V, Devlieghere F, Villani F, Björkroth J, Ercolini D (2015) Lactic acid bacteria and their controversial role in fresh meat spoilage. Meat Sci 109:66–74. https://doi.org/10.1016/j.meatsci.2015.04.014

Quigley EMM (2012) Prebiotics and probiotics. Nutr Clin Pract 27(2):195–200. https://doi.org/10.1177/0884533611423926

Ramírez JC, Ulloa P, Velázquez M, Ulloa J, Arce F (2011) Bacterias lácticas: importancia en alimentos y sus efectos en la salud. Rev Fuente 2(7):16. Retrieved from http://www.hablemosclaro.org/repositorio/biblioteca/b_305_bacterias_lacticas_importancia_en_alimentos.pdf

Randazzo C, Restuccia C, Mancini A, Muccilli S, Gatti M, Caggia C (2016) Ragusana donkey milk as a source of lactic acid bacteria and yeast strains of dairy technological interest. Int J Dairy Sci Process 3:38–46

Rastogi A, Shukla S (2013) Amaranth: a new millennium crop of nutraceutical values. Crit Rev Food Sci Nutr 53(2):109–125. https://doi.org/10.1080/10408398.2010.517876

Ray B (2004) Fundamental food microbiology. CRC Press, New York. https://doi.org/10.1007/s13398-014-0173-7.2

Reis JA, Paula AT, Casarotti SN, Penna ALB (2012) Lactic acid bacteria antimicrobial compounds: characteristics and applications. Food Eng Rev 4(2):124–140. https://doi.org/10.1007/s12393-012-9051-2

Rolfe RD (2000) The role of probiotic cultures in the control of gastrointestinal health. J Nutr 130 (2):396S–402S. https://doi.org/10.1093/jn/130.2.396S

Rook GAW, Brunet LR (2005) Microbes, immunoregulation, and the gut. Gut 54(3):317–320. https://doi.org/10.1136/gut.2004.053785

Ross R, Morgan S, Hill C (2002) Preservation and fermentation: past, present and future. Int J Food Microbiol 79(1–2):3–16. https://doi.org/10.1016/S0168-1605(02)00174-5

Ruiz MJ, Colello R, Padola NL, Etcheverría AI (2017) Efecto inhibitorio de Lactobacillus spp. sobre bacterias implicadas en enfermedades transmitidas por alimentos. Rev Argent Microbiol 49:174. https://doi.org/10.1016/j.ram.2016.10.005

Rutala W, Weber D (2008) Disinfection and sterilization in health care facilities: what clinicians need to know. Clin Infect Dis 39(5):702–709. https://doi.org/10.1086/423182

Sáez C (2015) Desequilibrios en la microbiota intestinal, posible origen de algunos cánceres. La vanguardia

Salvetti E, Fondi M, Fani R, Torriani S, Felis GE (2013) Evolution of lactic acid bacteria in the order Lactobacillales as depicted by analysis of glycolysis and pentose phosphate pathways. Syst Appl Microbiol 36:291. https://doi.org/10.1016/j.syapm.2013.03.009

Savadogo A, Ouattara C, Bassole I, Traore S (2006) Bacteriocins and lactic acid bacterias - a mini review. J Biotechnol 5(9):678–683

Shibamoto T (2014) Diacetyl: occurrence, analysis, and toxicity. J Agric Food Chem 62 (18):4048–4053. https://doi.org/10.1021/jf500615u

Smid EJ, Kleerebezem M (2014) Production of aroma compounds in lactic fermentations. Annu Rev Food Sci Technol 5(1):313. https://doi.org/10.1146/annurev-food-030713-092339

Smith DR, Smyth AP, Strauss WM, Moir DT (1993) Incorporation of copy-number control elements into yeast artificial chromosomes by targeted homologous recombination. Mamm Genome 4:141–147

Soa SSY, Wana MLY, El-Nezami H (2017) Probiotics-mediated suppression of cancer. Curr Opin Oncol 29(1):62–72. https://doi.org/10.1097/CCO.0000000000000342

Sobrino-López A, Martín-Belloso O (2008) Use of nisin and other bacteriocins for preservation of dairy products. Int Dairy J 18(4):329–343. https://doi.org/10.1016/j.idairyj.2007.11.009

Starek-Swiechowicz B, Starek A (2014) Diacetyl exposure as a pneumotoxic factor: a review. Rocz Panstw Zakl Hig 65(2):87–92

Sun Z, Harris HMB, McCann A, Guo C, Argimón S, Zhang W et al (2015) Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. Nat Commun 6(1):8322. https://doi.org/10.1038/ncomms9322
Supharoek S, Ponhong K, Siriangkhawut W, Grudpan K (2017) Employing natural reagents from turmeric and lime for acetic acid determination in vinegar sample. J Food Drug Anal 26:583–590. https://doi.org/10.1016/j.jfda.2017.06.007
Tamang J, Thapa N, Tamang B, Rai A, Chettri R (2015) Microorganisms in fermented foods and beverages. In: Health benefits of fermented foods and beverages. CRC Press, Boca Raton, pp 1–110. https://doi.org/10.1201/b18279-2
Tyler CA, Kopit L, Doyle C, Yu AO, Hugenholtz J, Marco ML (2016) Polyol production during heterofermentative growth of the plant isolate Lactobacillus florum 2F. J Appl Microbiol 120 (5):1336. https://doi.org/10.1111/jam.13108
Vafaeie F (2016) Critical review on probiotics and its effect on cancer. Cancer Press 2(2):30–34
Vandenplas Y, Benninga M (2009) Probiotics and functional gastrointestinal disorders in children. J Pediatr Gastroenterol Nutr 48(Suppl 2):S107–S109. https://doi.org/10.1097/MPG.0b013e3181a1603a
Venugopalan V, Dinesh MS, Geetha KS (2010) Enhancement of antimicrobial potential of Phyllanthus niruri by fermentation. J Herb Med Toxicol 4:167–175
Viana de Souza J, Silva Dias F (2017) Protective, technological, and functional properties of select autochthonous lactic acid bacteria from goat dairy products. Curr Opin Food Sci 13:1–9. https://doi.org/10.1016/j.cofs.2017.01.003
Vijayakumar J, Aravindan R, Viruthagiri T (2008) Recent trends in the production, purification and application of lactic acid. Chem Biochem Eng Q 2(2):245–264
Wilson KH, Perini F (1988) Role of competition for nutrients in suppression of Clostridium difficile by the colonic microflora. Infect Immun 56(10):2610–2614
Wollowski I, Rechkemmer G, Pool-zobel BL (2018) Protective role of probiotics and prebiotics in colon cancer. Am J Clin Nutr 73:451s
Yadav H, Jain S, Sinha PR (2008) Oral administration of dahi containing probiotic Lactobacillus acidophilus and Lactobacillus casei delayed the progression of streptozotocin-induced diabetes in rats. J Dairy Res 75(2):189–195. https://doi.org/10.1017/S0022029908003129
Yang S, Lin C, Sung CT, Fang J (2014) Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Front Microbiol 5:1–10. https://doi.org/10.3389/fmicb.2014.00241
Zacharof MP, Lovitt RW (2012) Bacteriocins produced by lactic acid bacteria a review article. APCBEE Proc 2:50–56. https://doi.org/10.1016/J.APCBEE.2012.06.010
Zacharof MP, Lovitt W (2014) Bacteriocins produced by lactic acid bacteria - a review. Acta Period Technol 45:271–283. https://doi.org/10.2298/APT1445271V
Zannini E, Waters DM, Coffey A, Arendt EK (2016) Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. Appl Microbiol Biotechnol 100:1121–1135. https://doi.org/10.1007/s00253-015-7172-2
Zarour K, Vieco N, Pérez-ramos A (2017) Lactic acid bacteria. In: Microbial production of food ingredients and additives. Elsevier, Saint Louis. https://doi.org/10.1016/B978-0-12-811520-6/00004-0
Zúñiga M, Gómez-escoín CL, González-candelas F (2011) Evolutionary history of the OmpR/IIIA family of signal transduction two component systems in Lactobacillaceae and Leuconostocaceae. BMC Evol Biol 11:34. https://doi.org/10.1186/1471-2148-11-34

Chapter 7
Probiotics in Livestock and Poultry Nutrition and Health

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Abstract The use of probiotics has gained immense interests in animal agriculture around the world due to the myriad of health and production benefits, especially in the context of more natural and antibiotic-free animal production. They have been widely evaluated in animal nutrition to improve the balance of beneficial gut microbiome (eubiosis) and eliminate the detrimental gut pathogens (dysbiosis), which results in a range of advantages such as enhanced functioning of gastrointestinal tract, improved immunity at the gut as well as systemic levels, and better health status of both ruminants and non-ruminants. Consequently, these beneficial effects positively influence overall production performance and farm profitability. The use of probiotics in ruminants has primarily focused on improving ruminal fermentation efficiency such as stabilisation of pH and enhanced fibre digestion, reduction of methane production in the rumen, thereby impacting production performance. Among the several species of probiotic organisms studied, yeasts have been most widely explored followed by bacterial probiotics in ruminant nutrition. In non-ruminants, bacterial probiotics dominate over yeast in augmenting performance measures. Furthermore, probiotics have also shown to reduce incidences of intestinal diseases, faecal shedding of gut pathogens, and improving the gut barrier functions and quality of meat and milk in food animals. This chapter discusses various species of probiotics, their beneficial effects and mode of actions in enhancing efficiency of animal production.

Keywords Probiotic · Ruminant · Non-ruminant · Immunity · Production performance

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7.1 Introduction

The world population is expected to grow over 9.7 billion persons by 2050. In view of feeding extra 2 billion people in the next 30 years with an ongoing escalated demand for foods of animal origins, it is necessary to intensify the industrial animal production systems in a more efficient manner. This can only be possible with proper strategies for breeding and selection, optimised nutrition along with disease control and prevention, which maximises the production and makes it sustainable. Following the ban and restriction on usage of in-feed antibiotics in European Union and other countries owing to possible emergence of antibiotic resistance against pathogens (Van Boeckel et al. 2019), it has become imperative for animal nutritionists worldwide to search for natural and alternative feed additives for sustainable production (Huyghebaert et al. 2011; Lillehoj et al. 2018; Patra 2019). One such category gaining increasing importance is natural microbial feed additives—the "probiotics". The initial idea of probiotics was described by Metchnikoff (1907) as "friendly microbes", which was further conceptualised by Lilly and Stillwell (1965) and later, Parker (1974) coined the term "probiotics" (sometimes also termed as "direct-fed microbials"). It is currently defined as "live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO 2002). Therefore, probiotics are essentially non-pathogenic, non-toxic microorganisms that are classified as "generally recognised as safe" ingredients by the United States Food and Drug Administration.

Digestion and absorption of nutrients in the gut are central to supplying essential nutrients for synthesis of quality animal products (milk, meat, and eggs). Thus, it is extremely important to achieve a healthy balance of gut microbiota enabling optimum gastrointestinal (GI) functions (Yeoman et al. 2012). In this context, probiotics have proven to be of immense value in animal agriculture by means of improving balance of beneficial gut microbiome (eubiosis) leading to a range of benefits such as enhanced functioning of GI tract, improved immunity and health status, which positively influence overall production performance and farm profitability. In this way, probiotics have found successful application in animal food production systems represented by ruminants (cattle, buffaloes, sheep, and goats) as well as non-ruminants (poultry and pigs). This chapter discusses multifaceted beneficial functions of probiotics and their mechanisms of actions in enhancing efficiency of animal production.

7.2 Probiotic Microorganisms

There are many beneficial microbes in nature, but a number of criteria need to be fulfilled for qualifying them to designate as probiotics (Table 7.1). Depending on the source of origin, probiotics could either be of autochthonous (isolated from host) or allochthonous (isolated from another host) type (Seghouani et al. 2017) and could be

Table 7.1 Characteristics of an ideal probiotic for animal feeding[a]

	Characteristic	Reference
1	Origin from host animal	Nisbet (2002) and De Angelis et al. (2006)
2	Non-pathogenic	Hodgkinson et al. (2017), Kerry et al. (2018), and Tewari et al. (2019)
3	Withstand processing and storage conditions	Soares et al. (2019)
4	Resist both very low pH (gastric acid) and high pH (bile and pancreatic juice of small intestine)	Sarkar (2018) and Samedi and Charles (2019)
5	Adhere to gastrointestinal tract epithelium or mucus	Park et al. (2016), Fernández et al. (2018), and Reuben et al. (2019)
6	Persist in the intestinal tract	Park et al. (2016)
7	Produce various inhibitory compounds (bacteriocin and colicin, etc.)	Sakata et al. (2003) and Vieco-Saiz et al. (2019)
8	Modulate immune response	Lee et al. (2011), Park et al. (2016), Vuong et al. (2016), Ma and Suzuki (2018), Wang et al. (2018), Willson et al. (2018), and Ognik et al. (2019)
9	Alter microbial activities	Collado et al. (2007), Wang et al. (2018), and Ognik et al. (2019)

[a]Adapted and modified from Simmering and Blaut (2001) and Patterson and Burkholder (2003)

of either single microbial strain or multi-strain probiotics. Moreover, probiotic products could also be a mixture of bacterial, yeast, and fungal microorganisms with proven synergistic action on hosts (Birkelo 2003). Several micro-organisms have been identified with claimed probiotic activities along with their different modes of action (Tables 7.2 and 7.3). It is evident that bacterial probiotics fall primarily into two categories: lactic acid utilisers and lactic acid producers in ruminants, whereas *Saccharomyces cerevisiae* is the prominent yeast explored in both ruminants and non-ruminants. Nevertheless, while there are many yeasts or yeast derivatives investigated in animal nutrition (Table 7.4), only active dry yeast and yeast culture are commonly used in field (Shurson 2018). In addition, few ethanol industry-derived feed ingredients including dried distillers grains with solubles and distillers wet grains also contain fraction of yeast cells and associated nutraceutical compounds (Shurson 2018). The supplementation of probiotics results in a multitude of responses in an animal system, which in combination offers benefits for improving production efficiency, health, and welfare of animals (Fig. 7.1).

7.3 Effect on Disease Incidence

Probiotics added to the diet maintain or increase the populations of beneficial microorganisms while minimising the abundances of non-essential and pathogenic microorganisms such as *Escherichia coli*, *Salmonella,* and *Clostridium perfringens*

Table 7.2 List of probiotic microorganisms studied in ruminant production

Microorganism	Application	Key reference[a]	Mode(s) of action
Lactic acid producers			
Lactobacillus plantarum, L. acidophilus, L. casei, L. salivarius, L. gallinarum, L. reuteri, and *Streptococcus bovis*	Calves, sheep, dairy and feedlot (beef) cattle	Nollet et al. (1998), Ohya et al. (2001), Bertin et al. (2017), and Doyle et al. (2019)	• Ruminal provision of lactic acid • Stimulation of lactate utilisers • Competitive exclusion • Stabilisation of rumen pH • Direct antibacterial action • Reduction in faecal shedding of *E. coli* O157 • Improving immune function • Reduction in enteric methane production (?)
Enterococcus faecium	Calves, sheep, dairy and feedlot cattle	Emmanuel et al. (2007), Oetzel et al. (2007), Qadis et al. (2014), and Chiquette et al. (2015)	
Bifidobacterium pseudolongum and *B. thermophilum*	Calves	Abe et al. (1995), and Krehbiel et al. (2003)	
Streptococcus bovis	Calves	Ohya et al. (2001)	
Leuconostoc mesenteroides subsp. *mesenteroides*	Sheep	Mwenya et al. (2004)	
Lactic acid utilisers			
Megasphaera elsdenii (formerly known as *Peptostreptococcus elsdenii*)	Sheep, dairy and feedlot cattle	Aikman et al. (2009, 2011), Leeuw et al. (2009), and Henning et al. (2010)	• Utilisation of lactate in rumen • Synthesis of propionate from lactate • Reduction in methane production and regulation of pH
Propionibacterium freudenreichii, P. jensenii, and *P. acidipropionici*	Calves, buffaloes, dairy and feedlot cattle	Lehloenya et al. (2008a, b), Ahmed et al. (2013), Vyas et al. (2014), and Azzaz et al. (2019)	
Selenomonas ruminantium subsp. *lactilytica*	Lambs	Wiryawan and Brooker (1995)	
Others			
Prevotella bryantii	Dairy cattle and sheep	Chiquette et al. (2012), and Fraga et al. (2018)	• Moderating rumen fermentation
Bacillus subtilis, B. licheniformis and *B. coagulans*	Calves, sheep, dairy and beef cattle	Qiao et al. (2010), Sun et al. (2013), Jia et al. (2018), and Musa et al. (2019)	• Supporting growth of other rumen microbes • Moderating rumen fermentation • Improved feed degradation
Ruminococcus flavefaciens	Dairy buffaloes	Kumar and Sirohi (2013)	• Moderating rumen fermentation
Pediococcus acidilactici	Calves	Frizzo et al. (2011)	• Faster development of rumen function
Escherichia coli	Calves	Ohya et al. (2001)	• Competitive exclusion

(continued)

Table 7.2 (continued)

Microorganism	Application	Key reference[a]	Mode(s) of action
Yeast			
Saccharomyces cerevisiae and *S. cerevisiae* subsp. *boulardii*	Calves, goat kids, buffaloes, sheep, dairy and beef cattle	Kamalamma et al. (1996), Keyser et al. (2007), Pal et al. (2010); Finck et al. (2014), Anand Laxmi et al. (2016), Geng et al. (2016), Bach et al. (2018, 2019), and Feye et al. (2019)	• Oxygen scavenging in rumen • Enhanced lactate utilisation • Supporting growth of other microbes in rumen • Supplying growth factors, hydrolytic enzymes and B vitamins • Increasing feed intake • Immunomodulation • Reducing invasion of *Salmonella* • Regulation of genes encoding rumen epithelial barrier • Modulating colon microbiome
Fungi			
Aspergillus oryzae and *A. niger*	Calves, sheep, dairy and beef cattle	Yu et al. (1997), Rojo et al. (2005), and Piamphon et al. (2017)	• Stimulating cellulolytic bacteria • Promoting lactate utilisation
Trichosporon sericeum	Sheep	Mwenya et al. (2004)	• Moderating rumen pH
Piromyces sp.	Buffaloes	Paul et al. (2006) and Tripathi et al. (2007)	• Enhancing fibre digestion

Adapted and modified from Seo et al. (2010) and McAllister et al. (2011)
[a]Selective references are included, not an exhaustive list

(Clavijo and Flórez 2017; Bhogoju et al. 2018; Markowiak and Śliżewska 2018; Price et al. 2020), which are responsible for diseases and adverse conditions including anorexia, necrotic enteritis, gut dysbiosis, diarrhoea, dysregulation of the immune system, and reduce chronic inflammation (Nava et al. 2005; Dahiya et al. 2006; O'Hara et al. 2006; Neish 2009). Probiotics help in improvement of animal health status by altering gut microbiome in a favourable direction, i.e., enhancing the number of beneficial gut microbiota. For example, inclusion of *Bacillus subtilis* improved intestinal health in broiler chickens (Fritts et al. 2000) and improved performance in birds with necrotic enteritis (Tactacan et al. 2013). *B. subtilis* also showed reduction of *Salmonella* in broiler chickens (Knap et al. 2011). Inclusion of *Bacillus* probiotic either in single (*B. cereus* var. toyoi; Vilà et al. 2009) or in combination (*B. amyloliquefaciens, B. licheniformis,* and *B. pumilus*; Price et al.

Table 7.3 List of probiotic microorganisms studied in non-ruminant production

Microorganism	Application	Key reference[a]	Mode(s) of action
Lactobacillus spp.	Poultry and pig	Patterson and Burkholder (2003), Nakphaichit et al. (2011), Neal-McKinney et al. (2012), Pakbaten et al. (2019), and Vieco-Saiz et al. (2019)	• Stimulation of adaptive immunity • Alteration of the caecal microbiome • Production of inhibitory metabolites such as organic acids and bacteriocins
Bifidobacteria	Poultry	Patterson and Burkholder (2003) and Jung et al. (2008)	• Competition for colonisation sites • Competition for nutrients • Reduction of toxic compounds • Immuno-stimulation
Bacillus spp.	Poultry and pig	Lee et al. (2011); Liu et al. (2012); Tellez et al. (2012), Lei et al. (2013), Jayaraman et al. (2017), Park and Kim (2014), Bai et al. (2018), Zong et al. (2019), Mingmongkolchai and Panbangred (2018), Upadhaya et al. (2019), and Price et al. (2020)	• Rapid activation of innate host immune responses • Protection of intestinal barrier function • Production of antimicrobial compounds against pathogens • Reduction in pathogen colonisation • Improvement in feed conversion and greater weight gain
Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium and *Bifidobacterium bifidium*	Poultry	Talebi et al. (2008)	• Stimulation of adaptive immunity • Alteration of the caecal microbiome • Production of inhibitory metabolites such as organic acids and bacteriocins
Lactobacillus, Bifidobacterium, Leuconostoc, Enterococcus, Lactococcus, Bacillus, Saccharomyces, Aspergillus and *Pediococcus*	Poultry and pig	Mountzouris et al. (2007) and Getachew (2016)	• Rapid activation of innate host immune responses • Production of inhibitory metabolites such as bacteriocins

(continued)

Table 7.3 (continued)

Microorganism	Application	Key reference[a]	Mode(s) of action
Enterococcus faecium	Poultry	Samli et al. (2007), Veizaj-Delia et al. (2010), Zhao et al. (2013), and Yu et al. (2019)	• Meat quality and antioxidant activity of muscle
Aspergillus oryzae	Poultry	Lee et al. (2006) and FEEDAP (2016)	• 6-phytase and other metabolites
Saccharomyces cerevisiae	Poultry and pig	Karaoglu and Durdag (2005) and Elghandour et al. (2020)	• Competitive exclusion of pathogenic bacteria • Production of antimicrobial agents • Balancing the gut microbiome • Stimulation of host adaptive immune system • Improving gut morphological structure
Clostridium butyricum	Poultry and pig	Yang et al. (2012); Zhao et al. (2013), Takahashi et al. (2018), and Zong et al. (2019)	• Improving intestinal barrier functions • Increasing growth performance and alleviating diarrhoea

[a]Selective references are included, not an exhaustive list

2020) in the diet resulted in uniform reduction of *Salmonella enteritidis* in the caeca of layers.

Corr et al. (2009) and Fuller (2012) after going through the published literature summarised that the modes of action responsible for growth inhibition and reduction of abundances of pathogens in the GI tract include (1) production of bacteriocins and colicins with antibacterial effects (Cotter et al. 2005; Chichlowski et al. 2007; Sakata et al. 2003; Vieco-Saiz et al. 2019), antimicrobial compounds/peptides, e.g., volatile fatty acids (VFA; Van Immerseel et al. 2004; Talebi et al. 2008), and defensins (Zhu et al. 2000; Toure et al. 2003; Furrie et al. 2005), and inhibitors of virulence gene expression (Mack et al. 2003); (2) competitive exclusion of pathogens competing for binding sites in the intestine (Sherman et al. 2005; Tsai et al. 2005; Collado et al. 2007; Wang et al. 2018; Ognik et al. 2019; Table 7.5) or enhancing the epithelial barrier function through blocking of epithelial surface receptors or causing conformational changes in the tight junction of the epithelia (Mack et al. 1999; Furrie et al. 2005) or stimulation of synthesis of mucins to form a physical barrier along the epithelial monolayer (Nurmi et al. 1992; Madsen et al. 2001; Mattar et al. 2002; Callaway et al. 2008; Corr et al. 2009); (3) stimulation of immune responses through increased synthesis of immunoglobulin A (IgA) and anti-inflammatory cytokines and controlling the production of pro-inflammatory cytokines (Haghighi et al. 2005;

Table 7.4 Types of various yeast products employed in animal production

Type of yeast	Description
1. Viable yeast products	
(a) Active dry yeast	Most commonly used in feed industry worldwide; typically contains 15–25 billion live yeast cells/g, i.e., colony forming units (CFU)
(b) Diluted yeast products	Active dry yeast diluted with inert carriers such as rice hulls and distillers solubles; contains relatively lesser concentration of yeast ~5 billion CFU/g
2. Yeast culture	A combination of yeast biomass plus fermentation metabolites (peptides, alcohols, esters, organic acids, and certain undefined metabolites)
3. Nutritional yeast products	Comprises of dried yeast, brewer's dried yeast, torula (*Candida utilis*) dried yeast, and whey yeast. Brewer's dried yeast (>45% protein) is commonly used in animal feeding
4. Speciality yeast products	
(a) Irradiated yeast	A source of vitamin D; not commonly used today
(b) Selenium yeast	A source of highly bioavailable organically complexed selenium (selenomethionine)
(c) Chromium (Cr) yeast	A source of trivalent Cr and anti-stress nutrient for animals
(d) Phaffia (*Phaffia rhodozyma*) yeast	A source of red carotenoid pigment astaxanthin; a feed additive used in aquaculture
5. Fractionated yeast products	Comprises of molasses yeast condensed solubles, yeast hydrolysates, yeast extracts and cell walls (glucan and mannan)
6. Yeast fermented corn ethanol co-products	Comprises of dried distillers grains with solubles, distillers wet grains, thin stillage, and condensed distillers solubles. These contain varying proportions of yeast cell and nutraceutical components.

Summarised from Shurson (2018)

Hodgkinson et al. 2017); (4) down-regulating the virulence gene or protein expressions in pathogens of GI tract (Medellin-Peña et al. 2007; Yang et al. 2014), and (5) secretion of enzymes such as amylase, alteration of GI pH, and microbiota to favour an increased activity of intestinal enzymes and digestibility of nutrients (Van Immerseel et al. 2004; Bhogoju et al. 2018). Short chain fatty acids such as lactate, acetate, propionate, and butyrate produced by various gut microorganisms are inhibitory to certain pathogens including the members of family Enterobacteriaceae (van Der Wielen et al. 2000) mostly by lowering extracellular and intracellular pH (Van Immerseel et al. 2004).

The species and strains of probiotics play a major role for imparting the desired traits to be improved. For monogastric animals, the role of probiotics is more pronounced and easily observed than the ruminants. The choice of probiotic species is utmost important, e.g., *Bacillus* species (*B. subtilis*, *B. cereus*, and *B. clausii*) have been commonly used as direct-fed microbials (Zhang et al. 2013; Park and Kim 2014; Bai et al. 2017) due to their capacity of sporulation (Chaucheyras-Durand and Durand 2010). Dietary *Bacillus subtilis* as probiotic has shown to exert its beneficial effect by decreasing intestinal pH, enhancing the intestinal antioxidant status, boosting gut-associated immune system, and activating the intestinal intraepithelial lymphocytes (Nurmi et al. 1992; Neish 2009; Park et al. 2019).

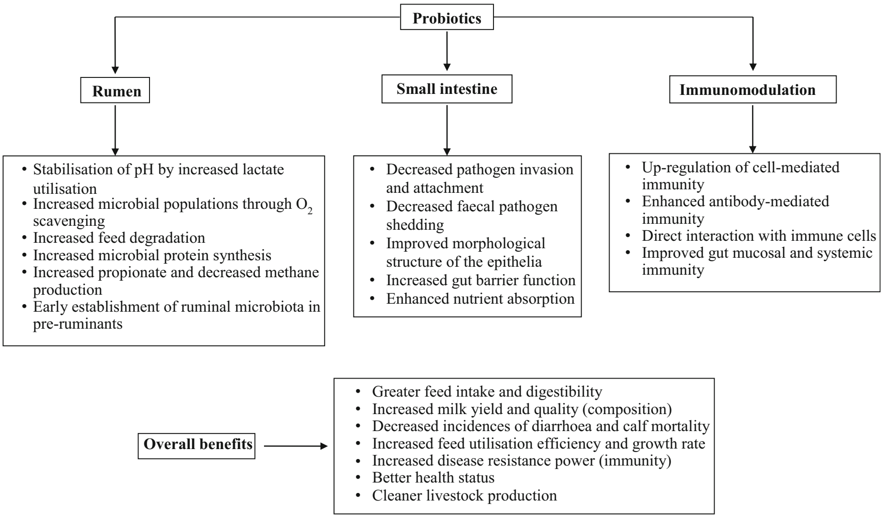

Fig. 7.1 Potential beneficial attributes of probiotic supplementation in animal production

Table 7.5 Summary of various effects for decreasing disease incidence by probiotics

Probiotic organism	Inhibitory effect by	Antagonistic effect against	Source
Enterococcus faecium, Pediococcus pentosaceus, and *Bacillus subtilis*	Bacteriocins	*C. perfringens* and *Listeria monocytogenes*	Teo and Tan (2005)
Lactobacillus salivarius	Bacteriocins	*Campylobacter jejuni*	Stern et al. (2006)
Bacillus subtilis	Competitive exclusion	*E. coli*	Molnár et al. (2011)
Clostridium butyricum	Increased populations of *Lactobacillus* and *Bifidobacterium*	*Salmonella* spp. and *C. perfringens*	Yang et al. (2012)
E. faecium, P. acidilactici, L. salivarius, and *L. reuteri*	Competitive exclusion	*Campylobacter jejuni*	Ghareeb et al. (2012)
Lactobacillus crispatus	Competitive exclusion	*E. coli* O157: H7 and *S. typhimurium*	Chen et al. (2007)
Lactobacillus spp.	Competitive exclusion	*Campylobacter jejuni*	Pogačar et al. (2020)

7.4 Effect on Immunity

Probiotics boost immune responses and help combat enteric pathogens (Patterson and Burkholder 2003; Fernández et al. 2018; Ognik et al. 2019). Gut allows nutrient absorption as well as restricts pathogens and antigens regulating the barrier function conferred by the junctional protein complexes and immune systems in epithelia (Patra et al. 2019; Patra 2019, 2020). Probiotics can modulate innate and acquired immune systems through metabolites produced by them, their cell wall components (i.e., yeast cell wall complex polysaccharides such as mannan-oligosaccharides and β-glucans and probiotic bacterial peptidoglycan) and by DNA (Oelschlaeger 2010; Alugongo et al. 2017). It is aided by the fact that they can interact directly with the recognition receptors of the gut epithelial or immune cells (Oelschlaeger 2010; Shurson 2018). Adhesion of probiotic bacteria to the epithelial cells or soluble factors released from the cells can trigger signalling cascades in immune cells leading to improved host immunity by interacting with the cell- and antibody-mediated (e.g., IgA, IgE, IgG and IgM) immune systems, decreased production of pro-inflammatory cytokines (e.g., IL-2 and IL-10), protecting epithelial barrier integrity by minimising apoptosis, enhancing association of dendritic cell to T-cells as well as T-cells to lymph nodes with a greater toll-like receptor signalling (Oelschlaeger 2010; McAllister et al. 2011).

Supplementation with *Bacillus subtilis* natto to calves increased IgG and gamma interferon levels in blood (Sun et al. 2010); *S. cerevisiae* and its metabolites up-regulated immune-related gene expressions associated with recognition of pathogen-associated molecular patterns (e.g., toll-like receptor (TLR) 1, TLR2, and TLR6), T-cell differentiation (e.g., ICAM1, RORC, TBX21, STAT6, and CXCR3), and others such as CASP1 in beef steers (Adeyemi et al. 2019); and *B. amyloliquefaciens* increased IL10 content, but decreased tumour necrosis factor alpha level in the small intestines of piglets (Li et al. 2018). It has been reported that addition of *Lactobacillus plantarum* B2984 in the diet of piglets enhanced serum antibody responses to *S. typhimurium* infection (Naqid et al. 2015). Dietary inclusion of *B. subtilis*-based probiotics stimulated the immune system, and improved anti-oxidant status in poultry (La Ragione and Woodward 2003; Lee et al. 2011). Further, *B. subtilis* has reported to augment macrophage functions, i.e., nitric oxide (NO) production and phagocytosis in broiler chickens (Lee et al. 2011). In addition, yeast culture has also been shown to have performance and immunomodulatory effects in broiler in chickens (Gao et al. 2008).

As observed by different workers, dietary probiotics help in increasing the number of IgA-producing cells (Hodgkinson et al. 2017) or improve serum Ig concentrations (Koenen et al. 2004; Yang et al. 2012). The improvements can be attributed to the immunomodulatory activities of probiotics by stimulation of the gut-associated immunity (Brisbin et al. 2008; Harris et al. 2019). The intestinal mucosal innate immune system produces antimicrobial peptides like β-defensins, which can kill various intestinal pathogens, when microorganisms penetrate into the intestinal epithelium or mucus layer (Liévin-Le Moal and Servin 2006; Gadde et al.

2017) by disrupting cell membrane permeability leading to cell lysis (Brisbin et al. 2010). They also enhance antibody-mediated or cell-mediated immune responses (expressing different cytokine expressions in T cells; Brisbin et al. 2010). However, the effect of probiotics on immunity cannot be generalised because some of the studies have reported that probiotic supplementation did not influence immunity response (Mountzouris et al. 2010), probably due to the absence of immune-regulatory factors in the probiotic strains.

7.5 Probiotics to Improve Ruminal Functions

7.5.1 Effects on pH and Microorganisms in the Rumen

Rumen represents a large anaerobic fermentation vat harbouring diverse groups of microbial consortia, mainly bacteria, protozoa, and fungi, besides archaea and others (Krause et al. 2013). These microbial communities in the rumen carry out >0.8 of total digestion of complex feed carbohydrates (van Vuuren et al. 2012) into energy yielding VFA. As rumen microbial digestion is crucial for ruminant productivity, it is equally necessary to ensure all time healthy functioning of rumen. Nonetheless, recent genetic progress for high milk production demands a large quantity of cereal grains to feed cows that lead to either acidosis or sub-acute ruminal acidosis (SARA) due to lowering of rumen pH from 6.7 to 5.5 or further low by lactic acid (Calsamiglia et al. 2012). Furthermore, SARA presents far-reaching consequences by jeopardising not only ruminal microbial functions, but also causing milk fat depression, disruption of the ruminal epithelial barrier, laminitis, environmental mastitis, and reproduction leading to substantial financial losses in dairying (Abdela 2016; Aschenbach et al. 2019).

The positive role of yeast in improving ruminal pH and fermentation in ruminants is well established. Lactic acid producing probiotic species (e.g., *Lactobacillus, Enterococcus, Bifidobacterium,* and *Streptococcus*) promote the growth of lactic acid utilisers (*Megasphaera, Propionibacterium*, and *Selenomonas,* etc.), thereby accelerating lactate metabolism (McAllister et al. 2011), i.e., lactic acid is converted into gluconeogenic VFA propionate via acrylate and/or succinate pathway (Jeyanathan et al. 2014). Live yeast cells of *Saccharomyces cerevisiae* furnish certain essential micronutrient growth factors (B vitamins, peptides, and amino acids) to lactate utilisers and fibre digesters (Robinson and Erasmus 2009). In addition, yeast is known to moderate ruminal pH by outcompeting S. *bovis* for soluble sugar (glucose) utilisation that limits sugar availability to lactic acid producers (Vohra et al. 2016). Furthermore, yeasts help strengthen anaerobic environment by decreasing redox potential in the rumen through scavenging the traces of oxygen that enters into the rumen during feed ingestion and mastication (Chaucheyras-Durand et al. 2008). This provides a more favourable condition for strictly anaerobic cellulolytic ruminal bacteria such as *Fibrobacter*, *Ruminococcus*, and *Butyrivibrio* sp. (Vohra et al. 2016) to multiply and attach on forage particles

Table 7.6 Summary of meta-analysis evaluating the effect of addition of commercial probiotic yeast (*Saccharomyces cerevisiae*) products on ruminant performance

Animal	Type of yeast	Data set	Key conclusion	Reference
Dairy cattle	Viable yeast products and yeast culture	22 experiments	A modest benefit on output of milk (0.9 kg/d; 2.7%) and NE_L (5.3%)	Robinson and Erasmus (2009)
Dairy cattle	Viable yeast products	110 papers with 157 experiments and 376 treatments	Increase in DMI (0.44 g/kg BW), milk yield (1.2 g/kg BW) and milk fat (0.05%) along with positive rumen environment (↑ in pH by 0.03, ↓in lactic acid by 0.9 m*M* and ↑ in VFA by 2.17 m*M*) and ↑ in OM digestion by 0.8%	Desnoyers et al. (2009)
Dairy cattle	Live yeast	14 trials with 160 observations	No effect on DMI; 3% improvement in feed efficiency as well as yield of milk protein and fat increased	De Ondarza et al. (2010)
Dairy cattle	Yeast culture products	36 studies with 69 comparisons	Milk yield response of 0.98–1.37 kg/d (mean: 1.18 kg/d) across lactation with DMI of +0.62 kg/d in early and − 0.78 kg/d in other phases of lactation, respectively. Milk fat (0.06 kg/d) and protein yield (0.03 kg/d) were also increased	Poppy et al. (2012)
Feedlot (beef) cattle	Yeast fermentation products	18 experiments	Increase in DMI, final BW and ADG by 1%, 2.9 kg and 6.5%, respectively	Wagner et al. (2016)

ADG average daily gain, *BW* body weight, *DMI* dry matter intake, NE_L net energy for lactation, *OM* organic matter

(Roger et al. 1990). In a detailed meta-analysis involving viable yeast, Desnoyers et al. (2009) concluded that these products enhance ruminal pH by 0.03 units, decrease lactic acid by 0.9 mM, and increase VFA production by 2.17 mM in dairy cattle (Table 7.6). Furthermore, yeast can also stabilise ruminal pH by stimulating the growth of *Entodinomorph* protozoa, which facilitate engulfment of starch granules, thus minimising lactate production in addition to consumption of lactate and outcompeting *S. bovis* for glucose uptake by protozoa (Vohra et al. 2016). Also, the strain of probiotics can have a differential influence on rumen fermentation pattern in cattle (Chung et al. 2012). Indeed, under conditions of ruminal acidosis, it has been demonstrated that probiotic yeasts have promising role in minimising lactic acid accumulation, while buffering the excess acid by sodium bicarbonate (Aslan et al. 1995; Marden et al. 2008). Overall, it is clear that probiotics exhibit positive effects by improving ruminal health during dietary challenge of grain-induced pH fluctuation.

7.5.2 Methane Production

Ruminants emit methane (a potent greenhouse gas), which is produced as a part of normal fermentative feed digestion in the rumen representing a substantial loss of up to 15% of energy intake depending upon the diets. Mitigating a part of enteric methane is useful to ruminants as it conserves the energy that can be otherwise utilised for productive purposes, and thus supporting cleaner and environmental-friendly ruminant production. Therefore, several dietary strategies have been suggested to mitigate enteric methane production in ruminants (Patra 2016).

Probiotic yeast supplementation support a shift in hydrogen utilisation from methanogenesis to reductive acetogenesis (Chaucheyras-Durand and Durand 2010). In addition, growth of lactate utilisers (*Megasphaera elsdenii*) producing propionic acid leads to a decrease in molar proportion of methane in rumen. Further, a reduction in methane to the extent of 6–20% in vitro and 3–7% in vivo has been noticed across various studies involving *S. cerevisiae* (Jeyanathan et al. 2014). A recent review by Doyle et al. (2019) concluded from the small number of in vitro studies that lactic acid bacteria can decrease in vitro methane production effectively depending upon the strains, but the efficacy of lactict acid bacteria to lower in vivo methane production is not convincing at the present time due to the lack of robust animal studies. Probiotics may reduce methane production with the following mechanisms: (1) alter ruminal fermentation such as stimulation of lactate utilisers leading to increased propionate production with a resultant decrease in methane production, (2) directly inhibit ruminal methanogens by bacteriocins, and (3) inhibit specific ruminal bacteria that produce hydrogen or methyl-containing compounds required for methanogenesis (Doyle et al. 2019). Certain types of bacteriocins produced by probiotic bacteria have shown to have direct inhibitory effects on methanogens in vitro (Jeyanathan et al. 2014). Hence, there is a possibility to explore probiotics as an ecological tool to abate methane emissions in ruminants.

7.6 Effect on Performance Indicators

Probiotic supplementation in ruminants was initially suggested for stressed or diseased animals when dysbiosis of the gut microbiota occurs (NRC 2001). However, more studies have proved it useful to supplement on regular basis impacting nutrient intake, digestion, production performance, and economic efficiency.

7.6.1 Non-ruminants

In monogastric animals, as feeding accounts for about 70–75% of the total costs, optimisation of diet is necessary to provide all the essential nutrients for optimum health and performance. Intestinal microbiome plays an important role in gut health and homeostasis, host metabolism, interact with gut-associated lymphoid (immune) system, pathogen inhibition, digestion of complex substrates including non-starch polysaccharides and influence overall production traits (Fuller 2012; Pan and Yu 2014; Borda-Molina et al. 2018).

Probiotics aid in improving the performance traits such as gain in body weight for growing animals (Kocher 2006; Veizaj-Delia et al. 2010), broiler chickens (Marshall and Levy 2011), pigs (Meng et al. 2010; Park et al. 2019), improving feed conversion efficiency for meat (Atela et al. 2019) and egg production (Peralta-Sánchez et al. 2019). The first few weeks of life after birth plays a major role in composition of gastrointestinal microbiota and other factors of young animals and birds' performance during subsequent stages of life. Probiotics help to stabilise the GI microbiome, thereby preventing the occurrence of many diseases like coccidiosis and diarrhoea, etc. In this regard, *B. subtilis* supplementation has been shown to improve broiler performance (Fritts et al. 2000), meat quality (Pelicano et al. 2003; Liu et al. 2012), as well as performance in birds affected with necrotic enteritis (Tactacan et al. 2013). *B. amyloliquefaciens* has been shown to improve feed conversion ratio (FCR; i.e., feed-to-gain ratio) in broiler chickens (Flores et al. 2019). Besides, dietary addition of probiotics improves production performance by stimulating the immune system, improving the antioxidant status, and synthesis of endogenous antimicrobial peptides in the intestine (La Ragione and Woodward 2003; Lee et al. 2011). Several studies demonstrated that supplementation of diets with different probiotics could improve the growth performance of chickens (Yang et al. 2012; Zhang et al. 2012) and pigs (Meng et al. 2010; Park et al. 2019). Supplementation of *Enterococcus faecium* (0.5% of the total diet) reduced the counts of *Salmonella* and also increased body weight gain and breast muscle yield in broilers (Gheisar et al. 2016).

Probiotics act by enhancing the activity of certain digestive enzymes like proteases, lipases, and amylases (Fuller 2001) and improving mucosa ultrastructure (Gheisar et al. 2016), thereby aiding in digestion and absorption of nutrients (Savaiano et al. 1984). However, some contrasting reports are available where dietary probiotics showed no effects on the growth performance of broiler chickens (Mountzouris et al. 2007; Lee et al. 2010) and pigs (Hu et al. 2019; Zhang et al. 2019). These discrepancies may be attributed to the probiotic strains, dosage, processing of feeds containing probiotics, age of animals, composition of diet, and hygiene status (Lee et al. 2010; Zhang et al. 2012).

The effect of certain probiotics on improvement of FCR can be better explained by the study of Singh et al. (2014), which reported that the metagenomic analysis of faecal samples of high FCR animals had higher abundances of the genera of *Lactobacillus, Bacteroides, Acinetobacter, Streptococcus,* and *Clostridium*, whereas low FCR animals had more abundances of *Escherichia, Salmonella*, and *Shigella*. It suggests that the establishment of a good gut microbiome is needed for overall performance improvement in the animals (Stanley et al. 2012; Markowiak and Śliżewska 2018).

7.6.2 Ruminants

7.6.2.1 Feed Intake and Digestion

One of the prominent effects of probiotics is their ability to enhance the voluntary feed dry matter intake (DMI). The optimum DMI and digestibility are vital for supplying nutrients essential for animal growth, milk and meat production. Considerably less research has been performed on bacterial probiotics than yeast in this aspect. When a combination of *Enterococcus* and yeast was fed, cows had higher DMI (Nocek and Kautz 2006), whereas, no effect on DMI was noticed by others for bacterial (Ghorbani et al. 2002; Raeth-Knight et al. 2007) and yeast (Kamalamma et al. 1996; Keyser et al. 2007) probiotics. It appears that basal diet, species, and strain of probiotics influence the effect of probiotics on DMI. However, meta-analysis of studies considering various yeast products concluded a mean daily increase in DMI response of 0.44 kg (Desnoyers et al. 2009) and 0.62 kg (Poppy et al. 2012) in dairy cows, and 1% increase in feedlot cattle (Wagner et al. 2016).

Improvement in digestibility of complex forage carbohydrates such as neutral detergent fibre (NDF) confers practical benefits because 0.17 kg DMI and 0.25 kg 4% fat-corrected milk production are increased per unit increase of NDF digestibility (Oba and Allen 1999). It is proposed that a higher fibre digestion with probiotics is a consequence of microbial population shift towards cellulolysis, as described in the previous section. In this direction, improvement in fibre digestion upon probiotic yeast supplementation has been demonstrated (Guedes et al. 2008; Tang et al. 2008; Pal et al. 2010), while few studies reported a better protein digestion as well (Desnoyers et al. 2009; Pal et al. 2010; Bitencourt et al. 2011). Fungal culture (*Orpinomyces* sp. and *Piromyces* sp.) administration has shown to improve DM digestion in growing buffaloes (Tripathi et al. 2007). Nonetheless, few researchers failed to register any changes with probiotic yeast supplementation on nutrient digestibility (Yoon and Stern 1995; Kamalamma et al. 1996). Therefore, the effect of probiotics on digestibility has been inconsistent across multiple studies, which is influenced by different factors such as dose, diet, species, strains, and physiological status of animals (Patra 2012).

7.6.2.2 Milk Yield and Composition

Literature reveals mixed effects of bacterial and fungal probiotics on milk production performance and efficiency. In this regard, Moallem et al. (2009) and Maamouri et al. (2014) found an increase in milk production by 4 and 8%, respectively, with higher fat and protein content when dairy cows received *S. cerevisiae* in diets. Similarly, Lehloenya et al. (2008a, b) also noted better milk production performance in Holstein cows on feeding a combination of yeast culture and propionibacteria. Also, dairy cows produced more milk components on receiving corn grains treated with *Aspergillus oryzae* (Yu et al. 1997). In addition, Ayad et al. (2013) observed greater lactation persistency with yeast supplementation. Beneficial response seems less evident in low producing crossbred cows to supplemental yeast culture (Kamalamma et al. 1996). Nevertheless, meta-analysis studies on different probiotic yeast products revealed positive effects on milk production and composition in dairy cows (Robinson and Erasmus 2009; Desnoyers et al. 2009; Poppy et al. 2012). Yeast could also improve feed efficiency by 3% in dairy cows (De Ondarza et al. 2010). Overall, it is reasonable that the improved lactation responses could be a consequence of increased DMI and digestibility, thereby ensuring increased availability of nutrient precursors for the synthesis of milk and milk components.

7.7 Faecal Shedding of Gut Pathogens

Faecal shedding of potential zoonotic pathogens like *E. coli, Clostridia,* and *Salmonella* represents a serious food-borne public health risk associated with cattle production (Chaucheyras-Durand and Durand 2010). Probiotic supplementation can confer benefits in reducing the shedding of these gut pathogenic microbes into environment and contamination to meat and milk. In this direction, the results of a systematic review and meta-analysis found that probiotic combination of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* was more efficacious in decreasing the prevalence of faecal *E. coli* O157 in feedlot cattle (Wisener et al. 2014). Similarly, *B. subtilis* and *B. licheniformis* addition to the diet reduced *E. coli* shedding in laying hens (Upadhaya et al. 2019). The mechanisms underlying the elimination of pathogens from intestinal tract have been attributed to competitive exclusion, production of bacteriocins, and stabilising tight junction proteins of intestinal epithelium (McAllister et al. 2011). In pigs, adhesion of pathogens (e.g., *Salmonella*, *Clostridium*, and *E. coli* strains) to intestinal mucosa has been reported to reduce by *Bifidobacterium lactis* Bb12 and/or *Lactobacillus rhamnosus* LGG (Collado et al. 2007). Moreover, probiotics can inhibit pathogens to invade the gut epithelial cells (Oelschlaeger 2010). Therefore, probiotics offer an opportunity to minimise digestive carriage of human pathogens from animals, ensuring safe farm to fork operation.

7.8 Probiotics in Pre-ruminant Nutrition

Rearing young ruminants such as calves constitute an important activity in dairying as these represent future stocks of heifers or cows, and it is recognised that calf rearing practices impact lifetime milk productivity of cows (Heinrichs and Heinrichs 2011). Young ruminants do not have fully developed rumen for microbial fermentation.

Probiotic bacteria and yeast exhibit differentiated actions in calves/pre-ruminants. Probiotic formulations containing mostly lactic acid bacteria have been studied in case of pre-ruminants. The lactic acid bacteria and spores of *Bacillus* sp. generally target small intestine, where they act primarily by limiting the colonisation of gut pathogens (*E. coli* and *Salmonella*) through competitive exclusion and improving immunity, thereby preventing diarrhoea (calf scour), faecal shedding of coliform bacteria, improved feed efficiency (i.e., gain-to-feed ratio) and growth, and reduced morbidity rate in calves (Krehbiel et al. 2003; McAllister et al. 2011). Further, a meta-analysis study has shown that lactic acid bacteria supplementation in calves can decrease diarrhoeal incidences (Signorini et al. 2012), improve body weight gain and feed efficiency (Frizzo et al. 2011).

Yeast-based probiotics exert their action by enabling early establishment of ruminal microbial communities like cellulolytic bacteria and ciliate protozoa (Chaucheyras-Durand et al. 2008), which results in early initiation of digestion of complex forage carbohydrates. Consequent to rapid development of functional rumen mediated through papillae length, width, and rumen wall thickness, an improvement in DMI, feed efficiency, and growth rate (Tripathi et al. 2007) as well as improved carcass (meat) quality attributes (Wagner et al. 2016) have been observed. It is noteworthy here that the beneficial effects are more pronounced in calves subjected to poor and stressful raising than under ideal managemental conditions (Alugongo et al. 2017).

7.9 Effect on Oxidative Stress

Body normally maintains an equilibrium between oxidants (produced during normal metabolic activities such as reactive oxygen and nitrogen species) and anti-oxidants (e.g., endogenous superoxide dismutase, peroxidase, catalase, and reduced glutathione). Increased reactive oxygen species are detrimental to health of animals. Dietary probiotics have been shown to nullify the adverse influence of oxidative stress up to a large extent by promoting antioxidant enzyme activities (Tabidi et al. 2013; Singh et al. 2014). This helps in preventing accumulation of excess reactive oxygen species and improving the health and production parameters (Pridmore et al. 2008; Wen et al. 2011) as found with probiotics like *B. subtilis* (Lee et al. 2011; Bai et al. 2017), and/or its combination with *Saccharomyces boulardii* (Rajput et al. 2013). The reduction in oxidative stress may be attributed to the stabilisation of intestinal microbiota and their metabolic activities (Pridmore et al. 2008).

7.10 Effect on Meat Quality

Supplementation of probiotics in diets has shown to improve meat quality parameters namely pH, tenderness, and colour in chickens (Pelicano et al. 2003; Kim and Yoon 2008; Zhou et al. 2010; Bai et al. 2017; Atela et al. 2019). However, the effect is not generalised as some strains of probiotics did not offer any encouraging benefits on meat quality (Kim and Yoon 2008; Zhang et al. 2012). This may be attributed to the species difference in probiotics and gut microbiome composition (Bai et al. 2017), which affect the pH of GI tract, influence growth of animals, protein and fatty acid composition of meat (Popova 2017).

7.11 Other Beneficial Effects of Probiotics

Apart from the above discussed advantages of probiotics, there are some less studied/minor effects of probiotics as listed in Table 7.7. These include improvement in nutraceutical value of milk by increased secretion of health-promoting conjugated linoleic acid, preventing mastitis in dairy cows, acting as silage inoculants, minimising mycotoxin adversity and alleviating mimosine toxicity in leucaena-fed animals (Hammond 1995).

Table 7.7 Other beneficial effects of probiotics in ruminants

Feature	Probiotic organism	Description	Reference
Conjugated linoleic acid production	*Butyrivibrio* sp.	Potential to improve nutraceutical/functional value of milk	Jaglan et al. (2019)
Minimising incidence of mastitis	Lactic acid bacteria	Still under experiment and sound scientific validation has not been established	Rainard and Foucras (2018)
As a silage additive/inoculant	Lactic acid bacteria	Improvement in nutritive and quality parameters of silage and in turn impacting positive animal performance	Bernardi et al. (2019)
Ameliorating mycotoxin menace	*S. cerevisiae*	Binding feed mycotoxins and reducing milk aflatoxin (AFM_1) excretion	Jiang et al. (2018)
Preventing mimosine toxicity	*Synergistes jonesii*	Degradation of toxic metabolites of *Leucaena,* thereby preventing mimosine toxicity	Hammond (1995)

7.12 Conclusion and Practical Considerations in Probiotic Supplementation

Although there are a plethora of additives available to boost productivity and efficiency, the supplementation of probiotics have become a preferred choice in animal food production systems due to their distinct benefits. In ruminants, enormous body of literature explored the use of probiotics, but the primary target has been the rumen and improving fermentation efficiency, thereby impacting production performance. Out of the several species of probiotic organisms investigated, the most studied category is yeast followed by bacterial probiotics in ruminant nutrition, where they may stabilise the ruminal pH and increase fibre digestion. In non-ruminants (particularly, poultry and pig), bacterial probiotics dominate over yeast in influencing performance attributes. Probiotics, either singly or in combination, belonging to genus *Bacillus, Lactobacillus, Streptococcus, Pediococcus, Enterococcus, Aspergillus*, *Saccharomyces*, and *Candida* are commonly used. When they are added to the diet in required quantities, they help in sustaining and improving the beneficial gut microbiota, enhancing host immunity, and inhibiting the pathogens in gut. These beneficial effects are not always obtained, which depends upon the species and strains of probiotics used in the diets.

In modern livestock production systems, feeding complete feeds to monogastric animals and concentrate to ruminants in steam-pelleted form is gaining popularity worldwide. When such feeds are used as vehicle for supplements including probiotics, heat or pellet stability becomes crucial because most of the yeast as well as lactic acid bacteria may be killed or deactivated during pelleting. Therefore, encapsulated and more temperature withstanding forms of commercial probiotics are the needs of today's feed industry, while on-top supplementation at farm level can go without requirement for any heat stability. In either of these cases, it is important to ensure the correct daily dose of particular probiotic required per animal for realising the potential benefits.

References

Abdela N (2016) Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: a review of past and recent research at global prospective. Achiev Life Sci 10:187–196

Abe F, Ishibashi N, Shimamura S (1995) Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. J Dairy Sci 78:2838–2846

Adeyemi JA, Harmon DL, Compart DMP, Ogunade IM (2019) Effects of a blend of *Saccharomyces cerevisiae*-based direct-fed microbial and fermentation products in the diet of newly weaned beef steers: growth performance, whole-blood immune gene expression, serum biochemistry, and plasma metabolome. J Anim Sci 97:4657–4667

Ahmed SA, Sirohi SK, Puniya AK, Sheel R (2013) Influence of addition of propionic acid producing bacteria on dry matter digestibility and rumen fermentation profiles in wheat straw containing total mixed ration in vitro. Indian J Anim Nutr 30:272–280

Aikman PC, Henning PH, Horn CH, Humphries DJ (2009) Use of *Megasphaera elsdenii* NCIMB41125 as a probiotic for early-lactation dairy cows: effects on rumen pH and fermentation patterns. J Dairy Sci 92:288–289

Aikman PC, Henning PH, Humphries DJ, Horn CH (2011) Rumen pH and fermentation characteristics in dairy cows supplemented with *Megasphaera elsdenii* NCIMB 41125 in early lactation. J Dairy Sci 94:2840–2849

Alugongo GM, Xiao J, Wu Z, Li S, Wang Y, Cao Z (2017) Utilization of yeast of *Saccharomyces cerevisiae* origin in artificially raised calves. J Anim Sci Biotechnol 8:34

Anand Laxmi A, Sehgal JP, Bharath Kumar BS, Gorakhnath (2016) Supplementation of fermented yeast culture augments the growth and reduces the age at puberty in male Murrah buffalo calves. Buffalo Bull 35:179–189

Aschenbach JR, Zebeli Q, Patra AK, Greco G, Amasheh S, Penner GB (2019) Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. J Dairy Sci 102:1866–1882

Aslan V, Thamsborg SM, Jørgensen RJ, Basse A (1995) Induced acute ruminal acidosis in goats treated with yeast (*Saccharomyces cerevisiae*) and bicarbonate. Acta Vet Scand 36:65–77

Atela JA, Mlambo V, Mnisi CM (2019) A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chickens. Anim Nutr 5:179–184

Ayad MA, Benallou B, Saim MS, Samadi MA, Meziane T (2013) Impact of feeding yeast culture on milk yield, milk components, and blood components in Algerian dairy herds. J Vet Sci Technol 4:135–140

Azzaz HH, El-Sherbiny M, Murad HA, Ebeid HM (2019) *Propionibacteria* in ruminant's diets: an overview. J Appl Sci 19:166–172

Bach A, Guasch I, Elcoso G, Chaucheyras-Durand F, Castex M, Fàbregas F, Garcia-Fruitos E, Aris A (2018) Changes in gene expression in the rumen and colon epithelia during the dry period through lactation of dairy cows and effects of live yeast supplementation. J Dairy Sci 101:2631–2640

Bach A, López-García A, González-Recio O, Elcoso G, Fàbregas F, Chaucheyras-Durand F, Castex M (2019) Changes in the rumen and colon microbiota and effects of live yeast dietary supplementation during the transition from the dry period to lactation of dairy cows. J Dairy Sci 102:6180–6098

Bai K, Huang Q, Zhang J, He J, Zhang L, Wang T (2017) Supplemental effects of probiotic *Bacillus subtilis* fmbJ on growth performance, antioxidant capacity, and meat quality of broiler chickens. Poult Sci 96:74–82

Bai K, Feng C, Jiang L, Zhang L, Zhang J, Zhang L, Wang T (2018) Dietary effects of *Bacillus subtilis* fmbj on growth performance, small intestinal morphology, and its antioxidant capacity of broilers. Poult Sci 97:2312–2321

Bernardi A, Härter CJ, Silva AW, Reis RA, Rabelo CH (2019) A meta-analysis examining lactic acid bacteria inoculants for maize silage: Effects on fermentation, aerobic stability, nutritive value and livestock production. Grass Forage Sci 74:596–612

Bertin Y, Habouzit C, Dunière L, Laurier M, Durand A, Duchez D, Segura A, Thévenot-Sergentet D, Baruzzi F, Chaucheyras-Durand F, Forano E. (2017) *Lactobacillus reuteri* suppresses *E. coli* O157: H7 in bovine ruminal fluid: Toward a pre-slaughter strategy to improve food safety?. PLoS One 12:e0187229

Bhogoju S, Nahashon S, Wang X, Darris C, Kilonzo-Nthenge A (2018) A comparative analysis of microbial profile of Guinea fowl and chicken using metagenomic approach. PLoS One 13: e0191029

Birkelo CP (2003) Pharmaceuticals, direct-fed microbials, and enzymes for enhancing growth and feed efficiency of beef. Vet Clin Food Anim 19:599–624

Bitencourt LL, Silva JR, Oliveira BM, Dias Júnior GS, Lopes F, Siécola Júnior S, Zacaroni OD, Pereira MN (2011) Diet digestibility and performance of dairy cows supplemented with live yeast. Sci Agric 68:301–307

Borda-Molina D, Seifert J, Camarinha-Silva A (2018) Current perspectives of the chicken gastrointestinal tract and its microbiome. Comput Struct Biotechnol J 16:131–139
Brisbin JT, Gong J, Sharif S (2008) Interactions between commensal bacteria and the gut-associated immune system of the chicken. Anim Health Res Rev 9:101–110
Brisbin JT, Gong J, Parvizi P, Sharif S (2010) Effects of lactobacilli on cytokine expression by chicken spleen and cecal tonsil cells. Clin Vaccine Immunol 17:1337–1343
Callaway TR, Edrington TS, Anderson RC, Harvey RB, Genovese KJ, Kennedy CN, Venn DW, Nisbet DJ (2008) Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim Health Res Rev 9:217–225
Calsamiglia S, Blanch M, Ferret A, Moya D (2012) Is subacute ruminal acidosis a pH related problem? Causes and tools for its control. Anim Feed Sci Technol 172:42–50
Chaucheyras-Durand F, Durand H (2010) Probiotics in animal nutrition and health. Benef Microbes 1:3–9
Chaucheyras-Durand F, Walker ND, Bach A (2008) Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. Anim Feed Sci Technol 145:5–26
Chen X, Xu J, Shuai J, Chen J, Zhang Z, Fang W (2007) The S-layer proteins of *Lactobacillus crispatus* strain ZJ001 is responsible for competitive exclusion against *Escherichia coli* O157: H7 and *Salmonella typhimurium*. Int J Food Microbiol 115:307–312
Chichlowski M, Croom J, McBride BW, Havenstein GB, Koci MD (2007) Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: a brief review of current knowledge. Int J Poult Sci 6:694–704
Chiquette J, Allison MJ, Rasmussen M (2012) Use of *Prevotella bryantii* 25A and a commercial probiotic during subacute acidosis challenge in mid lactation dairy cows. J Dairy Sci 95:5985–5995
Chiquette J, Lagrost J, Girard CL, Talbot G, Li S, Plaizier JC, Hindrichsen IK (2015) Efficacy of the direct-fed microbial *Enterococcus faecium* alone or in combination with *Saccharomyces cerevisiae* or *Lactococcus lactis* during induced subacute ruminal acidosis. J Dairy Sci 98:190–203
Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, Reading NC, Villablanca EJ, Wang S, Mora JR, Umesaki Y, Mathis D, Benoist C, Relman DA, Kasper DL (2012) Gut immune maturation depends on colonization with a host-specific microbiota. Cell 149:1578–1593
Clavijo V, Flórez MJ (2017) The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: a review. Poult Sci 97:1006–1021
Collado MC, Grześkowiak Ł, Salminen S (2007) Probiotic strains and their combination inhibit *in vitro* adhesion of pathogens to pig intestinal mucosa. Curr Microbiol 55:260–265
Corr SC, Hill C, Gahan CGM (2009) Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. Adv Food Nutr Res 56:1–15
Cotter PD, Hill C, Ross RP (2005) Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 3:777–788
Dahiya J, Wilkie D, Van Kessel A, Drew M (2006) Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim Feed Sci Technol 129:60–88
De Angelis M, Siragusa S, Berloco M, Caputo L, Settanni L, Alfonsi G, Amerio M, Grandi A, Ragni A, Gobbetti M (2006) Selection of potential probiotic lactobacilli from pig feces to be used as additives in pelleted feeding. Res Microbiol 157:792–801
De Ondarza MB, Sniffen CJ, Dussert L, Chevaux E, Sullivan J, Walker N (2010) Case study: multiple-study analysis of the effect of live yeast on milk yield, milk component content and yield, and feed efficiency. Prof Anim Sci 26:661–666
Desnoyers M, Giger-Reverdin S, Bertin G, Duvaux-Ponter C, Sauvant D (2009) Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J Dairy Sci 92:1620–1632
Doyle N, Mbandlwa P, Kelly WJ, Attwood G, Li Y et al (2019) Use of lactic acid bacteria to reduce methane production in ruminants, a critical review. Front Microbiol 10:2207

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (2016) Safety and efficacy of RONOZYME® WX (endo-1,4-beta-xylanase) as a feed additive for chickens and turkeys for fattening, minor poultry species for fattening, weaned piglets and pigs for fattening. EFSA J 14:e04564

Elghandour MM, Tan ZL, Abu Hafsa SH, Adegbeye MJ, Greiner R, Ugbogu EA, Cedillo Monroy J, Salem AZM (2020) *Saccharomyces cerevisiae* as a probiotic feed additive to non and pseudo-ruminant feeding: a review. J Appl Microbiol 128:658–674

Emmanuel DG, Jafari A, Beauchemin KA, Leedle JA, Ametaj BN (2007) Feeding live cultures of *Enterococcus faecium* and *Saccharomyces cerevisiae* induces an inflammatory response in feedlot steers. J Anim Sci 85:233–239

FAO (2002) Guidelines for the evaluation of probiotics in food. In: Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. 30 April–1 May 2002. Food and Agriculture Organization, London

Fernández S, Fraga M, Silveyra E, Trombert AN, Rabaza A, Pla M, Zunino P (2018) Probiotic properties of native *Lactobacillus* spp. strains for dairy calves. Benef Microbes 9:613–624

Feye KM, Carroll JP, Anderson KL, Whittaker JH, Schmidt-McCormack GR, McIntyre DR, Pavlidis HO, Carlson SA (2019) *Saccharomyces cerevisiae* fermentation products that mitigate food borne *Salmonella* in cattle and poultry. Front Vet Sci 6:107

Finck DN, Ribeiro FR, Burdick NC, Parr SL, Carroll JA, Young TR, Bernhard BC, Corley JR, Estefan AG, Rathmann RJ, Johnson BJ (2014) Yeast supplementation alters the performance and health status of receiving cattle. Prof Anim Sci 30:333–341

Flores CA, Duong T, Askelson TE, Dersjant-Li Y, Gibbs K, Awati A, Lee JT (2019) Effects of direct fed-microorganisms and enzyme blend co-administration on growth performance in broilers fed diets with or without antibiotics. J Appl Poult Res 28:1181–1188

Fraga M, Fernández S, Perelmuter K, Pomiés N, Cajarville C, Zunino P (2018) The use of *Prevotella bryantii* 3C5 for modulation of the ruminal environment in an ovine model. Braz J Microbiol 49:101–106

Fritts CA, Kersey JH, Motl MA, Kroger EC, Yan F, Si J, Jiang Q, Campos MM, Waldroup AL, Waldroup PW (2000) *Bacillus subtilis* C-3102 (Calsporin) improves live performance and microbiological status of broiler chickens. J Appl Poult Res 9:149–155

Frizzo LS, Soto LP, Zbrun MV, Signorini ML, Bertozzi E, Sequeira G et al (2011) Effect of lactic acid bacteria and lactose on growth performance and intestinal microbial balance of artificially reared calves. Livest Sci 140:246–252

Fuller R (2001) The chicken gut microflora and probiotic supplements. Poult Sci 38:189–196

Fuller R (2012) Probiotics: the scientific basis. Springer Science & Business Media, Dordrecht

Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT (2005) Synbiotic therapy (*Bifidobacterium longum/Synergy 1*) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut 54:242–249

Gadde U, Oh ST, Lee YS, Davis E, Zimmerman N, Rehberger T, Lillehoj HS (2017) The effects of direct-fed microbial supplementation, as an alternative to antibiotics, on growth performance, intestinal immune status, and epithelial barrier gene expression in broiler chickens. Probiotics Antimicrob Proteins 9:397–405

Gao J, Zhang HJ, Yu SH, Wu SG, Yoon I, Quigley J, Gao YP, Qi GH (2008) Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poult Sci 87:1377–1384

Geng CY, Meng QX, Ren LP, Zhou ZM, Zhang M, Yan CG (2016) Comparison of ruminal fermentation parameters, fatty acid composition and flavour of beef in finishing bulls fed active dry yeast (*Saccharomyces cerevisiae*) and yeast culture. Anim Prod Sci 58:841–847

Getachew T (2016) A review on effects of probiotic supplementation in poultry performance and cholesterol levels of egg and meat. J World Poult Res 6:31–36

Ghareeb K, Awad WA, Mohnl M, Porta R, Biarnés M, Böhm J, Schatzmayr G (2012) Evaluating the efficacy of an avian-specific probiotic to reduce the colonization of *Campylobacter jejuni* in broiler chickens. Poult Sci 91:1825–1832

Gheisar MM, Hosseindoust A, Kim IH (2016) Effects of dietary *Enterococcus faecium* on growth performance, carcass characteristics, faecal microbiota, and blood profile in broilers. Vet Med (Praha) 61:28–34

Ghorbani GR, Morgavi DP, Beauchemin KA, Leedle JA (2002) Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. J Anim Sci 80:1977–1985

Guedes CM, Goncalves D, Rodrigues MA, Dias-da-Silva A (2008) Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. Anim Feed Sci Technol 145:27–40

Haghighi HR, Gong J, Gyles CL, Hayes MA, Sanei B, Parvizi P, Gisavi H, Chambers JR, Sharif S (2005) Modulation of antibody-mediated immune response by probiotics in chickens. Clin Diagn Lab Immunol 12:1387–1392

Hammond AC (1995) *Leucaena* toxicosis and its control in ruminants. J Anim Sci 73:1487–1492

Harris EV, de Roode JC, Gerardo NM (2019) Diet–microbiome–disease: investigating diet's influence on infectious disease resistance through alteration of the gut microbiome. PLoS Pathog 15:e1007891

Heinrichs AJ, Heinrichs BS (2011) A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. J Dairy Sci 94:336–341

Henning PH, Horn CH, Leeuw KJ, Meissner HH, Hagg FM (2010) Effect of ruminal administration of the lactate-utilizing strain *Megasphaera elsdenii* (me) NCIMB 41125 on abrupt or gradual transition from forage to concentrate diets. Anim Feed Sci Technol 157:20–29

Hodgkinson AJ, Cakebread J, Callaghan M, Harris P, Brunt R, Anderson RC, Armstrong KM, Haigh B (2017) Comparative innate immune interactions of human and bovine secretory IgA with pathogenic and non-pathogenic bacteria. Dev Comp Immunol 68:21–25

Hu C, Xing W, Liu X, Zhang X, Li K, Liu J, Deng B, Deng J, Li Y, Tan C (2019) Effects of dietary supplementation of probiotic *Enterococcus faecium* on growth performance and gut microbiota in weaned piglets. AMB Express 9:33

Huyghebaert G, Ducatelle R, Van Immerseel F (2011) An update on alternatives to antimicrobial growth promoters for broilers. Vet J 187:182–188

Jaglan N, Kumar S, Choudhury PK, Tyagi B, Tyagi AK (2019) Isolation, characterization and conjugated linoleic acid production potential of bifidobacterial isolates from ruminal fluid samples of Murrah buffaloes. Anaerobe 56:40–45

Jayaraman S, Das PP, Saini PC, Roy B, Chatterjee PN (2017) Use of *Bacillus subtilis* PB6 as a potential antibiotic growth promoter replacement in improving performance of broiler birds. Poult Sci 96:2614–2622

Jeyanathan J, Martin C, Morgavi DP (2014) The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. Animal 8:250–261

Jia P, Cui K, Ma T, Wan F, Wang W, Yang D, Wang Y, Guo B, Zhao L, Diao Q (2018) Influence of dietary supplementation with *Bacillus licheniformis* and *Saccharomyces cerevisiae* as alternatives to monensin on growth performance, antioxidant, immunity, ruminal fermentation and microbial diversity of fattening lambs. Sci Rep 8:16712

Jiang Y, Ogunade IM, Kim DH, Li X, Pech-Cervantes A, Arriola KG, Oliveira AS, Driver JP, Ferraretto LF, Staples CR, Vyas D, Adesogan AT (2018) Effect of adding clay with or without a *Saccharomyces cerevisiae* fermentation product on the health and performance of lactating dairy cows challenged with dietary aflatoxin B1. J Dairy Sci 101:3008–3020

Jung SJ, Houde R, Baurhoo B, Zhao X, Lee BH (2008) Effects of galacto-oligosaccharides and a *Bifidobacteria lactis*-based probiotic strain on the growth performance and fecal microflora of broiler chickens. Poult Sci 87:1694–699

Kamalamma, Krishnamoorthy U, Krishnappa P (1996) Effect of feeding yeast culture (Yea-sacc[1026]) on rumen fermentation *in vitro* and production performance in crossbred dairy cows. Anim Feed Sci Technol 57:247–256

Karaoglu M, Durdag H (2005) The influence of dietary probiotic (*Saccharomyes cerevisiae*) supplementation and different slaughter age on the performance, slaughter and carcass properties of broilers. Int J Poult Sci 4:309–316

Kerry RG, Patra JK, Gouda S, Park Y, Shin HS, Das G (2018) Benefaction of probiotics for human health: a review. J Food Drug Anal 26:927–939

Keyser SA, McMeniman JP, Smith DR, MacDonald JC, Galyean ML (2007) Effects of *Saccharomyces cerevisiae* subspecies *boulardii* CNCM I-1079 on feed intake by healthy beef cattle treated with florfenicol and on health and performance of newly received beef heifers. J Anim Sci 85:1264–1273

Kim YJ, Yoon YB (2008) Effect of the feeding probiotics, illite, activated carbon, and hardwood vinegar on the meat quality and shelf-life in chicken thigh. Food Sci Anim Resour 28:480–485

Knap I, Kehlet AB, Bennedsen M, Mathis GF, Hofacre CL, Lumpkins BS, Jensen MM, Raun M, Lay A (2011) *Bacillus subtilis* (DSM17299) significantly reduces Salmonella in broilers. Poult Sci 90:1690–1694

Kocher A (2006) Interfacing gut health and nutrition: the use of dietary pre and probiotics to maximize growth performance in pigs and poultry. In: Barug D, de Jong J, Kies AK, Verstegen MWA (eds) Antimicrobial growth promoters. Where do we go from here? Wageningen Academic Publishers, Wageningen, p 289

Koenen ME, Kramer J, Hulst RVD, Heres L (2004) Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. Br Poult Sci 45:355–366

Krause DO, Nagaraja TG, Wright AD, Callaway TR (2013) Rumen microbiology: leading the way in microbial ecology. J Anim Sci 91:331–341

Krehbiel CR, Rust SR, Zhang G, Gilliland SE (2003) Bacterial direct-fed microbials in ruminant diets: performance response and mode of action. J Anim Sci 81(Suppl 2):120–132

Kumar B, Sirohi SK (2013) Effect of isolates of fibre degrading bacteria on body weight changes, milk production and its composition, nutrient intake and nutrient utilisation in lactating Murrah buffaloes. Afr J Biotechnol 12:3302–3308

La Ragione RM, Woodward MJ (2003) Competitive exclusion by Bacillus subtilis spores of *Salmonella enterica* serotype enteritidis and *Clostridium perfringens* in young chickens. Vet Microbiol 94:245–256

Lee K, Lee SK, Lee BD (2006) *Aspergillus oryzae* as probiotic in poultry-A review. Int J Poult Sci 5:1–3

Lee K, Lillehoj HS, Siragusa GR (2010) Direct-fed microbials and their impact on the intestinal microflora and immune system of chickens. J Poult Sci 47:106–114

Lee KW, Li G, Lillehoj HS, Lee SH, Jang SI, Babu US, Lillehoj EP, Neumann AP, Siragusa GR (2011) *Bacillus subtilis*-based direct-fed microbials augment macrophage function in broiler chickens. Res Vet Sci 91:e87–e91

Leeuw KJ, Siebrits F, Henning PH, Meissner HH (2009) Effect of *Megasphaera elsdenii* NCIMB 41125 drenching on health and performance of steers fed high and low roughage diets in the feedlot. South Afr J Anim Sci 39:337–348

Lei K, Li YL, Yu DY, Rajput IR, Li WF (2013) Influence of dietary inclusion of *Bacillus licheniformis* on laying performance, egg quality, antioxidant enzyme activities, and intestinal barrier function of laying hens. Poult Sci 92:2389–2395

Lehloenya KV, Krehbiel CR, Mertz KJ, Rehberger TG, Spicer LJ (2008a) Effects of propionibacteria and yeast culture fed to steers on nutrient intake and site and extent of digestion. J Dairy Sci 91:653–662

Lehloenya KV, Stein DR, Allen DT, Selk GE, Jones DA, Aleman MM, Rehberger TG, Mertz KJ, Spicer LJ (2008b) Effects of feeding yeast and *Propionibacteria* to dairy cows on milk yield and components, and reproduction. J Anim Physiol Anim Nutr 92:190–202

Li Y, Zhang H, Su W, Ying Z, Chen Y, Zhang L, Lu Z, Wang T (2018) Effects of dietary *Bacillus amyloliquefaciens* supplementation on growth performance, intestinal morphology, inflammatory response, and microbiota of intra-uterine growth retarded weanling piglets. J Anim Sci Biotechnol 9:22

Liévin-Le Moal V, Servin AL (2006) The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clin Microbiol Rev 19:315–337
Lillehoj H, Liu Y, Calsamiglia S, Fernandez-Miyakawa ME, Chi F, Cravens RL, Oh S, Gay CG (2018) Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet Res 49:76
Lilly DM, Stillwell RH (1965) Probiotics: growth-promoting factors produced by microorganisms. Science 147:747–748
Liu X, Yan H, Lv L, Xu Q, Yin C, Zhang K, Hu J (2012) Growth performance and meat quality of broiler chickens supplemented with *Bacillus licheniformis* in drinking water. Asian-Australas J Anim Sci 25:682–689
Ma T, Suzuki Y (2018) Dissect the mode of action of probiotics in affecting host-microbial interactions and immunity in food producing animals. Vet Immunol Immunopathol 205:35–48
Maamouri O, Selmi H, M'Hamd N (2014) Effects of yeast (*Saccharomyces cerevisiae*) feed supplement on milk production and its composition in Tunisian Holstein Friesian cows. Sci Agric Bohem 45:170–174
Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA (1999) Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. Am J Phys 276:G941–G950
Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA (2003) Extracellular MUC3 mucin secretion follows adherence of lactobacillus strains to intestinal epithelial cells *in vitro*. Gut 52:827–833
Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C (2001) Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 121:580–591
Marden JP, Julien C, Monteils V, Auclair E, Moncoulon R, Bayourthe C (2008) How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? J Dairy Sci 91:3528–3535
Markowiak P, Śliżewska K (2018) The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens 10:21
Marshall M, Levy SB (2011) Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24:718–733
Mattar A, Teitelbaum DH, Drongowski R, Yongyi F, Harmon C, Coran A (2002) Probiotics up-regulate MUC-2 mucin gene expression in a Caco-2 cell-culture model. Pediatr Surg Int 18:586–590
McAllister TA, Beauchemin KA, Alazzeh AY, Baah J, Teather RM, Stanford K (2011) The use of direct fed microbials to mitigate pathogens and enhance production in cattle. Can J Anim Sci 91:193–211
Medellin-Peña MJ, Wang H, Johnson R, Anand S, Griffiths MW (2007) Probiotics affect virulence-related gene expression in *Escherichia coli* O157: H7. Appl Environ Microbiol 73:4259–4267
Meng QW, Yan L, Ao X, Zhou TX, Wang JP, Lee JH, Kim IH (2010) Influence of probiotics in different energy and nutrient density diets on growth performance, nutrient digestibility, meat quality, and blood characteristics in growing-finishing pigs. J Anim Sci 88:3320–3326
Metchnikoff E (1907) The prolongation of life. Optimistic studies. William Heinemann, London
Mingmongkolchai S, Panbangred W (2018) *Bacillus* probiotics: an alternative to antibiotics for livestock production. J Appl Microbiol 124:1334–1346
Moallem U, Lehrer H, Livshitz L, Zachut M, Yakoby S (2009) The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J Dairy Sci 92:343–351
Molnár AK, Podmaniczky B, Kürti P, Tenk I, Glávits R, Virág G, Szabó Z (2011) Effect of different concentrations of *Bacillus subtilis* on growth performance, carcase quality, gut microflora and immune response of broiler chickens. Br Poult Sci 52:658–665

Mountzouris KC, Tsitrsikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K (2007) Evaluation of the efficacy of a probiotic containing lactobacillus, bifidobacterium, enterococcus, and pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult Sci 86:309–317

Mountzouris KC, Tsitrsikos P, Palamidi I, Arvaniti A, Mohnl M, Schatzmayr G, Fegeros K (2010) Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poult Sci 89:58–67

Musa BB, Duan Y, Khawar H, Sun Q, Ren Z, Elsiddig Mohamed MA, Abbasi IH, Yang X (2019) *Bacillus subtilis* B21 and *Bacillus licheniformis* B26 improve intestinal health and performance of broiler chickens with *Clostridium perfringens*-induced necrotic enteritis. J Anim Physiol Anim Nutr 103:1039–1049

Mwenya B, Santoso B, Sar C, Gamo Y, Kobayashi I, Arai TJ (2004) Effects of including 1–4 galacto-oligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. Anim Feed Sci Technol 115:313–326

Nakphaichit M, Thanomwongwattana S, Phraephaisarn C, Sakamoto N, Keawsompong S, Nakayama J, Nitisinprasert S (2011) The effect of including *Lactobacillus reuteri* KUB-AC5 during post-hatch feeding on the growth and ileum microbiota of broiler chickens. Poult Sci 90:2753–2765

Naqid IA, Owen JP, Maddison BC, Gardner DS, Foster N, Tchórzewska MA, La Ragione RM, Gough KC (2015) Prebiotic and probiotic agents enhance antibody-based immune responses to *Salmonella typhimurium* infection in pigs. Anim Feed Sci Technol 201:57–65

Nava GM, Bielke LR, Callaway TR, Castaneda MP (2005) Probiotic alternatives to reduce gastrointestinal infections: the poultry experience. Anim Health Res Rev 6:105–118

Neal-McKinney JM, Lu X, Duong T, Larson CL, Call DR, Shah DH, Konkel ME (2012) Production of organic acids by probiotic lactobacilli can be used to reduce pathogen load in poultry. PLoS One 7:e43928

Neish AS (2009) Microbes in gastrointestinal health and disease. Gastroenterology 136:65–80

Nisbet D (2002) Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine. Antonie Van Leeuwenhoek 81:481–486

Nocek JE, Kautz WP (2006) Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre-and postpartum dairy cattle. J Dairy Sci 89:260–266

Nollet L, Mbanzamihigo L, Demeyer D, Verstraete W (1998) Effect of the addition of *Peptostreptococcus productus* ATCC 35244 on reductive acetogenesis in the ruminal ecosystem after inhibition of methanogenesis by cell-free supernatant of *Lactobacillus plantarum* 80. Anim Feed Sci Technol 71:47–64

NRC (2001) Nutrient Requirements of Dairy Cattle, 7th revised edn. National Academy Press, Washington, DC

Nurmi E, Nuotio L, Schncitz C (1992) The competitive exclusion concept: development and future. Int J Food Microbiol 15:237–240

Oba M, Allen MS (1999) Effects of brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. J Dairy Sci 82:135–142

Oelschlaeger TA (2010) Mechanisms of probiotic actions–a review. Int J Med Microbiol 300:57–62

Oetzel GR, Emery KM, Kautz WP, Nocek JE (2007) Direct-fed microbial supplementation and health and performance of pre-and postpartum dairy cattle: a field trial. J Dairy Sci 90:2058–2068

Ognik K, Cholewińska E, Krauze M, Abramowicz K, Matusevicius P (2019) The effect of a probiotic preparation containing *Enterococcus faecium* DSM 7134 for chickens on growth performance, immune status, and the histology and microbiological profile of the jejunum. Anim Prod Sci 59:101–108

O'Hara AM, O'Regan P, Fanning Á, O'Mahony C, MacSharry J, Lyons A, Bienenstock J, O'Mahony L, Shanahan F (2006) Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. Immunology 118:202–215

Ohya T, Akiba M, Ito H (2001) Use of a trial probiotic product in calves experimentally infected with *Escherichia coli* O157. Japan Agric Res Quart 35:189–194
Pakbaten B, Heravi RM, Kermanshahi H, Sekhavati MH, Javadmanesh A, Ziarat MM (2019) Production of phytase enzyme by a bioengineered probiotic for degrading of phytate phosphorus in the digestive tract of poultry. Probiotics Antimicrob Proteins 11:580–587
Pal K, Paul SK, Biswas P, Patra AK, Bhunia T, Pakhira MC (2010) Responses of addition of yeast (*Saccharomyces cerevisiae*) from rice distillers grains with solubles with or without trace minerals on the performance of Black Bengal kids. Small Rumin Res 94:45–52
Pan D, Yu Z (2014) Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes 5:108–119
Park JH, Kim IH (2014) Supplemental effect of probiotic *Bacillus subtilis* B2A on productivity, organ weight, intestinal Salmonella microflora, and breast meat quality of growing broiler chicks. Poult Sci 93:2054–2059
Park YH, Hamidon F, Rajangan C, Soh KP, Gan CY, Lim TS, Abdullah WN, Liong MT (2016) Application of probiotics for the production of safe and high-quality poultry meat. Korean J Food Sci Anim Resour 36:567
Park S, Hong J, Francis D, Vega CJ, Htoo JK, Woyengo TA (2019) PSVII-42 growth performance, gut health, and immune responses of *Escherichia coli*-challenged weaned pigs fed probiotic-supplemented diets. J Anim Sci 97(Supplement 3):359–360
Parker RB (1974) Probiotics, the other half of the antibiotic story. Anim Nutr Health 29:4–8
Patra AK (2012) The use of live yeast products as microbial feed additives in ruminant nutrition. Asian J Anim Vet Adv 7:366–375
Patra AK (2016) Recent advances in measurement and dietary mitigation of enteric methane emissions in ruminants. Front Vet Sci 3:39
Patra AK (2019) Are nanomaterials potential new generation antimicrobial feed additives in livestock? Indian J Anim Health 58(Special):105–120
Patra AK (2020) Influence of plant bioactive compounds on intestinal epithelial barrier in poultry. Mini Rev Med Chem 20:566–577
Patra AK, Amasheh S, Aschenbach JR (2019) Modulation of gastrointestinal barrier and nutrient transport function in farm animals by natural plant bioactive compounds - a comprehensive review. Crit Rev Food Sci Nutr 59:3237–3266
Patterson JA, Burkholder KM (2003) Application of prebiotics and probiotics in poultry production. Poult Sci 82:627–631
Paul SS, Kamra DN, Sastry VRB, Sahu NP (2006) Effect of adding an anaerobic fungal culture isolated from a wild blue bull (*Boselaphus tragocamelus*) to rumen fluid from buffaloes on in vitro fibrolytic enzyme activity, fermentation and degradation of tannins and tannin-containing kachnar tree (*Bauhinia variegata*) leaves and wheat straw. J Sci Food Agric 86:258–270
Pelicano ER, De Souza PA, De Souza HB, Oba A, Norkus EA, Kodawara LM, De Lima TM (2003) Effect of different probiotics on broiler carcass and meat quality. Braz J Poult Sci 5:207–214
Peralta-Sánchez JM, Martín-Platero AM, Ariza-Romero JJ, Rabelo-Ruiz M, Zurita-González MJ, Baños A, Rodríguez-Ruano SM, Maqueda M, Valdivia E, Martínez-Bueno M (2019) Egg production in poultry farming is improved by probiotic bacteria. Front Microbiol 10:1042
Piamphon N, Wachirapakorn C, Bannasan K, Pornsopin P, Sotawong P, Gunun P (2017) Influence of *Aspergillus niger* or *Saccharomyces cerevisiae*-fermented napier grass *(Pennisetum purpureum)* mixed with fresh cassava root on blood parameters and nutrient digestibility in growing beef cattle. Pak J Nutr 16:776–781
Pogačar MŠ, Langerholc T, Mičetić-Turk D, Možina SS, Klančnik A (2020) Effect of lactobacillus spp. on adhesion, invasion, and translocation of *Campylobacter jejuni* in chicken and pig small-intestinal epithelial cell lines. BMC Vet Res 16:1–4
Popova T (2017) Effect of probiotics in poultry for improving meat quality. Curr Opinion Food Sci 14:72–77

Poppy GD, Rabiee AR, Lean IJ, Sanchez WK, Dorton KL, Morley PS (2012) A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae* on milk production of lactating dairy cows. J Dairy Sci 95:6027–6041

Price PT, Gaydos TA, Berghaus RD, Baxter V, Hofacre CL, Sims MD (2020) *Salmonella Enteritidis* reduction in layer ceca with a Bacillus probiotic. Vet World 13:184–187

Pridmore RD, Pittet AC, Praplan F, Cavadini C (2008) Hydrogen peroxide production by *Lactobacillus johnsonii* NCC 533 and its role in anti-*Salmonella* activity. FEMS Microbiol Lett 283:210–215

Qadis AQ, Goya S, Yatsu M, Kimura A, Ichijo T, Sato S (2014) Immune-stimulatory effects of a bacteria-based probiotic on peripheral leukocyte subpopulations and cytokine mRNA expression levels in scouring Holstein calves. J Vet Med Sci 76:677–684

Qiao J, Zhang B, Chen Y, Cao Y (2010) Codon optimization, expression and characterization of *Bacillus subtilis* MA139 β-1, 3-1, 4-glucanase in *Pichia pastoris*. Biologia 65:191–196

Raeth-Knight ML, Linn JG, Jung HG (2007) Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of Holstein dairy cows. J Dairy Sci 90:1802–1809

Rainard P, Foucras G (2018) A critical appraisal of probiotics for mastitis control. Front Vet Sci 5:251

Rajput IR, Li YL, Xu X, Huang Y, Zhi WC, Yu DY, Li W (2013) Supplementary effects of *Saccharomyces boulardii* and *Bacillus subtilis* B10 on digestive enzyme activities, antioxidation capacity and blood homeostasis in broiler. Int J Agric Biol 15:1560–8530

Reuben RC, Roy PC, Sarkar SL, Alam RU, Jahid IK (2019) Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC Microbiol 19:253

Robinson PH, Erasmus LJ (2009) Effects of analyzable diet components on responses of lactating dairy cows to *Saccharomyces cerevisiae* based yeast products: a systematic review of the literature. Anim Feed Sci Technol 149:185–198

Roger V, Fonty G, Komisarczuk-Bony S, Gouet P (1990) Effects of physicochemical factors on the adhesion to cellulose Avicel of the ruminal bacteria *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* subsp. *succinogenes*. Appl Environ Microbiol 56:3081–3087

Rojo R, Mendoza GD, González SS, Landois L, Bárcena R, Crosby MM (2005) Effects of exogenous amylases from *Bacillus licheniformis* and *Aspergillus niger* on ruminal starch digestion and lamb performance. Anim Feed Sci Technol 123-124:655–665

Sakata T, Kojima T, Fujieda M, Takahashi M, Michibata T (2003) Influences of probiotic bacteria on organic acid production by pig caecal bacteria *in vitro*. Proc Nutr Soc 62:73–80

Samedi L, Charles AL (2019) Evaluation of technological and probiotic abilities of local lactic acid bacteria. J Appl Environ Microbiol 7:9–19

Sarkar S (2018) Whether viable and dead probiotic are equally efficacious? Nutr Food Sci 48:285–300

Samli HE, Senkoylu N, Koc F, Kanter M, Agma A (2007) Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Arch Anim Nutr 61:42–49

Savaiano DA, Abou ElAnouar A, Smith DE, Levitt MD (1984) Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactase-deficient individuals. Am J Clin Nutr 40:1219–1923

Seghouani H, Garcia-Rangel CE, Füller J, Gauthier J, Derome N (2017) Walleye autochthonous bacteria as promising probiotic candidates against *Flavobacterium columnare*. Front Microbiol 8:1349

Seo JK, Kim SW, Kim MH, Upadhaya SD, Kam DK, Ha JK (2010) Direct-fed microbials for ruminant animals. Asian-Australas J Anim Sci 23:1657–1667

Sherman PM, Johnson-Henry KC, Yeung HP, Ngo PS, Goulet J, Tompkins TA (2005) Probiotics reduce enterohemorrhagic *Escherichia coli* O157: H7- and enteropathogenic *E. coli* O127: H6-induced changes in polarized T84 epithelial cell monolayers by reducing bacterial adhesion and cytoskeletal rearrangements. Infect Immun 73:5183–5188

Shurson GC (2018) Yeast and yeast derivatives in feed additives and ingredients: sources, characteristics, animal responses, and quantification methods. Anim Feed Sci Technol 235:60–76
Signorini ML, Soto LP, Zbrun MV, Sequeira GJ, Rosmini MR, Frizzo LS (2012) Impact of probiotic administration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. Res Vet Sci 93:250–258
Simmering R, Blaut M (2001) Pro-and prebiotics–the tasty guardian angels? Appl Microbiol Biotechnol 55:19–28
Singh KM, Shah TM, Reddy B, Deshpande S, Rank DN, Joshi CG (2014) Taxonomic and gene-centric metagenomics of the fecal microbiome of low and high feed conversion ratio (FCR) broilers. J Appl Genet 55:145–154
Soares MB, Almada CN, Almada CN, Martinez RC, Pereira EP, Balthazar CF, Cruz AG, Ranadheera CS, Sant'Ana AS (2019) Behavior of different *Bacillus* strains with claimed probiotic properties throughout processed cheese ("requeijão cremoso") manufacturing and storage. Int J Food Microbiol 307:108288
Stanley D, Denman SE, Hughes RJ, Geier MS, Crowley TM, Chen H, Haring VR, Moore RJ (2012) Intestinal microbiota associated with differential feed conversion efficiency in chickens. Appl Microbiol Biotechnol 96:1361–1369
Stern NJ, Svetoch EA, Eruslanov BV, Perelygin VV, Mitsevich EV, Mitsevich IP, Pokhilenko VD, Levchuk VP, Svetoch OE, Seal BS (2006) Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. Antimicrob Agents Chemother 50:3111–3116
Sun P, Wang JQ, Deng LF (2013) Effects of *Bacillus subtilis* natto on milk production, rumen fermentation and ruminal microbiome of dairy cows. Animal 7:216–222
Sun P, Wang JQ, Zhang HT (2010) Effects of *Bacillus subtilis natto* on performance and immune function of pre-weaning calves. J Dairy Sci 93:5851–5855
Tabidi MH, Mukhtar AM, Mohammed HI (2013) Effects of probiotic and antibiotic on performance and growth attributes of broiler chicks. Glob J Med Plant Res 1:136–142
Tactacan GB, Schmidt JK, Miille MJ, Jimenez DR (2013) A *Bacillus subtilis* (QST 713) spore-based probiotic for necrotic enteritis control in broiler chickens. J Appl Poult Res 22:825–831
Takahashi M, McCartney E, Knox A, Francesch M, Oka K, Wada K, Ideno M, Uno K, Kozłowski K, Jankowski J, Gracia MI (2018) Effects of the butyric acid-producing strain *Clostridium butyricum* MIYAIRI 588 on broiler and piglet zootechnical performance and prevention of necrotic enteritis. Anim Sci J 89:895–905
Talebi A, Amirzadeh B, Mokhtari B, Gahri H (2008) Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. Avian Pathol 37:509–512
Tang SX, Tayo GO, Tan ZL, Sun ZH, Shen LX, Zhou CS, Xiao WJ, Ren GP, Han XF, Shen SB (2008) Effects of yeast culture and fibrolytic enzyme supplementation on in vitro fermentation characteristics of low-quality cereal straws. J Anim Sci 86:1164–1172
Tellez G, Pixley C, Wolfenden RE, Layton SL, Hargis BM (2012) Probiotics/direct fed microbials for *Salmonella* control in poultry. Food Res Int 45:628–633
Teo AY, Tan HM (2005) Inhibition of *Clostridium perfringens* by a novel strain of *Bacillus subtilis* isolated from the gastrointestinal tracts of healthy chickens. Appl Environ Microbiol 71:4185–4190
Tewari S, David J, Gautam A (2019) A review on probiotic dairy products and digestive health. J Pharmacogn Phytochem 8:368–372
Toure R, Kheadr E, Lacroix C, Maroni O, Fliss I (2003) Production of antibacterial substances by bifidobacterial isolates from infant stool active against *Listeria monocytogenes*. J Appl Microbiol 95:1058–1069
Tripathi VK, Sehgal JP, Puniya AK, Singh K (2007) Effect of administration of anaerobic fungi isolated from cattle and wild blue bull *(Boselaphus tragocamelus)* on growth rate and fibre utilization in buffalo calves. Arch Anim Nutr 61:416–423

Tsai CC, Hsih HY, Chiu HH, Lai YY, Liu JH, Yu B, Tsen HY (2005) Antagonistic activity against *Salmonella* infection in vitro and in vivo for two *Lactobacillus* strains from swine and poultry. Int J Food Microbiol 102:185–194

Upadhaya SD, Rudeaux F, Kim IH (2019) Efficacy of dietary *Bacillus subtilis* and *Bacillus licheniformis* supplementation continuously in pullet and lay period on egg production, excreta microflora, and egg quality of hyline-brown birds. Poult Sci 98:4722–4728

Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, Gilbert M, Bonhoeffer S, Laxminarayan R (2019) Global trends in antimicrobial resistance in animals in low-and middle-income countries. Science 365:eaaw1944

van Der Wielen PW, Biesterveld S, Notermans S, Hofstra H, Urlings BA, van Knapen F (2000) Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl Environ Microbiol 66:2536–2540

Van Immerseel F, Fievez V, de Buck J, Pasmans F, Martel A, Haesebrouck F, Ducatelle R (2004) Microencapsulated short-chain fatty acids in feed modify colonization and invasion early after infection with *Salmonella enteritidis* in young chickens. Poult Sci 83:69–74

Van Vuuren AM, Calsamiglia S, Udén P (2012) Rumen health: a 360° analysis. Anim Feed Sci Technol 172:1–3

Veizaj-Delia E, Piu T, Lekaj P, Tafaj M (2010) Using combined probiotic to improve growth performance of weaned piglets on extensive farm conditions. Livest Sci 134:249–251

Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, Drider D (2019) Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Front Microbiol 10:57

Vilà B, Fontgibell A, Badiola I, Esteve-Garcia E, Jiménez G, Castillo M, Brufau J (2009) Reduction of *Salmonella enterica* var. Enteritidis colonization and invasion by *Bacillus cereus* var. *toyoi* inclusion in poultry feeds. Poult Sci 88:975–979

Vohra A, Syal P, Madan A (2016) Probiotic yeasts in livestock sector. Anim Feed Sci Technol 219:31–47

Vuong CN, Chou WK, Hargis BM, Berghman LR, Bielke LR (2016) Role of probiotics on immune function and their relationship to antibiotic growth promoters in poultry, a brief review. Int J Probiotics Prebiotics 11:1–7

Vyas D, McGeough EJ, Mohammed R, McGinn SM, McAllister TA, Beauchemin KA (2014) Effects of *Propionibacterium* strains on ruminal fermentation, nutrient digestibility and methane emissions in beef cattle fed a corn grain finishing diet. Animal 8:1807–1815

Wagner JJ, Engle TE, Belknap CR, Dorton KL (2016) Meta-analysis examining the effects of *Saccharomyces cerevisiae* fermentation products on feedlot performance and carcass traits. Prof Anim Sci 32:172–182

Wang Y, Dong Z, Song D, Zhou H, Wang W, Miao H, Wang L, Li A (2018) Effects of microencapsulated probiotics and prebiotics on growth performance, antioxidative abilities, immune functions, and caecal microflora in broiler chickens. Food Agric Immunol 29:859–869

Wen J, Sun JA, Zhou XX, Wei-Fen LI (2011) Effects of *Enterococcus faecium* on growth performance, immune and antioxidant function of piglets. Acta Agric Zhejiang 23:70–73

Willson NL, Nattrass GS, Hughes RJ, Moore RJ, Stanley D, Hynd PI, Forder RE (2018) Correlations between intestinal innate immune genes and cecal microbiota highlight potential for probiotic development for immune modulation in poultry. Appl Microbiol Biotechnol 102:9317–9329

Wiryawan KG, Brooker JD (1995) Probiotic control of lactate accumulation in acutely grain-fed sheep. Austr J Agric Res 46:1555–1568

Wisener L, Sargeant J, O'Connor A, Faires M, Glass-Kaastra S (2014) The use of direct-fed microbials to reduce shedding of *Escherichia coli* O157 in beef cattle: a systematic review and meta-analysis. Zoonoses Public Health 62:75–89

Yang CM, Cao GT, Ferket PR, Liu TT, Zhou L, Zhang L, Xiao YP, Chen AG (2012) Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens. Poult Sci 91:2121–2129

Yang X, Brisbin J, Yu H, Wang Q, Yin F, Zhang Y, Sabour P, Sharif S, Gong J (2014) Selected lactic acid-producing bacterial isolates with the capacity to reduce *Salmonella* translocation and virulence gene expression in chickens. PLoS One 9:e93022

Yeoman CJ, Chia N, Jeraldo P, Sipos M, Goldenfeld ND, White BA (2012) The microbiome of the chicken gastrointestinal tract. Anim Health Res Rev 13:89–99

Yoon IK, Stern MD (1995) Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: a review. Asian-Australas J Anim Sci 8:533–555

Yu P, Huber JT, Theurer CB, Chen KH, Nussio LG, Wu Z (1997) Effect of steam-flaked or steam-rolled corn with or without *Aspergillus oryzae* in the diet on performance of dairy cows fed during hot weather. J Dairy Sci 80:3293–3297

Yu L, Peng Z, Dong L, Wang H, Shi S (2019) *Enterococcus faecium* NCIMB 10415 supplementation improves the meat quality and antioxidant capacity of muscle of broilers. J Anim Physiol Anim Nutr 103:1099–1106

Zhang ZF, Cho JH, Kim IH (2013) Effects of *Bacillus subtilis* UBT-MO_2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. Livest Sci 155:343–347

Zhang L, Jin P, Qin S, Liu J, Yang Z, Zhao H, Sheng Q (2019) Effects of dietary supplementation with *S. platensis* and probiotics on the growth performance, immune response and the fecal *Lactobacillus* spp. and *E. coli* contents of weaned piglets. Livest Sci 225:32–38

Zhang ZF, Zhou TX, Ao X, Kim IH (2012) Effects of β-glucan and *Bacillus subtilis* on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize–soybean meal based diets. Livest Sci 150:419–424

Zhao X, Guo Y, Guo S, Tan J (2013) Effects of *Clostridium butyricum* and *Enterococcus faecium* on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. Appl Microbiol Biotechnol 97:6477–6488

Zhou X, Wang Y, Gu Q, Li W (2010) Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi yellow chicken. Poult Sci 89:588–593

Zhu WM, Liu W, Wu DQ (2000) Isolation and characterization of a new bacteriocin from *Lactobacillus gasseri* KT7. J Appl Microbiol 88:877–886

Zong X, Wang TH, Lu ZQ, Song DG, Zhao J, Wang YZ (2019) Effects of *Clostridium butyricum* or in combination with *Bacillus licheniformis* on the growth performance, blood indexes, and intestinal barrier function of weanling piglets. Livest Sci 220:137–142

Chapter 8
Probiotics for Human Health

Savitri and Prem Lata

Abstract Probiotics are beneficial microorganisms which when consumed live impart positive health effects to the consumer. Bacteria, yeast, and mold are the microorganisms that are used as probiotics but the majority of the microorganisms used as probiotics are bacteria from genus *Lactobacillus*, *Lactococcus*, *Bifidobacterium,* and *Streptococcus*. These are similar to those naturally found in the intestine and have an important role in improving the general health of human and other animals which provides protection from different kinds of ailments. Probiotics provide various health benefits such as improvement of gut health, boosting of immune response, reduction in serum cholesterol level and blood pressure through a variety of mechanisms thereby improving overall health of the consumers. Competition for binding sites with pathogens on the epithelial cells of GI tract thus preventing the infection, generation of certain metabolites like bacteriocins which reduces pathogenic bacterial growth, strengthening of the epithelial barrier, improved adhesion to intestinal mucosa, and resulting in the inhibition of pathogenic adhesion and immune system modulation are some key *mechanisms* of *probiotic action*. Thus these have the potential to treat many diseases like gastroenteritis, cancer, diarrhea, inadequate lactose digestion, irritable bowel syndrome, allergies, urogenital infections, cholesterolaemia, *Helicobacter pylori* infections, and inflammatory bowel disease (IBD). Due to their numerous claimed health benefits, probiotics are attaining more interest as alternatives for anti-inflammatory drugs or antibiotics that have led to the growth of probiotic market. Presently, a number of probiotic products with claimed therapeutic values are available in the market. Further, more research on probiotics and their therapeutic benefits is required which will provide scientific basis for using probiotics for prevention and treatment of many diseases. In this chapter, the various roles played by probiotics in maintaining and improving health of human beings will be discussed.

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8.1 Introduction

A large and diverse number of microbes inhabit the gastrointestinal tract (GIT) of mammals; where along with their genomes these constitute the microbiome of gut (Marchesi and Ravel 2015). Within the host, bacteria, archaea, fungi, protozoa, and viruses coexist and interact reciprocally and also with epithelial and immune cells of the host. The immune-mediated disorders like allergic diseases are increasing rapidly which is linked to the little exposure to microorganisms at the initial stages of life. The intestine, which is called the largest immune organ, contains most of the antibody-producing cells. Several studies showed that gut microbiota play an important role in maintaining the homeostasis, health, and disease in human. Gastrointestinal function, appetite, and immune response are some areas that are associated with microbiome of the gut (van de Wouw et al. 2017).

Probiotics are viable microorganisms that enhance the well-being of the host. Mostly, probiotics are bacteria which are similar to beneficial gut microorganisms of human. Probiotics in the form of capsules, tablets, liquid form, gel, paste, and powders as foods and dietary supplements and beauty products are available in market. Milk products (both fermented and unfermented) such as *tempeh*, *miso*, soy beverages, and different types of juices are examples of foods that contain probiotics. Probiotics are already present or added in the preparation of food items. A single bacterial strain or an association of more bacterial strains (consortium) is used as probiotics. Various health benefits are provided by probiotics as they improve gut health, act as an enhancer of the immune response, and reduce the serum cholesterol; they are also used in the treatment of different types of diarrhea, irritable bowel syndrome, urogenital infections, and improvement of lactose metabolism.

8.2 History of Probiotics

During the late nineteenth century, microbiologists discovered that the microbes which are present in the GI tract of disease-free human are different from those in persons with any disease. These microorganisms which are found in the GI tract provide beneficial effects and were termed as probiotics. In its truest sense, probiotics means "for life." Fundamentally, probiotics are microorganisms that put forth health promoting influence on humans as well as in animals.

Elie Metchnikoff during 1900 hypothesized that the utilization of fermented milk is the reason behind the healthy and long lives of Bulgarian peasants. This hypothesis leads to the development of idea of probiotics. During his study, he suggested that beneficial microorganisms contained in the yogurt protect the host from the detrimental effects of other pathogenic microorganisms (Metchnikoff and Metchnikoff 1908). He isolated various strains of lactobacilli from yogurt and named them *Lactobacillus bulgaricus*. According to his study, fermented milk products can be prepared by using pure cultures of *L. bulgaricus*. Metchnikoff hypothesized that lactobacilli eliminate pathogenic toxin-producing bacteria from the intestine. In 1912 at Saint Petersburg the first bacterial drug, Lactobacillin also promoted by Metchnikoff, was manufactured [http://www.probiotics-help.com/mutaflor.html]. Further, Rettger and colleagues extensively researched the mechanism of the probiotic effects and the use of intestine-derived species after Metchnikoff's death.

The very first clinical trials to check the effectiveness of probiotics in relieving constipation was carried out in the 1930s. Later in 1950s, a probiotic product was authorized as a drug for the treatment of scouring caused by *E. coli* among pigs by the United States Department of Agriculture (Orrhage et al. 1994). Many probiotic species have been used in the elimination and for the treatment of diverse range of diseases. The blood serum cholesterol can be reduced by consuming the fermented yogurt containing *Lactobacillus* sp. which opened a new area of research. Reduction in the serum cholesterol was also reported in the infants when cells of *Lactobacillus acidophilus* were added to their formula. As the commonly used antibiotics are becoming useless due to antibiotic resistance, so the World Health Organization in 1994 described probiotics as the subsequent vital immune defense system.

Probiotics play various roles, for instance, the production of antimicrobial compounds such as lactic acid which help in the preservation of milk and also produce flavor compounds like acetaldehyde in yogurt and cheese. Probiotics give a product with the organoleptic characters by the generation of certain metabolites (e.g., extracellular polysaccharides) and also help in bio-enrichment of food items by the synthesis of vitamins and free amino acids. In addition to basic nutritional value, probiotics are widely reported to provide various health benefits to the consumers (Benchimol and Mack 2004). These are also reported to control the level of serum cholesterol thus showing exceptional prophylactic or remedial properties. The remedial benefits include the prevention against a number of intestinal infections, and improving the lactose metabolism of lactose-containing foods.

8.3 Probiotic Microorganisms

The microorganisms used as probiotics are bacteria, yeast, and mold, but the majority of probiotics used are bacteria. The microbial strains used as probiotics include the different species of *Bacillus*, *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *E. coli*, a range of yeast species, and undefined varied cultures of microbes. In humans, *Lactobacillus* and *Bifidobacterium* species are most extensively used as probiotics, while species of *Enterococcus*, *Bacillus*, and *Saccharomyces* have been commonly used in farm animals. Among LAB, species of *Lactobacillus acidophilus, L. bulgaricus,* *L. brevis, L. casei, L. delbrueckii, L. fermentum, L. helveticus, L. johnsonii, L. lactis, L. rhamnosus, L. reuteri, L. plantarum, L. salivarius, L. sporogenes, L. farciminis,* and *L. paracasei* are used. *Streptococcus thermophilus, S. cremoris, Enterococcus faecalis, E. faecium, Bifidobacterium bifidum, B. breve, B. infantis, B. adolescentis, B. lactis, B. longum, Leuconostoc mesenteroides, Pediococcus* spp., *Propionibacterium* spp. are also reported to be used as probiotics. Yeasts and molds belonging to *Saccharomyces cerevisiae, Saccharomyces boulardii*, *Candida pintolopesii, Aspergillus niger,* and *A. oryzae* are also used in different probiotic products (Amara and Shibl 2015) and these microorganisms/products are used in prevention and curing of many diseases (Table 8.1).

8.4 Mechanism of Probiotic Action

Probiotics, mostly lactic acid bacteria and their food variants, provide various important nutritional and remedial health benefits to the consumers such as anticarcinogenic and antimutagenic activity (Lee et al. 2004). These provide health benefits to the consumers by a variety of mechanisms.

Following are the mechanisms through which probiotics confer health benefits to the organisms:

8.4.1 Competition with Pathogens

Probiotics maintain or restore the balance of host-microbial association and therefore reduce intrusion and colonization of pathogens. The endogenous microbes remain present in all the functional niches in our gut; thereby reducing the pathogenic foray and establishment in that ecological community. Probiotics can also reside in useful niches or they can change the local environment directly by secreting short-chain fatty acids (SCFAs), bacteriocins, lactic acid, and reactive oxygen species that suppress the growth of pathogens (Harper et al. 2018).

8.4.2 Production of Metabolites

Several species of the gut microbiome impart the production of SCFAs and various vitamins (nicotinic acid, thiamine biotin, vitamin B_{12}, folate, pyridoxine, and vitamin K) (Vandenplas et al. 2015). Moreover, probiotic microorganisms also produce butyrate which plays a major source of energy for enterocytes and is involved in the wear and tear of the gastrointestinal wall (Rios-Covian et al. 2016).

Table 8.1 Probiotics used in prevention and treatment of various diseases/disorders

Type of diseases or disorders	Probiotic strains used	References
Kidney/Urinary stones	*L. acidophilus* LA-14, *L. plantarum* PBS067, *B. longum* PBS078, *B. breve*PBS077, *Oxalobacter formigenes*	Giardina et al. (2014)
Atopic Diseases	*Lactobacillus casei, L. acidophilus, L. salivarius, B. bifidum, B. lactis, L. rhamnosus lactis, L. GG, L. fermentum,* and *Lactococcus lactis*	Doege et al. (2012)
Colic	*L. casei, L. rhamnosus, L. delbrueckii* subsp. *bulgaricus, S. thermophilus, L. reuteri* DSM 17938, *L. acidophilus, B. infantis, B. breve*	Nation et al. (2017)
Helicobacter pylori Infection	*Lactobacillus, Bifidobacterium,* and *L. johnsonii* *L. rhamnosus GG* *Bifidobacterium animalis* ssp. *lactis* (DSM15954) *L. reuteri* DSM 17938 Mixture of *L. bulgaricus* and *L. acidophilus* and *S. thermophilus* and *B. bifidum* and galacto-oligosaccharides *Streptococcus faecalis, L. acidophilus, Bacillus subtilis* *Bacillus clausii* (Enterogermina strains) *Saccharomyces boulardii* CNCM I-745 *L. reuteri* ATCC 6475 and *L. reuteri* DSM 17938	Yvan et al. (2015) Dang et al. (2014) Hauser et al. (2015) Manfredi et al. (2012) Du et al. (2012) Emara et al. (2014)
Acute and antibiotic associated diarrhea	*Lactobacillus rhamnosus, L. reuteri, L. rhamnosus GG*, and *L. acidophilus* *Saccharomyces boulardii, B. animalis* subsp. *lactis* alone or in mixture with *S. thermophilus*, *L. casei* Yogurt having *L. casei* DN114, *S. thermophilus* and *L. bulgaricus* *Bifidobacterium lactis* W18, *B. longum* W51, *B. bifidum* W23, *L. acidophilus* W37 and W55, *L. rhamnosus* W71, *Enterococcus faecium* W54, *L. salivarius* W24, *L. paracasei* W72, and *L. plantarum* W62 *L. rhamnosus GG, Enterococcus faecium* SF68, *L. bulgaricus, B. longum, L. acidophilus* and *Saccharomyces boulardii*	Phavichitr et al. (2013) Hempel et al. (2012) Koning et al. (2008) Tiwari et al. (2012)
Candida infection	*Lactobacillus rhamnosus, Lactobacillus reuteri, Propionibacterium freudenreichii*	Jørgensen et al. (2017)
Constipation	*Bifidobacterium* sp., *B. lactis, B. infantis, B. breve, L. casei, B. longum, L. rhamnosus, Streptococcus thermophilus, L. bulgaricus, L. acidophilus* *L. acidophilus* (KCTC 11906BP), *B. bifidum* (KCTC 12199BP), *Streptococcus thermophilus* (KCTC 11870BP), *B. lactis* (KCTC 11904BP), *B. longum* (KCTC 12200BP), and *L. rhamnosus* (KCTC 12202BP) Fructo-oligosaccharides (FOS) with the cultures of *L. acidophilus* (NCFM), *L. paracasei* (Lpc-37),	Sadeghzadeh et al. (2014) Yeun and Lee (2015) Waitzberg et al. (2013) Ojetti et al. (2014)

(continued)

Table 8.1 (continued)

Type of diseases or disorders	Probiotic strains used	References
	B. lactis (HN019), and *L. rhamnosus* (HN001) *L. reuteri* DSM 17938	
Irritable bowel syndrome	*B. bifidum* MIMBb75, *B. lactis, L. casei, L. acidophilus,* and *L. plantarum, S. cerevisiae* *L. acidophilus* NCIMB 30175, *L. plantarum* NCIMB 30173, *L. rhamnosus* NCIMB 30174, and *Enterococcus faecium* NCIMB 30176 fructo-oligosaccharides and *Bacillus coagulans* *L. delbrueckii* ssp. *bulgaricus* LBY-27, *L. acidophilus* LA-5, *L. animalis* ssp. *lactis* BB-12, *S. thermophilus* STY-31 *S. boulardii* CNCM I-745 *E. coli* DSM17252 *L. plantarum* 299v (DSM 9843) *B. animalis* DN-173010, *B. infantis* 35,624 in fermented milk (along with *L. bulgaricus* and *S. thermophilus*) *L. acidophilus* SDC 2012, 2013 *B. animalis* ssp. *lactis* Bb12 DSM 15954, *L. rhamnosus* LC705, *L. rhamnosus GG, Propionibacterium freudenreichii* ssp. *shermanii* JS DSM 7067 *L. plantarum* CECT 7485, *L. plantarum* CECT 7484, *Pediococcus acidilactici* CECT 7483 *Bacillus coagulans* GBI-30, 6086	Cayzeele-Decherf et al. (2017) Ducrotté et al. (2012) Ford et al. (2014) Sisson et al. (2014) Rogha et al. (2014) Jafari et al. (2014) Choi et al. (2011) Guglielmetti et al. (2011) Moayyedi et al. (2010) Agrawal et al. (2009) Sinn et al. (2008) Kajander et al. (2008) Lorenzo-Zúñiga et al. (2014) Dolin (2009)
Acute viral upper respiratory infections	*L. paracasei, L. rhamnosus, L. acidophilus, B. bifidum, L. casei Shirota, L. plantarum* and *B. animalis* ssp. *Lactis, Streptococcus salivarius*	Shida et al. (2017)
Modulation of gut – brain axis	*L. lactis* ssp. *lactis* and *B. animalis* ssp. *Lactis*	Liu et al. (2015)
Colon Cancer	Lactic acid bacteria	Kahouli et al. (2013)
Diabetes and Obesity	*L. rhamnosus* CGMCC1.3724, *L. gasseri* SBT2055, *L. acidophilus* NCFM	Sanchez et al. (2014)
Clostridium difficile–associated diarrhea	Yogurt with *L. bulgaricus* and *L. casei* DN114 and *S. boulardii* CNCM I-745, *S. thermophilus* *L. acidophilus* NCFM with *L. rhamnosus* HN001 *B. bifidum* with *L. acidophilus* (Cultech strains) *L. casei* LBC80R and *L. acidophilus* CL1285	Goldenberg et al. (2017) Lahtinen et al. (2012) Plummer et al. (2004) Johnson et al. (2012)

(continued)

Table 8.1 (continued)

Type of diseases or disorders	Probiotic strains used	References
Hepatic encephalopathy	Combination of different strains of *L. delbrueckii* ssp. *bulgaricus*, *L. acidophilus, L. casei, L. plantarum, B. breve, B. longum, B. infantis,* and *S. salivarius* ssp. *thermophilus* Yogurt containing *L. acidophilus, L. bulgaricus, L. casei, S. thermophilus,* and *bifidobacteria*	Lunia et al. (2014) Shukla et al. (2011)
NAFLD	Yogurt containing *S. thermophilus* and *L. bulgaricus* supplemented with *B. lactis* Bb12 and *L. acidophilus* La5 Combination of *L. acidophilus, L. rhamnosus, L. casei, B. longum, S. thermophilus, L. bulgaricus,* and *B. breve* with fructo-oligosaccharides	Nabavi et al. (2014) Eslamparast et al. (2014)
NASH	*B. longum* W11 + FOS *S. thermophilus* and *L. bulgaricus*	Malaguarnera et al. (2012) Aller et al. (2011)
IBD—pouchitis and lcerative colitis	Mixture containing strains of *L. delbrueckii* ssp. *bulgaricus, L. plantarum, L. acidophilus, L. casei, B. breve, B. longum*, *B. infantis,* and *S. salivarius* ssp. *thermophilus*	Gionchetti et al. (2007)
Lactose intolerance	Yogurt containing alive cultures of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*	EFSA (2010)
Acute gastroenteritis	*L. rhamnosus GG* *S. boulardii* CNCM I-745 *L. acidophilus* *L. reuteri* DSM 17938 *B. bifidum* and *L. acidophilus* *B, infantis* and *L. acidophilus* *E. coli* Nissle 1917 *L. rhamnosus* GC, *L. casei* strain *Shirota*, *L. acidophilus, B. bifidum* and *S. thermophilus, L. reuteri, E. faecium* SF68, *L. delbrueckii* var. *bulgaricus, S. boulardii*	Szajewska et al. (2014) Szajewska and Skórka (2009) Klanifar et al. (2009) Urbańska et al. (2016) Lee et al. (2001) Canani et al. (2007) Rafeey et al. (2008) Tiwari et al. (2012)
Traveler's diarrhea	*L. fermentum* strain KLD, *L. acidophilus, L. rhamnosus GG, L. bulgaricus,* and *S. boulardii*	Tiwari et al. (2012)

8.4.3 Immunomodulatory Effects

Since so long, probiotics are known to have diverse positive effects on the immune system. These probiotic strains have the property of inducing IL-12 and also have natural killer (NK) cell immunity and thus considered to possess immune-stimulatory effects (Aziz and Bonavida 2016). Other species are immune-regulatory due to their ability to regulate T cell pathways and stimulation of interleukin IL-10 (Azad et al. 2018). The specific probiotic species or strains have a specific effect with some species performing as pro-inflammatory and others as anti-inflammatory agents on the immune system.

8.4.4 Removal of Contaminants/Heavy Metals

Some probiotics have the ability to lower the risk of ingested heavy metals and hazardous chemicals. Cell wall of probiotic bacteria plays an important role in the removal of metals. The bacterial cell wall binds to the metals by using three main mechanisms, firstly, by ion exchange reactions with teichoic acid and peptidoglycan, secondly, by nucleation reactions causing precipitation, and thirdly, by forming complexes with oxygen and nitrogen ligands (Mueller et al. 1989). For example, *Pediococcus pentosaceus* destroy fumonisins, cluster of mycotoxins produced by fungi (Vandenplas et al. 2015).

8.4.5 Metabolism of Xenobiotics and Drugs

Research on gut microbiome revealed that *p*-cresol metabolite of gut microbe inhibits the liver enzyme hepatic sulfotransferase by competitive inhibition thus reducing the ability of the liver to metabolize paracetamol. The similar role in drug and xenobiotic metabolism played by the microorganisms present in gut could have a significant effect on therapy options in the times to come (Jandhyala et al. 2015). Furthermore, several studies showed that pollutants from dietary and environmental chemicals interfere with gut bacterial function and therefore can induce a pro-inflammatory response and affect host health (Defois et al. 2018). Supplementation of probiotics can further help gut flora to effectively metabolize various drugs and even xenobiotics.

8.4.6 Bile Acid Metabolism

Primary bile acids are deconjugated and dehydrated by some species of the gut microbiota—like *Bacteroides intestinalis*—and convert them into secondary bile acids (Jandhyala et al. 2015). Secondary bile acids are responsible for inhibition of spore germination in *Clostridium difficile* and, hence, restrain the growth of vegetative forms of *C. difficile*.

8.5 Effects on Health

Probiotics have a variety of beneficial health effects on human as well as on animals. Probiotics have potential to treat certain diseases like gastroenteritis, cancer, diarrhea, inadequate lactose digestion, irritable bowel syndrome, allergies, cholesterolaemia, *Helicobacter pylori* infections, and IBD. Other than these effects, probiotics furthermore stimulate immune system by a variety of mechanisms thereby improving overall health of the consumers. The positive effects of probiotics on some diseases/physiological conditions are being discussed in the following sections:

8.5.1 Hypertension

Several studies revealed that the probiotics and their products play an important role in improving the cholesterol level in the body that helps in balancing the blood pressure (Guo et al. 2011). Probiotics decrease the blood pressure via different mechanisms, for instance, by insulin resistance, lowering in the level of blood sugar, and by regulation of rennin-angiotensin system which further results in decrease in the blood or serum cholesterol. In hypertensive conditions, probiotic supplementation might be helpful in reducing blood pressure. Various clinical studies and primary studies on animals showed that the probiotics and their products take part in controlling the blood pressure. During a clinical trial on hypertensive patients, there is considerable decrease in both systolic and diastolic blood pressure when patients were supplemented with fermented milk containing starter culture of bacteria, *Lactobacillus helveticus,* and yeast, *Saccharomyces cerevisiae* (Hata et al. 1996). A study revealed that two tripeptides (isoleucine-proline-proline and valine-proline-proline) function as angiotensin-I-converting enzyme inhibitors and helps in the reduction of blood pressure. These tripeptides were isolated from the milk-based medium in which starter culture of *Saccharomyces cerevisiae* and *Lactobacillus helveticus* was used (Figueiredo et al. 2018). Many probiotic strains like *Lactobacillus acidophilus, L. bulgaricus, L. casei, L. delbrueckii, L. helveticus, L. kefiri*, *L. rhamnosus*, *L. rhamnosus GG, Bifidobacterium longum, Bifidobacterium breve, Streptococcus thermophiles, Saccharomyces cerevisiae* are used for anti-hypertension treatment (Rerksuppaphol and Rerksuppaphol 2015; Ekhlasi et al. 2017).

In hypertensive patients, the administration of powdered probiotic cell extracts results in a reduction in heart rate and blood pressure. The role of consumption of probiotic on heart disease by lowering the blood pressure and blood lipid levels can be assessed by long-term and well-controlled human studies. As risk of heart disease is widespread so the regular consumption of probiotics may act as preventive measure against heart disease.

8.5.2 Urogenital Infections

The abnormal vaginal condition that is comprised by vaginal discharge which is due to the overgrowth of atypical bacteria in the vagina is called bacterial vaginosis. Urogenital infections happen because of alteration in vaginal environment where concentration of lactobacilli is less or absent. The intestinal tract is the prevalent cause of pathogens for urogenital infection in women. Vaginal infections are mainly due to *Trichomonas*, *Candida, Mycoplasma hominis,* and *Gardnerella vaginalis*. The causative organisms of urinary tract infections are *Escherichia coli*, *Chlamydia,* and *Candida, Proteus* spp., *Staphylococcus* spp., and *Klebsiella* spp. (Lim et al. 2009). Vaginal infections lead to many problems including low birth-weight neonate, immature delivery, abdomen infections which can cause sterility, and sexually transmitted diseases.

The major microbial factor which is responsible for the protection of urogenital cells is *Lactobacillus* spp. *Lactobacilli* are main bacteria associated with vaginal health where they reduce the growth of bacterial pathogens (Cadieux et al. 2002; Reid and Bruce 2001; McLean and Rosenstein 2000). These species regulate the occurrence, expansion, establishment, and perseverance of non-endogenous microbes in vagina. Probiotic microorganisms, viz. *Lactobacillus acidophilus, L. crispatus, L. rhamnosus, L. reuteri, L. gasseri, L. vaginalis,* and *Streptococcus thermophilus,* are used for the elimination of recurring bacterial vaginosis (Ya et al. 2010; Siroli et al. 2017). The consumption of products containing these microorganisms can effectively help in treating UTIs. The thought of oral probiotic use is based on the fact that pathogens that cause most of the urinary tract infections progress from the rectum to the vagina and the mesentery. Probiotics help in the reduction of urogenital infection by the stimulation of immune response, and produce some organic acids that reduce the vaginal pH, competitive exclusion, and generation of certain antimicrobial substances (bacteriocins and hydrogen peroxide).

Alternative medicines are beneficial to those with chronic vaginal symptoms (Nyirjesy et al. 1997), while intake of probiotics such as lactobacilli can prevent the occurrence of vaginal infection, by the recolonization of lactobacilli in the vaginal tract. Although the application of lactobacilli in UTIs (urinary tract infections) has been studied, but until now the oral administration method is not used (Reid et al. 1998).

The dried lactobacilli (⍰10^9 colony-forming units/dose) were given weekly through intravaginally to premenopausal women and it was observed that the urinary tract infection reduced to 5 percent in a year (Reid and Bruce 1995). In placebo-controlled trials including 38 women, vaginal lactobacilli were found to decrease the possibility of urinary tract infection (Reid 1995). These studies suggested that *lactobacilli* play a major role to prevent urogenital infections in women by suppressing pathogenic bacteria.

8.5.3 Lactose Intolerance

Major carbohydrate present in milk is lactose. For the absorption of lactose from milk, sufficient lactase activity should be present in the small intestine. This enzyme is present in children, however, reduced in adults in the majority of the people (Daliria and Lee 2015). Several studies showed that lactose absorption from yogurt is improved in lactose deficient in lactase activity (Mustapha et al. 1997) having milk with *Lactobacillus acidophilus* culture than with normal milk.

Yogurt containing starter cultures of *Lactobacillus delbrueckii* subsp. *L. bulgaricus,* and *Streptococcus thermophilus* are reported to effectively improve lactose break down in lactose maldigesters by lactase (β-galactosidase) enzyme present in the bacteria. Various studies showed that lactic acid present in the yogurt reduces the lactose intolerance problem in lactase-deficient persons. The activity of enzyme lactase or β-galactosidase in small intestine is enhanced by the presence of lactic acid bacteria in fermented milk (Marteau et al. 1990).

Various studies were conducted on human, in which the intake of yogurt containing live culture was compared with that of a pasteurized product, resulting in improved digestion and absorption of lactose in yogurt containing live cultures (Labayen et al. 2001). Studies showed that *Yakult,* in which mixture of *Lactobacillus casei shirota* and the bacterium *Bifidobacterium breve* were used, had satisfactory effects on patients with lactose intolerance (Vonk et al. 2012).

8.5.4 Cholesterol Removal

Cholesterol plays a significant role to perform several functions in human. Cholesterol is a constituent of cell membrane that functions as a precursor to many vitamins and hormones. However, an increase in the level of both low-density lipoprotein cholesterol and high-density lipoprotein cholesterol is a threat factor for various cardiovascular diseases. The risk of heart attack is three times more in patients with increased blood cholesterol level as compared to the persons having normal blood lipid value (Ghosh 2012). Cholesterol is synthesized within the human body to maintain the minimum level and diet also affects the serum cholesterol level, while the effects vary from person to person. Several human studies suggested that the probiotic products have the potential to lower the level of blood serum cholesterol.

Probiotic bacteria are reported to decrease the cholesterol levels via different mechanisms such as assimilation of cholesterol by microorganisms, de-conjugation of bile acids, and by attachment of cholesterol to the cell wall of bacteria. By using cholesterol, liver produces bile acids that are released into the intestine, and from the intestine, they are absorbed again and returned to the liver. Probiotic bacteria of the intestine deconjugate these bile acids as a result decreases the total blood cholesterol (Bordoni et al. 2013). Certain animal studies evidenced that greater the number of bacteria in gut, greater elimination of bile acids is there (Mott et al. 1973). Probiotic bacteria degrade the bile acids and reduce the reabsorption of bile salts. Due to the reduction in reabsorption, cholesterol storage in liver begins to reduce.

A study on animals suggested that high level of cholesterol was observed in the excreta of conventional animals than germ-free animals, which is due to the existence of microbes in the gut of conventional animals affecting the serum cholesterol levels. During a trial on human, a decrease in blood cholesterol and low lipid levels by 4.4 and 5.4%, respectively, was observed when treated with yogurt containing starter culture of *L. acidophilus* for various weeks as compared with control (Schaafsma et al. 1998). Mann (1977) suggested that 3-hydroxy-3-methyl glutaric acid (HMG) found in fermented milk suppresses the enzyme, i.e., hydroxymethyl glutaryl CoA reductase that is used in the synthesis of cholesterol. It was also observed that the probiotics may suppress cholesterol synthesis in liver and its transportation to liver by generating the metabolites exclusively short-chain fatty acids (Pereira and Gibson 2002). Several in vitro studies showed that the cultures of probiotics are capable of removing cholesterol from culture media hence more emphasis has been given to this aspect of the probiotics. Removal of cholesterol is due the bile salt hydrolase enzyme produced by probiotic bacteria which hydrolyzed the acids (Parvez et al. 2005).

8.5.5 Cancer

Cell growth and cell division are controlled by abnormal genes and certain variations or stimulation of these abnormal genes cause cancer. Generally, cancer does not result from these abnormal cells as they exceed the normal ones. Most of the abnormal cells were recognized by the immune system itself and these are demolished. The prevalence of abnormal cells increases due to many processes or exposures; therefore, the chance of cancer can be decreased by reducing these exposures. Out of many exposures, chemical exposures are more likely risky exposures. Carcinogens can be produced by the microbes of the GI tract during digestion process. Further, the enzymes like Beta-glucuronidase and nitroreductase enzymes produced by enteropathogens, for example, *E. coli* and *Clostridium perferinges* transform pro-carcinogens into carcinogens. Probiotic bacteria reduce the exposure to chemical carcinogens by certain mechanisms including detoxification of engulfed carcinogens, and reduce the metabolic processes of bacteria that can produce carcinogens by changing the conditions of the intestine, generation of compounds that reduces the expansion of tumor cells, producing the metabolic products such as butyrate that enhances the capability of cell to die as it is supposed to die, and activating the immune system to provide protection against the production of cancer cells (Sanders 2009).

Several studies carried out involving humans have revealed that probiotics might inhibit the possibility of colon cancer by deactivating the mutagenic compounds, generating antimutagenic compounds, decreasing the assimilation of mutagens in the intestine, suppressing the conversion of pro-carcinogens to carcinogens, decreasing the proliferation of procarcinogenic bacteria, and improving immune functions (Gill and Guarner 2004). Epidemiological and population-based studies have showed that the utilization of fermented dairy foods with starter cultures lactobacilli or bifidobacteria decreases the occurrence of breast and colon cancer. Bifidobacterium decreases the pH of intestine through the generation of organic acids, specifically lactic acid that creates a bactericidal environmental for these enteropathogens. Bifidobacterium has antitumor properties, i.e., property to kill tumor cells caused by phagocyte activation (Sekine et al. 1994).

A study revealed that the utilization of *L. casei* strain Shirota might detain the reappearance of cancer in bladder. *L. casei* Shirota reduces the growth of tumor by stimulating the immune system, an increase in the number of T-helper cells and NK cells were reported in patients with colorectal cancer during the use of *L. casei* Shirota. According to a hypothesis, lactobacilli bind to the mutagens in the intestine and reduce the assimilation of these compounds, hence, prevent or delay the tumor development (Murch 2001; Isolauri 2004).

In the western world, main reason of death from cancer is colorectal cancer (CRC). Environmental factors, mainly the diet, are associated with approximately 70% of CRC. In vitro studies of human and animals revealed that fermented milk with probiotic cultures has protecting effects against CRC (Saikali et al. 2004). A study revealed that the probiotic yogurt prepared with starter culture of *Lactobacillus acidophilus* 145 and *Bifidobacterium longum* 913 considerably reduced fecal water genotoxicity in comparison to the yogurt without probiotics (Oberreuther-Moschner et al. 2004). Although, the probiotic treatment also enhances oxidative damage; this may be due to the pro-oxidative activity or activation of some defense systems.

A study on rats was done and it was observed that the use of product containing *L. acidophilus* leads to the decrease in the overall number of colon cancer cells (De Santis et al. 2000). Another study on animals was conducted where colon cancer was artificially induced and the treatment with *Lactobacillus GG* decreases the prevalence and many tumors. It was observed that *Bifidobacterium longum* also reduces the occurrence of mammary tumors, liver, colon, small intestinal tumors in rats (Orrhage et al. 1994).

The anticancerous activity was reported in the extract of *L. casei*, *L. helveticus*, and *L. acidophilus* when applied in the treatment of sarcomas in mice. During in vitro study, it was observed that fermented milk with starter culture of *L. delbrueckii* ssp. *bulgaricus* exhibits antimutagenic activities against 4-NQO (4-Nitroquinoline 1- oxide), a typical mutagen (Hosono et al. 1986).

8.5.6 Irritable Bowel Syndrome (IBS)

IBS is a persistent and frequent gastrointestinal disorder. Irritable bowel syndrome is characterized by abdominal pain, bloating, flatulence, and diarrhea. These conditions are not easy to treat and thus have a considerable influence on the lives of the patients. IBS is caused by different factors such as food ingestion, abnormal absorption of nutrients, and psychosomatic disorders and these factors affect the motor function of the gastrointestinal tract. Probiotics showed significant consequences on irritable bowel syndrome in the gut.

Different clinical trials revealed that the consumption of different species of lactic acid bacteria decreases the stomach ache, bloating, flatulence, and constipation. A study suggested that *Saccharomyces boulardii* decreases diarrhea, although not efficient in reducing other symptoms of IBS (Marteau et al. 2001).

A study on patients with IBS illustrated a satisfactory increase in relief of general symptoms of irritable bowel syndrome and of stomach ache with time when they receive consortia of *L. acidophilus* LA 102, *S. thermophilus* LA 104, *Lactococcus lactis* LA 103, *B. longum* LA 101 (Drouault-Holowacz et al. 2008). In another study, there was a significant decrease in symptoms of IBS in the experimental group as compared to the control group where experimental group was fed with a milk drink containing a mixture of probiotic strains (Kajander et al. 2008). Various studies revealed that *L. rhamnosus* GG is less effective for IBS, while *L. plantarum* 299 V had a pronounced positive effect on IBS (Niedzielin et al. 2001).

8.5.7 Allergy

During the last 50–60 years, the occurrence of allergy has increased in Western societies. An allergy is a response of immune system against foreign substances. There are two types of allergies: an antibody mediated allergy and a cell mediated allergy. When an individual is exposed to a stimulus at a higher dose, hypersensitivity reactions occur. The allergic reaction is caused by foreign substances (usually proteins) called allergens. The allergens enter into the human body by number of ways, for instance, through breathing, intake of food, and skin contact, or enter during the insect biting and are capable of reaching the immune system (Weiner et al. 2011). Atopy is a genetic tendency which occurs in childhood or adolescence to induce the production of IgE antibodies when foreign substances come in contact to immune system and produce signs like asthma, rhinoconjunctivitis, and atopic dermatitis. It has been observed that in every case of allergy, IgE mechanisms are not attributable (Fiocchi et al. 2012). The mechanisms of allergic diseases include genetic factors, relatedness of allergen exposure, host, and other environmental stimuli, for example, microflora of intestine and contagious agents (Chiang et al. 2012).

Several studies suggested that the allergy and their associated diseases are developed due to the insufficient exposure to environmental microbes. Allergies are related to the alteration in the ratio of T-helper 1 cytokine which leads to the stimulation of Th2 cytokine that results in the production of IgE and the liberation of different interleukins, i.e., IL-4, IL-5, and IL-13 (Michail 2009). Consumption of probiotics changes the microenvironment of gastrointestinal tract by changing the local microflora and change in the production of cytokine. Studies showed that probiotics change the Toll-like receptors (TLRs) and various recognition proteins present on intestinal lining which results in the stimulation of dendritic cells and Th1 cytokine response which results in the suppression of Th2 responses (Winkler et al. 2007).

Several studies have shown that probiotics exert the positive outcome on the allergic diseases through the development of mucosal barrier and stimulation of the immune system. Several studies on atopic eczema and food allergy suggested that probiotic bacteria play an important role in down-regulating inflammation during hypersensitivity reactions (Pohjavuori et al. 2004; Isolauri 2004). Supplementation of *Lactobacillus rhamnosus GG* during the perinatal stage in infants with eczema decreased the occurrence of disease by one half. In newly born baby, the very first bacteria which occupy the gastrointestinal tract can set up a stable niche and these bacteria are important in regulation of immune system and therefore also in developing atopic disorders. Studies on atopic children revealed that probiotics upregulate anti-inflammatory cytokines like interleukin-10.

Toh et al. (2012) suggested that the probiotics result in the stimulation of B and T cell responses specific to allergens and mucosal IgA level which reduces the effect of allergic diseases. Other studies also proposed that the association between the bacteria and host can stimulate the growth of T regulatory cells, production of cytokines like IL-10, and alteration of growth factor-beta. Toll-like receptors, a network of genes, various signaling molecules, and an increased IgA level are involved in these interactions making them quite complicated. Through these mechanisms, probiotics change the immune responses (adaptive and innate) against disease (Gourbeyre et al. 2011; McLoughlin and Mills 2011).

Probiotics are also useful for treatment of food allergies as they increase internal barriers of the intestine, reduce gut wall inflammation, and decrease the concentration of IgE in the serum (del Miraglia and De Luca 2004; Kalliomaki and Isolauri 2004). Defense barrier of the GI tract, i.e., non-immunologic and immunologic, is affected by probiotics which results in an increase in the inflammation in the case of food allergy. Consumption of *Bifidobacteria* and *Lactobacilli* enhanced the production of IgA in Peyer's patches as IgA is effective against potentially harmful antigens. Probiotics are also useful in facilitating the relief from milk protein related allergy, as these degrade the protein present in milk to smaller polypeptides or amino acids. By adding *Lactobacillus* GG in the meals of neonates on hydrolyzed whey formula, signs of eczema could be decreased (Majamaa and Isolauri 1997).

8.5.8 Antibiotic Associated Diarrhea

Antibiotic associated diarrhea (AAD) is described as diarrhea that happens due to the imbalance of microflora of GI tract caused by antibiotics. The origins of AAD are based on the alteration in the microflora of gut. The changes in the microflora of intestine reduce the number of anaerobic fecal bacteria and therefore, the breakdown of carbohydrates that causes the variations in their digestion resulting in the reduction in the assimilation of short chain fatty acids causing osmotic diarrhea (Doron et al. 2008). It was found that AAD exists in 5% to 39% of cases when patients are treated for 2 months. Antibiotics like aminopenicillins, clindamycin, and cephalosporins which act on bacteria are related with a higher possibility of AAD (Wistrom et al. 2001).

AAD can be started as mild diarrhea, however, if untreated it may further lead to a life-threatening disease called pseudomembranous colitis. In the majority of cases, the reason behind the diarrhea is unrevealed, although in the most severe forms and in patients with persistent illness, for instance, persons having inflammatory bowel disease, cystic fibrosis, and cancer, the disease-causing agent is *Clostridium difficile*. It has been observed that the frequency of AAD is lowered by the use of probiotics and their fermented products such as yogurt (Hill et al. 2014). The consumption of probiotics depends upon the theory that AAD occurs as a result of dysbiosis which is induced by antibiotics usage and that the probiotic interference also changes the microbiota of intestine. The strains of *Lactobacillus acidophilus, L. bulgaricus, L. rhamnosus,* and *Saccharomyces boulardii* have been used to restrain AAD.

Nosocomial diarrhea is prevalent worldwide in children and is a major problem. It is suggested that *Streptococcus thermophilus*, *Lactobacillus rhamnosus* GG, and *Bifidobacterium bifidum* are effective for newborn baby mainly for the elimination of acute diarrhea. Several studies suggested that several probiotics, comprising *Saccharomyces boulardii, Lactobacillus rhamnosus* GG*, L. reuteri, Bifidobacteria* spp., and others, have considerable benefits for diarrhea, travelers' diarrhea and diarrhea associated with rotaviruses (Marteau et al. 2001; Benchimol and Mack 2004; Vanderhoof 2000). Promising probiotic strains used in the treatment of diarrhea in children consist of *Lactobacillus* spp., *L. casei, L. reuteri, Bifidobacterium bifidum, Saccharomyces boulardii,* and *Streptococcus thermophilus* (Tomas et al. 2004).

Probiotics also compete for binding sites with pathogens to the intestinal lining thus preventing the infection. By the generation of certain metabolites, namely bacteriocins, probiotics reduce the growth of pathogens (del Miraglia and De Luca 2004). Several studies showed that the probiotics block the attachment of pathogenic bacteria to enterocytes. This blocking is thought to be intervened by increasing the expression of MUC2 and MUC3, intestinal mucins.

During the meta-analysis of 21 studies which include 4780 patients, it was observed that the *S. boulardii* reduces the chance of antibiotic associated diarrhea in children as well as in adult from 19 to 8.5%, with a risk ratio of 0.47. A study includes 82 randomized clinical trials with different probiotics (generally *Lactobacillus* spp., singly or in combination with *bifidobacteria*, *S. boulardii*, or *enterococci*), and a reduction in the antibiotic associated diarrhea with a risk ratio of 0.58 was observed. Most extensively used probiotics for curing the acute diarrhea are *S. boulardii, L. acidophilus, L. bulgaricus, L. rhamnosus, L. paracasei, B. longum,* and *B. breve* (Olveira and González-Moleroa 2016).

Floch et al. (2015) observed that probiotics also reduce the prevalence of illness caused by *C. difficile*, particularly when *S. boulardii* is administered. In another meta-analysis it was found that only four probiotic strains like *S. boulardii*, *L. casei* DN114001, a combination of *Bifidobacterium bifidum* and *L. acidophilus,* a mixture of *L. acidophilus*, *L. casei*, and *L. rhamnosus* considerably reduce the occurrence of diarrhea caused by *C. difficile* (McFarland 2015).

8.5.9 Inflammatory Bowel Diseases (IBD)

A group of diseases described as chronic inflammation of the gastrointestinal tract (specially the large or small intestine) are called inflammatory bowel diseases. The IBD are mainly of two types: Crohn's disease and ulcerative colitis (Iqbal et al. 2014). Chronic disease mainly damages the lower part of the small intestine and can also be seen anywhere along the lining of intestine, while damages to colon is called ulcerative colitis. In ulcerative colitis, the inflammation is limited to the intestinal epithelium, whereas in Crohn's disease the inflammation involves the entire intestinal wall (Palumbo et al. 2016). Abdominal pain, weight loss, intestinal ulcers, bleeding, and diarrhea are the symptoms of both of the cases, but narrowing of intestinal wall, fistulas, and fissures are frequent in Crohn's disease. A significant enhancement in signs of IBD, pouchitis, and ulcerative colitis with utilization of specific strains of *Lactobacilli* has been reported. Studies showed that lactic acid bacteria cause the reduction in gut pH by producing organic acids that can enhance intestinal mobility and relieve constipation.

Various studies revealed that inflammatory bowel diseases are positively modulated by gut microbiota or probiotics. With the supplementation of probiotics, prebiotics, and synbiotics, inflammatory bowel disorder can be treated (Spiller 2016). It has been reported that probiotic strains of *Lactobacillus rhamnosus* GG and *S. boulardii* enhance the level of IgA in the GI tract thus help in prevention as well as treatment of this disease.

Production of short chain fatty acids (SCFAs) mainly, acetate, butyrate, and propionate could induce positive effects in IBD. The SCFAs are recognized to perform a vital function in maintaining colonic homeostasis. These are known to improve the colonic function and also have anti-inflammatory effects (Curro et al. 2017). Hence, the consumption of dietary fibers (prebiotics) alone, or along with probiotics to boost the generation of SCFAs, might be effective for remedial approaches.

8.5.10 Antioxidant Effects

During regular metabolism, there is generation of free radicals in the human body, but the production of radicals increases significantly when our cells are unveiled to xenobiotic compounds from food and external environment. The free radicals are not neutralized by the antioxidant system of the organism efficiently thus resulting into the lethal changes (like cell death) by the oxidation of membranous lipids, enzymes/proteins, and DNA. The free radicals cause damage to the cellular and sub-cellular level which plays a significant function in the development of cancer, heart diseases, allergies, arteriosclerosis, and other diseases. The patients with Alzheimer's disease were also detected with the immense destruction of essential molecules in the brain by oxidation (Filipcik et al. 2006).

The defense reactions of antioxidant in the body are comprised not only of internal antioxidants but also of external antioxidants from the food items such as vitamins C and E, carotenoids, phytoestrogens, folates, flavonoids, and selenium. Probiotics are known to have properties of trapping the reactive forms of oxygen. Kaizu et al. (1993) suggested that the deficiency of vitamin E improved by the intracellular extract from *Lactobacillus* sp. in vitamin E-deficient rats. Probiotic bacteria, *L. delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus,* suppress the per-oxidation of lipids by removing the free radicals like hydroxyl or hydrogen peroxide.

Several bacteria found in fermented milk possess antioxidant activity which positively affects human health. Clinical studies also supported the antioxidant activity of goat fermented milk with *Lactobacillus fermentum* ME-3 strain (Songisepp et al. 2005). A group of healthy individuals were subjected to consume either sour or non-fermented 150 g of milk per day for 21 days. It was found that sour milk has extended the resistance of lipoprotein to oxidation, increased antioxidant activity of blood, and reduces the level of oxidized LDL and peroxide lipoproteins.

Certain antioxidant factors are generated by *lactobacilli* in the GI tract of human. Most of the milk bacteria by producing superoxide dismutase or glutathione eliminate the excess oxygen free radicals. The various researches are going on using milk bacteria as an option of food supplements to improve the antioxidant activity in human (Songisepp et al. 2005).

8.6 Probiotics in Oral Health

Different microorganisms like protozoa, fungi, viruses, archea, and bacteria are present in human mouth and are source of various diseases. The bacteria are generally responsible for two types of diseases: tooth decay and gum diseases. Strains of *Lactobacillus* and *Bifidobacterium* are extensively used as probiotics for oral health. A study revealed that the utilization of *Streptococcus salivarius* K12 reduces the development of dental plaque and furthermore decreases the accumulation of *Streptococcus mutans* (Burton et al. 2013). The suppression of periodontal pathogens has also been observed with the consumption of probiotic strains of *Streptococcus uberis* and *S. oralis*.

Furthermore, the consumption of probiotics can reduce the synthesis of halitosis (bad breath) and the volatile sulfur compounds. Bowen (2013) suggested that the cases of gum diseases are fewer as compared to the dental caries; however, the consumption of probiotics is found to be useful in controlling the oral diseases. Further researches are required to recognize the mechanism through which probiotics colonize and have an effect on oral cavity so that the understanding about the role of probiotics in improving oral health could be enhanced.

8.7 Probiotics and the Immune System

Utilization of probiotics like *Lactobacillus acidophilus* and *L. casei* strengthens both mucosal immunity and systemic immunity in the host. It was found that the probiotic species such as *Lactobacillus* induces the phagocytosis and stimulates the activation of T and B cells and IgM, IgA, and IgG antibodies production. Moreover, probiotics do not stimulate a considerable immune response as seen in the presence of pathogen so the consumption of probiotics on daily basis can be safe (Katelaris et al. 1995).

During the digestion process, larger particles of food are broken down into smaller particles by the enzymes so that nutrients can be assimilated by the body. Due to these enzymes, nutrients become easily available to the body and would not suffer from severe deficiencies caused by the absence of these nutrients (Land et al. 2005). Several digestive diseases, varying from gentle to harsh conditions, take place because of the non-digestible food present in the intestine. After colonization of probiotic bacteria in the gut, they take part in the digestion of food. These bacteria produce certain enzymes which increase the digestion of food, so that it can be easily assimilated. Therefore, the microflora of intestine plays an important role in the digestion, assimilation, and improvement in human health. Probiotics that are mainly present in intestine include *Lactobacillus acidophilus* and *L. casei.* These kinds of bacteria facilitate the digestion and absorption of food in the intestine and their intake balances the intestinal microflora. Consequently, they supply a number of good bacteria to the body and assist in absorption of nutrients, food digestion, and boost up the digestive system (Madsen et al. 2001). Probiotics also hamper the growth of pathogens so encourage the human health. Probiotics compete with infectious agents for food and hence for growth and expansion.

Many studies have proven that probiotics like *L. rhamnosus* strain GG and *L. plantarum* exhibit the capacity to block the adhesion of enteropathogenic *Escherichia coli* in the gastrointestinal tract (Wilson and Perini 1988). These bacteria also suppress the growth of infectious bacteria by producing the certain compounds, for example, organic acid (acetic and lactic acids) having strong inhibitory effect against pathogenic bacteria and antimicrobial compounds known as bacteriocins (Bermudez-Brito et al. 2012). Various probiotic species such as *L. acidophilus* (lactacin B), *Bifidobacterium bifidum* NCFB (bifidocin B), *L. plantarum* (plantaricin), and *Lactococcus lactis* (nisin) produce a variety of bacteriocins (Nielsen et al. 2010).

8.8 Anti-obesity Activities of Probiotics

Health is directly affected by the accumulation of excessive fat that leads to the obesity. The accumulation of fat is due to the increase in energy availability, little physical activity, and an imbalanced diet which causes disparity in energy intake and expenditure (Kobyliak et al. 2016). The intestinal flora from obese mice was when transplanted into germ-free mice they might replicate the obese phenotype and become more proficient at extracting energy from food and activating the lipogenesis.

Probiotic bacteria are known to have various properties that provide good health to the host by regulating intestinal microflora. In most cases, the sympathetic nervous system promotes the weight loss by lipolytic and thermogenic responses (Karimi et al. 2015). A study revealed that *Lactobacillus gasseri* BNR17 plays role in weight loss by limiting the secretion of leptin from adipocyte tissue (Kang et al. 2013). Various other probiotic species like *L. casei, L. acidophilus,* and *B. longum* are found to have hypocholesterolemic effects (Karimi et al. 2015).

8.9 Probiotic Products with Claimed Health Benefits

Due to the various health benefits, probiotics are gaining interest among consumers and probiotic consumption has become a global retail market with products including probiotic supplements, fermented milk products, and non-dairy based probiotic products. Among dairy based probiotic products, yogurt alone accounts for 75% of the total probiotic consumption. A range of probiotic products, dietary supplements, and therapeutic products are available in the global market (Table 8.2). The global probiotic market is expected to rise to around US$63 Billion by 2022. The main reason for this rise is the effectiveness of probiotic products in maintaining health of the consumers and disease treatment. Further, increasing health concerns, population explosion, and efficacy of probiotic products in providing health benefits are expected to complement the growth of probiotic products over the period of time.

8.10 Conclusion

Probiotic microorganisms have become well-known from the last few decades as a consequence of the continuous research that provided scientific basis for positive impacts of probiotics on human health. In addition, the rising health awareness among the people in rapidly aging global population provides an opportunity for probiotic products and ingredients to capture a large market share. Probiotic supplements are not only practiced for the prevention/treatment certain disorders but these also provide certain ingredients in the body to complement the diet. Although, a lot of scientific evidences are available on positive health effects of probiotic microorganisms, still further research is needed for strengthening the utilization of probiotics for preventive medicine and reducing the chance of many diseases.

Table 8.2 Microorganisms used in different products prepared throughout world

Species	Name of product	Company	Country
Bifidobacterium bifidum No.1 or *B. bifidum* 791	Bifidumbacterin Bifidumbacterin forte	Biomed Metchnikoff JSC, FSUC "SIC "Microgen," Patrner LTD	Russia
B. bifidum No.1 and Lysozyme	Bifilis	Ferment, LTD	London
Lactobacillus plantarum or *L. fermentum*	Lactobacterin	Biomed Metchnikoff JSC, FSUC "SIC "Microgen" IM-Bio	Russia
Enterococcus faecium L3	Laminolact	Avena, LTD	Watton, UK
L. acidophilus	Acilact	Lekko, LTD	Russia
Bacillus cereus IP 5832	Bactisubtil	Aventis Pharma International	France
L. acidophilus D-75, D-76	Vitaflor	State Institute of Fine pure Biochemicals	New York
Escherichia coli M-17	Colibacterin	FSUC "SIC "Microgen"	Russia
L. acidophilus, *B. infantis*, *E. faecium*	Linex	Sandoz, Lek	Slovenia
*B. bifidum*No.1 and *E. coli* M-17	Bificol	Biomed Metchnikoff JSC, FSUC "SIC" "Microgen"	Russia
B. longum, *E. faecium* SF68	Bifiform	Ferrosan	Denmark
L. rhamnosus HN001 (DR20) *L. acidophilus* NCFM *B. lactis* HN019 (DR10)	Sold as ingredient	Danisco	Madison, Wisconsin
B. animalis ssp. *lactis* BB-12	–	Chr. Hansen	Denmark
L. fermentum VRI003 (PCC)	Sold as ingredient	Probiomics	Eveleigh, Australia
B. animalis ssp. *lactis* HN019 (DR10)	Howaru Bifido	Danisco	Denmark
L. paracasei CRL 431 *L. acidophilus* LA5	Sold as ingredient	Chr. Hansen	Milwaukee, Wisconsin
B. longum BB536	–	Morinaga Milk Industry	Japan
B. breve strain Yakult *L. casei* strain Shirota	Yakult	Yakult	Tokyo, Japan
E. coli Nissle 1917	Mutaflor	Ardeypharm	Germany

(continued)

Table 8.2 (continued)

Species	Name of product	Company	Country
B. animalis DN173 010 ("*Bifidus regularis*")	Activia yogurt	Dannon	Tarrytown, New York
L. acidophilus LA-5	-	Chr. Hansen	Denmark
L. johnsonii Lj-1	LC1	Nestle	Lausanne, Switzerland
L. casei DN114001	Actimel/DanActive	Danone	France
L. rhamnosus 271	Sold as ingredient	Probi AB	Lund, Sweden
L. casei F19	Cultura	Arla Foods	Denmark
L. rhamnosus GG ("LGG")	Danimals Culturelle; Dannon	The Dannon Company Valio Dairy	Tarrytown, New York Helsinki, Finland
*L. paracasei*St11	Lactobacillus fortis	Nestlé	Switzerland
L. salivarius UCC118	–	University College Cork	Cork, Ireland
Lactococcus lactis L1A	–	Norrmejerier	Sweden
L. acidophilus LB	Sold as ingredient	Lacteol Laboratory	Houdan, France
L. reuteri ATTC55730	–	BioGaia Biologics	Sweden
L. rhamnosus GM-020 *L. paracasei* GMNL-33 *L. paracasei* 33	Sold as ingredient	Gen Mont Biotech	Taiwan
*L. rhamnosus*LB21	Verum	Norrmejerier	Sweden
Bacillus coagulans BC30	Sustenex, Digestive Advantage and sold as ingredient	Ganeden Biotech Inc.	Cleveland, Ohio
B. breve, Yakult	Bifiene	Yakult	Japan
S. cerevisiae boulardii	Florastor	Biocodex	Creswell, Oregon
B. infantis 35,264	Align	Procter and Gamble	Mason, Ohio
Bacillus coagulans GBI-30, 6086	GanedenBC30	Ganeden Biotech	USA
L. acidophilus R0052 *L. rhamnosus* R0011	Sold as ingredient	Institut Rosell	Montreal, Canada
E. coli M-17	ProBactrix	BioBalance	New Zealand
B. lactis Bb-12	Sold as ingredient	Chr. Hansen	Milwaukee, Wisconsin
L. acidophilus DDS-1	–	Nebraska Cultures	Walnut Creek, US

(continued)

Table 8.2 (continued)

Species	Name of product	Company	Country
L. casei DN-114001 ("L. casei Immunitas")	DanActive fermented milk	Danone	Paris, France
L. acidophilus NCFM	–	Danisco	Denmark
L. rhamnosus GR-1 *L. reuteri* RC-14	Femdophilus	Urex Biotech Jarrow Formulas Chr. Hansen	London, Ontario, Canada Los Angeles, California Milwaukee, Wisconsin
L. casei CRL431	–	Chr. Hansen	Denmark
L. plantarum 299 V	Sold as ingredient; Good Belly juice product	NextFoods Probi AB	Boulder, Colo-rado Lund, Sweden
L. casei Shirota	Yakult	Yakult	Japan
L. reuteri ATCC 55,730 ("*L. reuteri Protectis*")	BioGaia Probiotic chewable tablets or drops	BioGaia	Stockholm, Sweden
L. johnsonii La1	–	Nestlé	Switzerland
Lactococcus lactis L1A *L. rhamnosus* LB21	Sold as ingredient	Essum AB	Umea, Sweden
*L. plantarum*299V	GoodBelly/ProViva/TuZen	NextFoods Probi Ferring	–
B. longum BB536	Sold as ingredient	Morinaga Milk Industry Co. Ltd.	Zama-City, Japan
L. rhamnosus ATCC 53013	Vifit and others	Valio	Finland
L. paracasei F19	Sold as ingredient	Medipharm	Des Moines, Iowa
Saccharomyces cerevisiae (*boulardii*) lyo	DiarSafe and others	Wren Laboratories and others	United States
L. plantarum OM	Sold as ingredient	Bio-Energy Systems, Inc.	Kalispell, Montana
L. gasseri EB01 *L. rhamnosus* PBO1	EcoVag	Bifodan	Denmark
S. rattus JH145 *Streptococcus oralis* KJ3 *S. uberis* KJ2	ProBiora3 EvoraPlus	Oragenics Inc.	Alachua, Florida

References

Agrawal A, Houghton LA, Morris J, Reilly B, Guyonnet D, Goupil Feuillerat N, Schlumberger A, Jakob S, Whorwell PJ (2009) Clinical trial: the effects of a fermented milk product containing *Bifidobacterium lactis* DN-173 010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation. Aliment Pharmacol Ther 29:104–114. https://doi.org/10.1111/j.1365-2036.2008.03853.x

Aller R, De Luis DA, Izaola O, Conde R, Gonzalez Sagrado M, Primo D, De La Fuente B, Gonzalez J (2011) Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci 15:1090–1095

Amara AA, Shibl A (2015) Role of probiotics in health improvement, infection control and disease treatment and management. Saudi Pharmaceut J 23:107–114. https://doi.org/10.1016/j.jsps.2013.07.001

Azad MAK, Sarker M, Wan D (2018) Immunomodulatory effects of probiotics on cytokine profiles. Biomed Res Int 2018:1–10. https://doi.org/10.1155/2018/8063647

Aziz N, Bonavida B (2016) Activation of natural killer cells by probiotics. For Immunopathol Dis Therap 7:41–55. https://doi.org/10.1615/ForumImmunDisTher.2016017095

Benchimol EI, Mack DR (2004) Probiotics in relapsing and chronic diarrhea. J Pediatr Hematol Oncol 26:515–517. https://doi.org/10.1097/01.mph.0000133291.11443.a1

Bermudez-Brito M, Plaza-Diaz J, Munoz-Quezada S, Gómez-Llorente C, Gil A (2012) Probiotic mechanisms of action. Ann Nutr Metab 61:160–174. https://doi.org/10.1159/000342079

Bordoni A, Amaretti A, Leonardi A, Boschetti E, Danesi F, Matteuzzi D, Roncaglia L, Raimondi S, Rossi M (2013) Cholesterol lowering probiotics: In vitro selection and in vivo testing of bifidobacteria. Appl Microbiol Biotechnol 9:8273–8281. https://doi.org/10.1007/s00253-013-5088-2

Bowen DM (2013) Probiotics and oral health. J Dent Hyg 87:5–9

Burton JP, Drummond BK, Chilcott CN, Tagg JR, Thomson WM, Hale JD, Wescombe PA (2013) Influence of the probiotic *Streptococcus salivarius* strain M18 on indices of dental health in children: A randomized double-blind, placebo-controlled trial. J Med Microbiol 62:875–884. https://doi.org/10.1099/jmm.0.056663-0

Cadieux P, Burton J, Gardiner G, Braunstein I, Bruce AW, Kang CY, Reid G (2002) *Lactobacillus* strains and vaginal ecology. JAMA 287:1940–1941. https://doi.org/10.1001/jama.287.15.1935

Canani RB, Cirillo P, Terrin G, Cesarano L, Spagnuolo MI, De Vincenzo A, Albano F, Passariello A, De Marco G, Manguso F, Guarino A (2007) Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. BMJ 335:340. https://doi.org/10.1136/bmj.39272.581736.55

Cayzeele-Decherf A, Pélerin F, Leuillet S, Douillard B, Housez B, Cazaubiel M, Jacobson GK, Jüsten P, Desreumaux P (2017) *Saccharomyces cerevisiae* CNCM I-3856 in irritable bowel syndrome: an individual subject meta-analysis. World J Gastroenterol 23:336–344. https://doi.org/10.3748/wjg.v23.i2.336

Chiang CH, Lin MW, Chung MY, Yang UC (2012) The association between the IL-4, ADRb2 and ADAM 33 gene polymorphisms and asthma in the Taiwanese population. J Chin Med Assoc 75:635–643. https://doi.org/10.1016/j.jcma.2012.08.012

Choi CH, Jo SY, Park HJ, Chang SK, Byeon JS, Myung SJ (2011) A randomized, double-blind, placebo-controlled multicenter trial of *Saccharomyces boulardii* in irritable bowel syndrome: effect on quality of life. J Clin Gastroenterol 45:679–683. https://doi.org/10.1097/MCG.0b013e318204593e

Curro D, Ianiro G, Pecere S, Bibbo S, Cammarota G (2017) Probiotics, fiber and herbal medicinal products for functional and inflammatory bowel disorders. Br J Pharmacol 174:1426–1449. https://doi.org/10.1111/bph.13632

Daliria EB, Lee BH (2015) New perspectives on probiotics in health and disease. Food Sci Human Wellness 4:56–65. https://doi.org/10.1016/j.fshw.2015.06.002

Dang Y, Reinhardt JD, Zhou X, Zhang G (2014) The effect of probiotics supplementation on *Helicobacter pylori* eradication rates and side effects during eradication therapy: a meta-analysis. PLoS One 9:e111030. https://doi.org/10.1371/journal.pone.0111030

De Santis A, Famularo G, De Simone C (2000) Probiotics for the hemodynamic alterations of patients with liver cirrhosis. Am J Gastroenterol 95:323–324

Defois C, Ratel J, Garrait G, Denis S, Le Goff O, Talvas J, Mosoni P, Engel E, Peyret P (2018) Food chemicals disrupt human gut microbiota activity and impact intestinal homeostasis as revealed by in vitro systems. Sci Rep 8:11006. https://doi.org/10.1038/s41598-018-29376-9

del Miraglia GM, De Luca MG (2004) The role of probiotics in the clinical management of food allergy and atopic dermatitis. J Clin Gastroenterol 38:84–85. https://doi.org/10.1097/01.mcg.0000133293.18576.d2

Doege K, Grajecki D, Zyriax BC, Detinkina E, Zu Eulenburg C, Buhling KJ (2012) Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood–a meta-analysis. Br J Nutr 107:1–6. https://doi.org/10.1017/S0007114511003400

Dolin BJ (2009) Effects of a proprietary *Bacillus coagulans* preparation on symptoms of diarrhea-predominant irritable bowel syndrome. Methods Find Exp Clin Pharmacol 31:655–659. https://doi.org/10.1358/mf.2009.31.10.1441078

Doron SI, Hibberd PL, Gorbach SL (2008) Probiotics for prevention of antibiotic-associated diarrhea. J Clin Gastroenterol 42:58–63. https://doi.org/10.1097/MCG.0b013e3181618ab7

Drouault-Holowacz S, Bieuvelet S, Burckel A, Cazaubiel M, Dray X, Marteau P (2008) A double blind randomized controlled trial of a probiotic combination in 100 patients with irritable bowel syndrome. Gastroenterol Clin Biol 32:147–152. https://doi.org/10.1016/j.gcb.2007.06.001

Du YQ, Su T, Fan JG, Lu YX, Zheng P, Li XH, Guo CY, Xu P, Gong YF, Li ZS (2012) Adjuvant probiotics improve the eradication effect of triple therapy for *Helicobacter pylori* infection. World J Gastroenterol 18:6302–6307. https://doi.org/10.3748/wjg.v18.i43.6302

Ducrotté P, Sawant P, Jayanthi V (2012) Clinical trial: *Lactobacillus plantarum* 299v (DSM 9843) improves symptoms of irritable bowel syndrome. World J Gastroenterol 18:4012–4918. https://doi.org/10.3748/wjg.v18.i30.4012

EFSA Panel on Dietetic Products N and A (NDA) (2010) Scientific Opinion on the substantiation of health claims related to live yoghurt cultures and improved lactose digestion (ID 1143, 2976) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 8:1763–1781

Ekhlasi G, Zarrati M, Agah S, Hosseini AF, Hosseini S, Shidfar S, Aarbshahi SS, Razmpoosh E, Shidfar F (2017) Effects of symbiotic and vitamin E supplementation on blood pressure, nitric oxide and inflammatory factors in non-alcoholic fatty liver disease. EXCLI J 16:278–290. https://doi.org/10.17179/excli2016-846

Emara MH, Mohamed SY, Abdel-Aziz HR (2014) *Lactobacillus reuteri* in management of *Helicobacter pylori* infection in dyspeptic patients: a double-blind placebo-controlled randomized clinical trial. Ther Adv Gastroenterol 7:4–13. https://doi.org/10.1177/1756283X13503514

Eslamparast T, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hekmatdoost A (2014) Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. Am J Clin Nutr 99:535–542. https://doi.org/10.3945/ajcn.113.068890

Figueiredo NR, Dinkar AD, Khorate MM (2018) Probiotics in human health. Int J Orofac Biol 2:1–5. https://doi.org/10.4103/ijofb.ijofb_1_18

Filipcik P, Cente M, Ferencik M, Hulin I, Novak M (2006) The role of oxidative stress in the pathogenesis of Alzheimer's disease. Bratislavske lekarske listy 107:384–394

Fiocchi A, Burks W, Bahna SL, Bielory L, Boyle RJ, Cocco R, Dreborg S, Goodman R, Kuitunen M, Haahtela T, Heine RG (2012) Clinical use of probiotics in pediatric allergy (CUPPA): a world allergy organization position paper. World Allerg Organiz J 5:148–167. https://doi.org/10.1097/WOX.0b013e3182784ee0

Floch MH, Walker WA, Sanders ME, Nieuwdorp M, Kim AS, Brenner DA, Qamar AA, Miloh TA, Guarino A, Guslandi M, Dieleman LA, Ringel Y, Quigley EM, Brandt LJ (2015)

Recommendations for probiotic use-2015update: Proceedings and consensus opinion. J Clin Gastroenterol 49:69–73. https://doi.org/10.1016/j.iccn.2010.07.001

Ford AC, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BM, Moayyedi P (2014) Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. Am J Gastroenterol 109:1547–1561. https://doi.org/10.1038/ajg.2014.202

Ghosh AR (2012) Appraisal of probiotics and prebiotics in gastrointestinal infections. Webmed Central Gastroenterol 3:WMC003796. https://doi.org/10.9754/journal.wmc.2012.003796

Giardina S, Scilironi C, Michelotti A, Samuele A, Borella F, Daglia M, Marzatico F (2014) In vitro anti-inflammatory activity of selected oxalate-degrading probiotic bacteria: potential applications in the prevention and treatment of hyperoxaluria. J Food Sci 79:384–390. https://doi.org/10.1111/1750-3841.12344

Gill HS, Guarner F (2004) Probiotics and human health: a clinical perspective. Postgrad Med J 80:516–526. https://doi.org/10.1136/pgmj.2003.008664

Gionchetti P, Rizzello F, Morselli C, Poggioli G, Tambasco R, Calabrese C, Brigidi P, Vitali B, Straforini G, Campieri M (2007) High-dose probiotics for the treatment of active pouchitis. Dis Colon Rectum 50:2075–2084. https://doi.org/10.1007/s10350-007-9068-4

Goldenberg JZ, Yap C, Lytvyn L, Lo CK, Beardsley J, Mertz D, Johnston BC (2017) Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. Cochrane Database Syst Rev 12:CD006095. https://doi.org/10.1002/14651858.CD006095.pub4

Gourbeyre P, Denery S, Bodinier M (2011) Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. J Leukoc Biol 89:685–695. https://doi.org/10.1189/jlb.1109753

Guglielmetti S, Mora D, Gschwender M, Popp K (2011) Randomised clinical trial: Bifidobacterium bifidum MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life--a double-blind, placebo-controlled study. Aliment Pharmacol Ther 33:1123–1132. https://doi.org/10.1111/j.1365-2036.2011.04633.x

Guo Z, Liu XM, Zhang QX, Shen Z, Tian FW, Zhang H, Sun ZH, Zhang HP, Chen W (2011) Influence of consumption of probiotics on the plasma lipid profile: a meta-analysis of randomised controlled trials. Nutrition. Metabol Cardiovasc Dis 21:844–850. https://doi.org/10.1016/j.numecd.2011.04.008

Harper A, Naghibi M, Garcha D (2018) The role of bacteria, probiotics and diet in irritable bowel syndrome. Foods 7:13. https://doi.org/10.3390/foods7020013

Hata Y, Yamamoto M, Ohni M, Nakajima K, Nakamura Y, Takano T (1996) A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. Am J Clin Nutr 64:767–771. https://doi.org/10.1093/ajcn/64.5.767

Hauser G, Salkic N, Vukelic K, JajacKnez A, Stimac D (2015) Probiotics for standard triple *Helicobacter pylori* eradication: a randomized, double-blind, placebo-controlled trial. Medicine 94:e685. https://doi.org/10.1097/MD.0000000000000685

Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R, Johnsen B, Shekelle PG (2012) Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA 307:1959–1969. https://doi.org/10.1001/jama.2012.3507

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC (2014) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514. https://doi.org/10.1038/nrgastro.2014.66

Hosono A, Kashina T, Kada T (1986) Antimutagenic properties of lactic acid-cultured milk on chemical and fecal mutagens. J Dairy Sci 69:2237–2242. https://doi.org/10.3168/jds.S0022-0302(86)80662-2

Iqbal MZ, Qadir MI, Hussain T, Janbaz KH, Khan YH, Ahmad B (2014) Probiotics and their beneficial effects against various diseases. Pak J Pharm Sci 27:405–415

Isolauri E (2004) Dietary modification of atopic disease: Use of probiotics in the prevention of atopic dermatitis. Curr Allergy Asthma Rep 4:270–275. https://doi.org/10.1007/s11882-004-0070-9

Jafari E, Vahedi H, Merat S, Momtahen S, Riahi A (2014) Therapeutic effects, tolerability and safety of a multi-strain probiotic in Iranian adults with irritable bowel syndrome and bloating. Arch Iran Med 17:466–470

Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN (2015) Role of the normal gut microbiota. World J Gastroenterol 21:8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787

Johnson S, Maziade PJ, McFarland LV, Trick W, Donskey C, Currie B, Low DE, Goldstein EJ (2012) Is primary prevention of *Clostridium difficile* infection possible with specific probiotics? Int J Infect Dis 16:e786–e792. https://doi.org/10.1016/j.ijid.2012.06.005

Jørgensen MR, Kragelund C, Jensen PØ, Keller MK, Twetman S (2017) Probiotic *Lactobacillus reuteri* has antifungal effects on oral *Candida* species in vitro. J Oral Microbiol 9:1274582. https://doi.org/10.1080/20002297.2016.1274582

Kahouli I, Tomaro-Duchesneau C, Prakash S (2013) Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives. J Med Microbiol 62:1107–1123. https://doi.org/10.1099/jmm.0.048975-0

Kaizu H, Sasaki M, Nakajima H, Suzuki Y (1993) Effect of antioxidative lactic acid bacteria on rats fed a diet deficient in vitamin E. J Dairy Sci 76:2493–2499. https://doi.org/10.3168/jds.S0022-0302(93)77584-0

Kajander K, Myllyluoma E, Rajilić-Stojanović M, Kyrönpalo S, Rasmussen M, Järvenpää S, Zoetendal EG, De Vos WM, Vapaatalo H, Korpela R (2008) Clinical trial: multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. Aliment Pharmacol Ther 27:48–57. https://doi.org/10.1111/j.1365-2036.2007.03542.x

Kalliomaki MA, Isolauri E (2004) Probiotics and down-regulation of the allergic response. Immunology and Allergy. Clinics 24:739–752. https://doi.org/10.1016/j.iac.2004.06.006

Kang JH, Yun SI, Park MH, Park JH, Jeong SY, Park HO (2013) Anti-obesity effect of *Lactobacillus gasseri* BNR17 in high sucrose diet-induced obese mice. PLoS One 8:e54617. https://doi.org/10.1371/journal.pone.0054617

Karimi G, Sabran MR, Jamaluddin R, Parvaneh K, Mohtarrudin N, Ahmad Z, Khazaai H, Khodavandi A (2015) The anti-obesity effects of *Lactobacillus casei* strain *Shirota* versus Orlistat on high fat diet-induced obese rats. Food Nutr Res 59:29273. https://doi.org/10.3402/fnr.v59.29273

Katelaris PH, Salam I, Farthing MJ (1995) Lactobacilli to prevent traveler's diarrhea? N Engl J Med 333:1360–1361. https://doi.org/10.1056/NEJM199511163332016

Klanifar H, Farid R, Ahanchian H, Jabbari F, Moghiman T, Sistanian A (2009) Probiotics in the Treatment of Acute Diarrhea in Young Children. Iran J Med Sci 34:204–207

Kobyliak N, Conte C, Cammarota G, Haley AP, Styriak I, Gaspar L, Fusek J, Rodrigo L, Kruzliak P (2016) Probiotics in prevention and treatment of obesity: a critical view. Nutr Metabol 13:14. https://doi.org/10.1186/s12986-016-0067-0

Koning CJM, Jonkers DMAE, Stobberingh EE, Mulder L, Rombouts FM, Stockbrügger RW (2008) The effect of a multispecies probiotic on the intestinal microbiota and bowel movements in healthy volunteers taking the antibiotic amoxycillin. Am J Gastroenterol 103:178–189. https://doi.org/10.1111/j.1572-0241.2007.01547.x

Labayen I, Forga L, Gonzalez A, Lenoir-Wijnkoop I, Martinez JA (2001) Relationship between lactose digestion, gastrointestinal transit time and symptoms in lactose malabsorbers after dairy consumption. Aliment Pharmacol Ther 15:543–549. https://doi.org/10.1046/j.1365-2036.2001.00952.x

Lahtinen SJ, Forssten S, Aakko J, Granlund L, Rautonen N, Salminen S, Viitanen M, Ouwehand AC (2012) Probiotic cheese containing *Lactobacillus rhamnosus* HN001 and *Lactobacillus* *acidophilus* NCFM® modifies subpopulations of fecal *lactobacilli* and *Clostridium difficile* in the elderly. Age 34:133–143. https://doi.org/10.1007/s11357-011-9208-6

Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK (2005) *Lactobacillus sepsis* associated with probiotic therapy. Pediatrics 115:178–181. https://doi.org/10.1542/peds.2004-2137

Lee MC, Lin LH, Hung KL, Wu HY (2001) Oral bacterial therapy promotes recovery from acute diarrhea in children. Acta Paediatr Taiwan 42:301–305

Lee JW, Shin JG, Kim EH, Kang HE, Yim IB, Kim JY, Joo HG, Woo HJ (2004) Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. J Vet Sci 5:41–48

Lim IS, Lee HS, Kim WY (2009) The effect of lactic acid bacteria isolates on the urinary tract pathogens to infants in vitro. J Korean Med Sci 24:57–62. https://doi.org/10.3346/jkms.2009.24.S1.S57

Liu X, Cao S, Zhang X (2015) Modulation of gut microbiota-brain axis by probiotics, prebiotics, and diet. J Agric Food Chem 63:7885–7895. https://doi.org/10.1021/acs.jafc.5b02404

Lorenzo-Zúñiga V, Llop E, Suárez C, Álvarez B, Abreu L, Espadaler J, Serra J (2014) I. 31, a new combination of probiotics, improves irritable bowel syndrome-related quality of life. World J Gastroenterol 20:8709–8716. https://doi.org/10.3748/wjg.v20.i26.8709

Lunia MK, Sharma BC, Sharma P, Sachdeva S, Srivastava S (2014) Probiotics prevent hepatic encephalopathy in patients with cirrhosis: a randomized controlled trial. Clin Gastroenterol Hepatol 12:1003–1008. https://doi.org/10.1016/j.cgh.2013.11.006

Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C (2001) Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 121:580–591. https://doi.org/10.1053/gast.2001.27224

Majamaa H, Isolauri E (1997) Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol 99:179–185. https://doi.org/10.1016/S0091-6749(97)70093-9

Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, Mastrojeni S, Malaguarnera G, Mistretta A, Volti GL, Galvano F (2012) *Bifidobacterium longum* with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci 57:545–553. https://doi.org/10.1007/s10620-011-1887-4

Manfredi M, Bizzarri B, Sacchero RI, Maccari S, Calabrese L, Fabbian F, de' Angelis GL (2012) *Helicobacter pylori* infection in clinical practice: probiotics and a combination of probiotics+ lactoferrin improve compliance, but not eradication, in sequential therapy. Helicobacter 17:254–263. https://doi.org/10.1111/j.1523-5378.2012.00944.x

Mann GV (1977) A factor in yoghurt which lowers cholesteremia in man. Atherosclerosis 26:335–340. https://doi.org/10.1016/0021-9150(77)90086-7

Marchesi JR, Ravel J (2015) The vocabulary of microbiome research: a proposal. Microbiome 3. https://doi.org/10.1186/s40168-015-0094-5

Marteau P, Flourie B, Pochart F, Chastang C, Desjeux JF, Rambaud JC (1990) Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans. Br J Nutr 64:71–79. https://doi.org/10.1079/BJN19900010

Marteau P, de Vrese M, Cellier CJ, Schrezenmeir J (2001) Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr 73:430S–436S. https://doi.org/10.1093/ajcn/73.2.430s

McFarland LV (2015) Probiotics for the primary and secondary prevention of *C. difficile* infections: A meta-analysis and systematic review. Antibiotics 4:160–178. https://doi.org/10.3390/antibiotics4020160

McLean NW, Rosenstein IJ (2000) Characterisation and selection of a *Lactobacillus* species to re-colonise the vagina of women with recurrent bacterial vaginosis. J Med Microbiol 49:543–552. https://doi.org/10.1099/0022-1317-49-6-543

McLoughlin RM, Mills KH (2011) Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. J Allergy Clin Immunol 127:1097–1107. https://doi.org/10.1016/j.jaci.2011.02.012

Metchnikoff E, Metchnikoff II (1908) The Prolongation of Life: Optimistic Studies. Our post human future. Consequences of the Biotechnology Revolution. GP Putnam's Sons, New York. Available from: http://www. books. google. com/books

Michail S (2009) The role of probiotics in allergic diseases. Allergy, Asthma Clin Immunol 5:5. https://doi.org/10.1186/1710-1492-5-5

Moayyedi P, Ford AC, Talley NJ, Cremonini F, Foxx-Orenstein AE, Brandt LJ, Quigley EM (2010) The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. Gut 59:325–332. https://doi.org/10.1136/gut.2008.167270

Mott GE, Moore RW, Redmond HE, Reiser R (1973) Lowering of serum cholesterol by intestinal bacteria in cholesterol-fed piglets. Lipids 8:428–431. https://doi.org/10.1007/bf02531720

Mueller JG, Chapman PJ, Pritchard PH (1989) Creosote-contaminated sites. Their potential for bioremediation. Environ Sci Technol 23:1197–1201. https://doi.org/10.1021/es00068a003

Murch SH (2001) Toll of allergy reduced by probiotics. Lancet 357:1057–1059. https://doi.org/10.1016/S0140-6736(00)04305-1

Mustapha A, Jiang T, Savaiano DA (1997) Improvement of lactose digestion by humans following ingestion of unfermented acidophilus milk: influence of bile sensitivity, lactose transport, and acid tolerance of *Lactobacillus acidophilus*. J Dairy Sci 80:1537–1545. https://doi.org/10.3168/jds.S0022-0302(97)76083-1

Nabavi S, Rafraf M, Somi MH, Homayouni-Rad A, Asghari-Jafarabadi M (2014) Effects of probiotic yogurt consumption on metabolic factors in individuals with nonalcoholic fatty liver disease. J Dairy Sci 97:7386–7393. https://doi.org/10.3168/jds.2014-8500

Nation ML, Dunne EM, Joseph SJ, Mensah FK, Sung V, Satzke C, Tang ML (2017) Impact of *Lactobacillus reuteri* colonization on gut microbiota, inflammation, and crying time in infant colic. Sci Rep 7:15047. https://doi.org/10.1038/s41598-017-15404-7

Niedzielin K, Kordecki H, Ena Birkenfeld B (2001) A controlled, double-blind, randomized study on the efficacy of *Lactobacillus plantarum* 299V in patients with irritable bowel syndrome. Eur J Gastroenterol Hepatol 13:1143–1147

Nielsen DS, Cho GS, Hanak A, Huch M, Franz CM, Arneborg N (2010) The effect of bacteriocin-producing *Lactobacillus plantarum* strains on the intracellular pH of sessile and planktonic *Listeria monocytogenes* single cells. Int J Food Microbiol 141:S53–S59. https://doi.org/10.1016/j.ijfoodmicro.2010.03.040

Nyirjesy P, Weitz MV, Grody MHT, Lorber B (1997) Over-the-counter and alternative medicines in the treatment of chronic vaginal symptoms. Obstet Gynecol 90:50–53. https://doi.org/10.1016/S0029-7844(97)00242-1

Oberreuther-Moschner DL, Jahreis G, Rechkemmer G, Pool-Zobel BL (2004) Dietary intervention with the probiotics *Lactobacillus acidophilus* 145 and *Bifidobacterium longum* 913 modulates the potential of human faecal water to induce damage in HT29clone19A cells. Br J Nutr 91:925–932. https://doi.org/10.1079/BJN20041108

Ojetti V, Ianiro G, Tortora A, D'Angelo G, Di Rienzo TA, Bibbo S, Migneco A, Gasbarrini A (2014) The effect of *Lactobacillus reuteri* supplementation in adults with chronic functional constipation: a randomized, double-blind, placebo-controlled trial. J Gastrointestin Liver Dis 23:387–391. https://doi.org/10.15403/jgld.2014.1121.234.elr

Olveira G, González-Moleroa I (2016) An update on probiotics, probiotics and symbiotics in clinical nutrition. Endocrinol Nutr 63:482–494. https://doi.org/10.1016/j.endoen.2016.10.011

Orrhage K, Brismar B, Nord CE (1994) Effect of supplements with Bifidobacterium longum and Lactobacillus acidophilus on the intestinal microbiota during administration of clindamycin. Microb Ecol Health Dis 7:17–22. https://doi.org/10.3109/08910609409141570

Palumbo VD, Romeo M, Marino Gammazza A, Carini F, Damiani P, Damiano G, Buscemi S, Ignazio A, Monte L, Gerges-Geagea A (2016) The long-term effects of probiotics in the therapy

of ulcerative colitis: A clinical study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 160:372–377. https://doi.org/10.5507/bp.2016.044
Parvez S, Lee HC, Kim DS, Kim HY (2005) Bile salt hydrolase and cholesterol removal effect by *Bifidobacterium bifidum* NRRL 1976. World J Microbiol Biotechnol 22:455–459. https://doi.org/10.1007/s11274-005-9056-6
Pereira DI, Gibson GR (2002) Effects of consumption of probiotics and probiotics on serum lipid levels in humans. Crit Rev Biochem Mol Biol 37:259–281. https://doi.org/10.1080/10409230290771519
Phavichitr N, Puwdee P, Tantibhaedhyangkul R (2013) Cost-benefit analysis of the probiotic treatment of children hospitalized for acute diarrhea in Bangkok, Thailand. Southeast Asian J Trop Med Public Health 44:1065–1071
Plummer S, Weaver MA, Harris JC, Dee P, Hunter J (2004) *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhoea. Int Microbiol 7:59–62
Pohjavuori E, Viljanen M, Korpela R, Kuitunen M, Tiittanen M, Vaarala O, Savilahti E (2004) *Lactobacillus GG* effect in increasing IFN-gamma production in infants with cow's milk allergy. J Allergy Clin Immunol 114:131–136. https://doi.org/10.1016/j.jaci.2004.03.036
Rafeey M, Ostadrahimi A, Boniadi M, Ghorashi Z, Alizadeh M, Hadafey V (2008) *Lactobacillus acidophilus* Yogurt and Supplement in Children with Acute Diarrhea: A Clinical Trial. Res J Med Sci 2:13–18
Reid G (1995) Instillation of Lactobacillus and stimulation of indigenous organisms to prevent recurrence of urinary tract infections. Microecol Ther 23:32–45
Reid G, Bruce AW (1995) Low vaginal pH and urinary-tract infection. Lancet 346:1704. https://doi.org/10.1016/s0140-6736(95)92867-7
Reid G, Bruce AW (2001) Selection of Lactobacillus strains for urogenital probiotic applications. J Infect Dis 183:S77–S80. https://doi.org/10.1086/318841
Reid G, Bruce AW, Smeianov V (1998) The role of lactobacilli in preventing urogenital and intestinal infections. Int Dairy J 8:555–562. https://doi.org/10.1016/S0958-6946(98)00075-2
Rerksuppaphol S, Rerksuppaphol L (2015) A Randomized double blind controlled trial of Lactobacillus acidophilus Plus *Bifidobacterium bifidum* versus placebo in patients with hypercholesterolemia. J Clin Diagn Res 9:1–4. https://doi.org/10.7860/JCDR/2015/11867.5728
Rios-Covian D, Ruas-Madiedo P, Margolles A, Gueimonde M, de los Reyes-Gavilan CG, Salazar N (2016) Intestinal short chain fatty acids and their link with diet and human health. Front Microbiol 7:185. https://doi.org/10.3389/fmicb.2016.00185
Rogha M, Esfahani MZ, Zargarzadeh AH (2014) The efficacy of a synbiotic containing *Bacillus coagulans* in treatment of irritable bowel syndrome: a randomized placebo-controlled trial. Gastroenterol Hepatol Bed Bench 7:156–163
Sadeghzadeh M, Rabieefar A, Khoshnevisasl P, Mousavinasab N, Eftekhari K (2014) The effect of probiotics on childhood constipation: A randomized controlled double blind clinical trial. Int J Pediatr 2014:5. https://doi.org/10.1155/2014/937212
Saikali J, Picard V, Freitas M, Holt P (2004) Fermented milks, probiotic cultures, and colon cancer. Nutr Cancer 49:14–24. https://doi.org/10.1207/s15327914nc4901_3
Sanchez M, Darimont C, Drapeau V, Emady-Azar S, Lepage M, Rezzonico E, Ngom-Bru C, Berger B, Philippe L, Ammon-Zuffrey C, Leone P, Chevrier G, St-Amand E, Marette A, Doré J, Tremblay A (2014) Effect of *Lactobacillus rhamnosus* CGMCC1.3724 supplementation on weight loss and maintenance in obese men and women. Br J Nutr 111:1507–1519. https://doi.org/10.1017/S0007114513003875
Sanders ME (2009) How Do We Know When Something Called "Probiotic" Is Really a Probiotic? A Guideline for Consumers and Health Care Professionals. Funct Food Rev 1:3–12. https://doi.org/10.2310/6180.2009.00002
Schaafsma G, Meuling WJA, van Dokkum W, Bouley C (1998) Effects of a milk product, fermented by Lactobacillus acidophilus and with fructo-oligosaccharides added, on blood lipids in male volunteers. Eur J Clin Nutr 52:436–440. https://doi.org/10.1038/sj.ejcn.1600583

Sekine K, Watanabe-Sekine E, Ohta J (1994) Induction and activation of tumoricidal cells in vitro and (in vivo by the bacterial cell wall of B infants). Bifidobact Microflora 13:54–77. https://doi.org/10.12938/bifidus1982.13.2_65

Shida K, Sato T, Iizuka R, Hoshi R, Watanabe O, Igarashi T, Miyazaki K, Nanno M, Ishikawa F (2017) Daily intake of fermented milk with *Lactobacillus casei* strain *Shirota* reduces the incidence and duration of upper respiratory tract infections in healthy middle-aged office workers. Eur J Nutr 56:45–53. https://doi.org/10.1007/s00394-015-1056-1

Shukla S, Shukla A, Mehboob S, Guha S (2011) Meta-analysis: the effects of gut flora modulation using prebiotics, probiotics and synbiotics on minimal hepatic encephalopathy. Aliment Pharmacol Ther 33:662–671. https://doi.org/10.1111/j.1365-2036.2010.04574.x

Sinn DH, Song JH, Kim HJ, Lee JH, Son HJ, Chang DK, Kim YH, Kim JJ, Rhee JC, Rhee PL (2008) Therapeutic effect of *Lactobacillus acidophilus*-SDC 2012, 2013 in patients with irritable bowel syndrome. Dig Dis Sci 53:2714–2718. https://doi.org/10.1007/s10620-007-0196-4

Siroli L, Patrignani F, Serrazanetti DI, Parolin C, Palomino Ñ, Rogers A, Vitali B, Lanciotti R (2017) Determination of antibacterial and technological properties of vaginal lactobacilli for their potential application in dairy products. Front Microbiol 8:166. https://doi.org/10.3389/fmicb.2017.00166

Sisson G, Ayis S, Sherwood RA, Bjarnason I (2014) Randomised clinical trial: A liquid multi-strain probiotic vs. placebo in the irritable bowel syndrome--a 12 week double-blind study. Aliment Pharmacol Ther 40:51–62. https://doi.org/10.1111/apt.12787

Songisepp E, Kals J, Kullisaar T, Mändar R, Hutt P, Zilmer M, Mikelsaar M (2005) Evolution of the functional efficacy of an anti-oxidative probiotic in healthy volunteers. Nutr J 4:22–31. https://doi.org/10.1186/1475-2891-4-22

Spiller R (2016) Irritable bowel syndrome: new insights into symptom mechanisms and advances in treatment. F1000Research 5:1e11. https://doi.org/10.12688/f1000research.7992.1

Szajewska H, Skórka A (2009) *Saccharomyces boulardii* for treating acute gastroenteritis in children: updated meta-analysis of randomized controlled trials. Aliment Pharmacol Ther 30:960–961. https://doi.org/10.1111/j.1365-2036.2009.04113.x

Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, Vandenplas Y, Weizman Z (2014) Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr 58:531–539. https://doi.org/10.1097/MPG.0000000000000320

Tiwari G, Tiwari R, Pandey S, Pandey P (2012) Promising future of probiotics for human health: Current scenario. Chronicl Young Sci 3:17–28. https://doi.org/10.4103/2229-5186.94308

Toh ZQ, Anzela A, Tang ML, Licciardi PV (2012) Probiotic therapy as a novel approach for allergic disease. Front Pharmacol 3:171. https://doi.org/10.3389/fphar.2012.00171

Tomas MS, Claudia Oter M, Ocana V, Elena Nader-Macias M (2004) Production of antimicrobial substances by lactic acid bacteria I: determination of hydrogen peroxide. Methods Mol Biol 268:337–346. https://doi.org/10.1385/1-59259-766-1:337

Urbańska M, Gieruszczak-Białek D, Szajewska H (2016) Systematic review with meta-analysis: *Lactobacillus reuteri* DSM 17938 for diarrhoeal diseases in children. Aliment Pharmacol Ther 43:1025–1034. https://doi.org/10.1111/apt.13590

van de Wouw M, Schellekens H, Dinan TG, Cryan JF (2017) Microbiota-gut-brain axis: modulator of host metabolism and appetite. J Nutr 147:727–745. https://doi.org/10.3945/jn.116.240481

Vandenplas Y, Huys G, Daube G (2015) Probiotics: an update. J Pediatr 91:6–21. https://doi.org/10.1016/j.jped.2014.08.005

Vanderhoof JA (2000) Probiotics and intestinal inflammatory disorders in infants and children. J Pediatr Gastroenterol Nutr 30:S34–S38

Vonk RJ, Reckman GA, Harmsen HJ, Priebe MG (2012) Probiotics and lactose intolerance. INTECH 7:149–160. https://doi.org/10.5772/51424

Waitzberg DL, Logullo LC, Bittencourt AF, Torrinhas RS, Shiroma GM, Paulino NP, Teixeira-da-Silva ML (2013) Effect of synbiotic in constipated adult women - a randomized, double-blind,

placebo-controlled study of clinical response. Clin Nutr 32:27–33. https://doi.org/10.1016/j.clnu.2012.08.010
Weiner HL, Da Cunha AP, Quintana F, Wu H (2011) Oral tolerance. Immunol Rev 241:241–259. https://doi.org/10.1111/j.1600-065X.2011.01017.x
Wilson KH, Perini F (1988) Role of competition for nutrients in suppression of *Clostridium difficile* by the colonic microflora. Infect Immun 56:2610–2614
Winkler P, Ghadimi D, Schrezenmeir J, Kraehenbuhl JP (2007) Molecular and cellular basis of microflora host interactions. J Nutr 137:S756–S772. https://doi.org/10.1093/jn/137.3.756S
Wistrom J, Norrby SR, Myhre EB, Eriksson S, Granstrom G, Lagergren L, Englund G, Nord CE, Svenungsson B (2001) Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: A prospective study. J Antimicrob Chemother 47:43–50. https://doi.org/10.1093/jac/47.1.43
Ya W, Reifer C, Miller LE (2010) Efficacy of vaginal probiotic capsules for recurrent bacterial vaginosis: a double blind, randomized, placebo-controlled study. Am J Obstet Gynecol 203:120.e1–120.e6. https://doi.org/10.1016/j.ajog.2010.05.023
Yeun Y, Lee J (2015) Effect of a double-coated probiotic formulation on functional constipation in the elderly: a randomized, double blind, controlled study. Arch Pharm Res 38:1345–1350. https://doi.org/10.1007/s12272-014-0522-2
Yvan V, Geert H, Georges D (2015) Probiotics: An update. J de Pediatria (Rio J) 91:6–21. https://doi.org/10.1016/j.jped.2014.08.005

Chapter 9
Influence of Probiotics Over AMPK-Dependent Health Activity: A Look into Its Molecular Mechanisms

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Abstract The impact of gut microbiota on the health of the host is undeniable. Different microbial genres and strains present effects over several axes of health. Two examples are the regulation of lipids metabolism and the process of cellular aging. These health aspects have a common point: the activity of the adenosine monophosphate-activated protein kinase; this enzyme may regulate both energy homeostasis and cellular senescence. In the recent years, these aspects have gained research interest, and this has produced a growing set of information that includes outlines of the molecular mechanistic routes that the kinase may use to regulate lipids homeostasis and cellular aging. Probiotic microorganisms of the genres *Bifidobacterium* and *Lactobacillus* present more frequently an effect over AMPK activity, but the molecular effectors and the complete molecular mechanism are not yet described. In the present chapter, a general vision of these types of pathways and the molecular targets impacted by AMPK activity is presented.

Keywords Probiotics · AMPK · Lipid metabolism · Senescence

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9.1 Introduction

The interconnection of different aspects of metabolism is an intricate context, difficult to understand entirely even for those who research the metabolism phenomenon (Mou and Xie 2017). The connection between energy-related pathways, gut microbiota balance, metabolism diseases, and cell lifespan is hard to see. Nevertheless, there is a critical point in each process that can help us to build an outlook on this situation. In this chapter, a summary of different energy-related pathways, telomere structure, and consumption of probiotics are mainly related to the prevention or improvement of the consequences of metabolic disease, linked all together by a regulatory enzyme that acts over the immune system, energy balance, oxidative stress, and cellular lifespan is presented.

The intake of probiotics represents health benefits in the population that consumes them adequately (Hill et al. 2014; de Oliveira et al. 2017). The main benefits of probiotics intake or supplementation are the right balance of gut microbiota (GM), regulation of microbial metabolism, and an adequate permeability and integrity of the gut membrane (de Oliveira et al. 2017; Mishra et al. 2019). It has also been reported that the consumption of some probiotic strains regulates anti-inflammatory signaling pathways (Xie et al. 2018; Zheng et al. 2018) and provides incremented production of beneficial metabolites by the GM. A healthy microbiota helps to correctly assimilate the nutrients from the foods, like vitamins and minerals, to keep energy levels in optimal conditions; some probiotic strains produce vitamins B and K necessary for energy production at a cellular level (Arena et al. 2014).

9.2 Probiotics, AMPK, and Metabolism

As we already mentioned, GM plays a crucial role in maintaining health, and probiotics have a significant influence in this process (Ley et al. 2006; Vijay-Kumar et al. 2010; Kim et al. 2013), where homeostasis of energy is the main issue. The metabolism of energy includes lipids uptake, clearance, synthesis, hydrolysis, and secretion in the liver, skeletal muscle, and adipose tissue (Koo 2013; Ansari et al. 2018). The energy status and its control in the whole cell are mediated by the AMP-activated protein kinase (AMPK) activated by a low rate of ATP/AMP (Hardie et al. 2012). Due to its characteristics, AMPK complex is crucial in the regulation of metabolism, cell growth, autophagy, cell polarity, and it has been reported that its activation may cell stress-related prevent senescence and cell aging (Hardie 2008; Hardie et al. 2012; Jo et al. 2018). An impairment in the homeostasis of lipidic metabolism is a conditioning factor in developing obesity, metabolic syndrome, and type 2 diabetes mellitus (T2D). All these mediated by an oxidative stress-related chronic state of inflammation (Ansari et al. 2018). These facts have recently provided relevance to research about the relationship between impairments and improvements in the GM, including the probiotics use, and the energetic homeostasis. The recent research made in this issue covers the analysis of the probiotic properties for more than 400 strains (Lee et al. 2018) but some members of the genres *Lactobacillus, Clostridium,* and *Bifidobacterium* have shown exciting results (Endo et al. 2013; Kim et al. 2018; Lee et al. 2018; Lew et al. 2018).

There are reported research showing that it is possible to attenuate the impact exerted on the energetic homeostasis by obesity and other factors in preclinic models. Obesity causes impairments in the metabolism by different ways including unbalance of hormonal signals (like leptin), hyperleptinemia and its deficiency; it causes an increased endotoxins blood levels (Brun et al. 2007) as well as nonalcoholic fatty liver diseases (Spruss et al. 2009; Endo et al. 2013). These conditions establish a systemic inflammation state. An excess of fats in the diet, and a prolonged intake of alcohol, also may trigger inflammation specifically in gut and liver and unbalance of the GM that in turn may conduce to obesity, anxiety, impaired insulin sensitiveness, impaired glucose metabolism, T2D, anxiety, and colitis, among other complications. By the other hand, these harmful factors also trigger oxidative stress that may lead to liver damage, immune, behavioral, and endocrine alterations, and cancer (Lu et al. 2006; Mutlu et al. 2009; Das 2010; Ananthakrishnan et al. 2014; Szabo 2015; Moya-Perez et al. 2015; Chung et al. 2017; Sanderlin et al. 2017; Chen et al. 2018; Hor et al. 2019; Jang et al. 2019). The clear link between obesity, inflammation, and oxidative stress is fat. The mechanism if these phenomena are generally related to impairments in lipids metabolism, these make AMPK a promising therapeutic target to treat or prevent these complications, due to its vital role in the regulation of glucose and lipids metabolism from insulin secretion in β cell to a whole-body level from the hypothalamus (Aguilera et al. 2008; Rourke et al. 2017; Boone-Villa et al. 2019).

9.2.1 A Look into the Molecular Mechanism

Regarding the GM, some probiotics may secrete compounds that increase or restore AMPK activity. For example, *Clostridium butyricum* is a normal populator of the gut in healthy mammals (Kumar et al. 2009) and its strain MIYARI 588 is used as probiotic due to its capability to produce butyrate (a fatty acid that improves inflammatory disorders in the intestine by an AMPK-dependent mechanism) that rise the lipid oxidation by controlling the mitochondrial biogenesis and reduce the lipogenesis by inhibiting the transcriptional factor SREBP-1c (sterol regulatory element-binding protein 1c) and the action of ROS (Reactive Oxygen Species), lowering the oxidative stress (Endo et al. 2013). Another important probiotic genre is *Bifidobacterium*; some members of this group have been reported to help in lowering weight gain, inflammation, gastritis, pathogens proliferation, enhancing immune response, and other health-beneficial effects (Moya-Perez et al. 2015; Li et al. 2016; Park et al. 2018; Kim et al. 2019). The mechanistic pathway used by Bifidobacterium strains is related to the inactivation of the pro-inflammatory cytokines TNF-α (Tumor Necrosis Factor α) and NF-κB (Nuclear factor-κB) that ameliorates the endotoxicity produced by lipopolysaccharides (LPS) and alcohol-dependent liver and gut injuries (Kwon et al. 2017; Kim et al. 2019). The studied genre with most members that present prebiotic activity is *Lactobacillus*. Strains like *L. sakei, L. fermentum*, *L. paracasei*, *L. rhamnosus, L. brevis, L. plantarum,* and *L. helveticus* are already used as probiotics in commercial supplements (Hor et al. 2019). However, in recent years, its properties and mechanistic approaches have been studied. Mechanical routes related to the inhibition of NF-κB, LPS, SREBP-1c, and TNF-α and activation of SIRT-1 (Sirtuin-1) have been reported for *L. sakei*, all these effects are mediated by a marked AMPK activation (Hor et al. 2019; Jang et al. 2019). Dietary supplementation of milk fermented with different *Lactobacillus* strains lowered the expression of lipogenic genes (as SREBP-1c and FAS—Fatty Acids Synthase) and incrementing the relative expression of lipids-oxidation related genes (as PPARα—Peroxisome Proliferator-Activated Receptor α or ChREBP—Cholesterol Response Element Binding Protein) as well as regulation of genes of the transport and metabolism of cholesterol (like HMGR—Hydroxyl methyl glutaryl CoA reductase, LXR—Liver X Receptor, and ABCA1—ATP-Binding Cassette A1-) (Kim et al. 2013; Wa et al. 2019). There is evidence that *L. rhamnosus* also improves insulin sensitivity by increasing the relative abundance of the mRNA of insulin-dependent transporter GLUT4 (Kim et al. 2013), which is translocated to the cell membrane by the activation of AMPK (Fujii et al. 2006). Similar to these effects, *L. plantarum* is shown to improve cholesterol assimilation, improve glucose tolerance and insulin sensitivity, reduce total triglyceride, and lipids accumulation on *in vitro* and *in vivo* models with marked activation of AMPK (Lee et al. 2018; Lew et al. 2018). In a more in-depth insight, the activation of this kinase and the avoiding of NF-κB activation after a high-fat diet (HFD) ameliorate the obesity, liver steatosis, and colitis produced by the excessive lipid ingestion in a murine model. Restoration of the GM composition also accompanies these effects of the treatment with this probiotic as a reduction of the population of the genres *Firmicutes* and *Proteobacteria* that reduces the production of LPS (Kim et al. 2019). A chronic ethanol consumption may trigger inflammation in gut and liver injuries, an increased presence of *Proteobacteria* in the gut, a rise in plasma levels of triglycerides, cholesterol, hepatic aminotransferases, and LPS by activation of NF-κB (Mutlu et al. 2009; Kim et al. 2018). All these perturbations may be dealing with the use of probiotics at main or additional treatment.

The evidence of the action of probiotics to face gut inflammation, liver injuries, and their related effects show that AMPK plays a central role in the process is continuously growing. This finding is supported by metformin, an anti-diabetic drug, and lovastatin, a reducing cholesterol drug, used as pharmacological AMPK-activating controls (Lew et al. 2018; Park et al. 2018; Hor et al. 2019). The general effect of these drugs in the experimental models used (HepG2 cells, HT-29 cells, SH-SY5Y cells, Caco-2 cells, HaCaT cells, DU145 cells, U2OS cells, C2C12 cells, and Sprague-Dawley rats) consists in counteracting the consequences of HFD or chronic alcohol exposure as triggering agents for inflammation and endotoxicity. When comparing with the control drugs, probiotics treatment exerts effects similar to those of metformin and lovastatin and, in some cases, in a higher extension; these actions are blunted by AMPK inhibitors (like Compound C) and enforced by AMPK activators (like AICAR) (Lew et al. 2018; Park et al. 2018; Hor et al. 2019).

The importance of adequate balance in the GM to maintain a plenary health is guaranteed. Even there is growing evidence on the effect of a probiotic-based treatment, mainly lactic acid bacterial strains, to face factors that can damage this balance, and that these microorganisms have AMPK-related mechanisms. There is scarce information about the chemical signal that probiotics use to activate the kinase and its molecular targets or upstreaming effector, except for butyrate from *C. butyricum*. Nevertheless, we count with detailed reports about the signaling routes modulated by probiotics on diverse gut and liver diseases (Table 9.1).

9.3 Probiotics, AMPK, and Telomere Length

As we stated before, another important activity of AMPK is to protect cells against aging and senescence caused by oxidative stress, among other factors. ROS, LPS, HDF, and alcohol may trigger a decrease in AMPK signaling that can cause cellular stress and contribute to cell aging and senescence (Hardie 2008; Hardie et al. 2012; Jo et al. 2018). AMPK-related metabolic pathways avoid aging and cellular senescence by maintaining the integrity of cell telomeres (López-Otín et al. 2013; Sahin et al. 2013). The structure of telomeres is built by a repetitive DNA sequence (TTAGGG) associated with the protein complex Shelterin; this multimer gives the genomic content stability, structure, and protection (Victorelli and Passos 2017). The shortening of telomeres in somatic cells leads to a finite number of cell division, stating a “stop mark” to its development. This deterioration of telomeres is caused by inactivity of the enzyme telomerase (TERT), a ribonucleoprotein with polymerase activity, that is responsible for maintaining the length and integrity of telomeric DNA and is only found in stem, embryonic, and cancer cells (Low and Tergaonkar 2013; Ali et al. 2016; Diman et al. 2016). It has been reported that telomere transcription may be regulated through transcription factor binding sites such as nuclear respiratory factor 1 (NRF1). This mechanism reveals the link between telomeres transcription of telomeres and AMPK-dependent metabolism as a compensatory mechanism to prevent deterioration induced by telomere dysfunction (Diman et al. 2016; Finley 2018; Jo et al. 2018).

9.4 Conclusion

To conclude, the relationship between the whole body’s health and balance of GM is a well-established fact (Egert et al. 2006; Fava et al. 2006; González Hernández et al. 2019; Precup and Vodnar 2019). Nevertheless, a growing gavage of new evidence that supports the relationship between GM and specific aspects of health has

Table 9.1 Effect of probiotics over AMPK pathways

Strain	Effect/activity	AMPK molecular target	Assay (clinic or preclinic)	Reference
Clostridium butyricum MIYARI 588	Rise oxidation of lipids and reduce lipogenesis Lower oxidative stress Release butyrate	SREBP-1c Mitochondrial biogenesis ROS	Preclinic Fischer 344 rats fed an amino acid-deficient diet	Endo et al. (2013)
Lactobacillus rhamnosus GG	Improve insulin sensitivity Increase lipids oxidative gene expression Reduce lipogenic genes expression	GLUT4 PPARα CPT1 ACC1 Adiponectin PEPCK G6Pase	Preclinic BALB/c mice feed HFD	Kim et al. (2013)
Lactobacillus rhamnosus GG	Prevents lipogenesis-related gene expression. Increase in fatty acids oxidation	SREBP-1c PPARα SCD-1 PGC-1α CPT1 ACC Bax Bcl-2	Preclinic C57BL/6 N mice with chronic alcohol intake H4IIE cells	Zhang et al. (2015)
Lactobacillus spp.	Reduce colitis Reduce tumor growth Inhibit macrophage infiltration Maintain epithelial integrity	ERK Cyclin D1 Bcl-2	Preclinic BALB/c mice fed HFD treated with metformin HCT-116 cells	Chung et al. (2017)
Lactobacillus plantarum Ln4	Improve insulin sensitivity Improve glucose tolerance	IRS2 Akt LPL CD36 CRP IGFBP-3	Preclinic 3 T3-L1 cells C57BL/6J mice fed HFD	Lee et al. (2018)
Lactobacillus plantarum DR7, *Lactobacillus casei, Lactobacillus reuteri*, and *Lactobacillus fermentum* DR9.	Reduce cholesterol accumulation Rise cholesterol assimilation	HMGCR	Preclinic HepG2 cells HT-29 cells	Lew et al. (2018)
Lactobacillus acidophilus NS1	Improve lipid metabolism and insulin sensitivity	SREBP-1/PPAR-α	Preclinic C57BL/6 mice fed HFD HepG2 cells	Park et al. (2018)

(continued)

Table 9.1 (continued)

Strain	Effect/activity	AMPK molecular target	Assay (clinic or preclinic)	Reference
Lactobacillus mali APS1	Reduce hepatic lipid accumulation Increase hepatic anti-oxidant activity Increase relations *Bacteroidetes/ Firmicutes* and reduce *Escherichia coli*	SIRT-1/PGC-1α/SERBP-1 Nrf-2/HO-1	Preclinic Sprague-Dawley rats fed HFD	Chen et al. (2018)
Lactobacillus acidophilus NS1	Reduces obesity Reduces hepatic lipid accumulation Improve insulin sensitivity Reduces lipogenesis Rises lipids oxidation	SREBP-1c PPARα FAS ACC CPT1 ACOX Akt TNF-α	Preclinic C57BL/6 mice feed HFD HepG2 cells	Park et al. (2018)
Lactobacillus fermentum DR9, *Lactobacillus paracasei* OFS 0291, *Lactobacillus sakei* Probio 65 and *Lactobacillus helveticus* OFS 1515	Prevent telomere shortening Inhibit pathogens growth	Senescence gen p53	Preclinic D-galactose-induced senescent Sprague-Dawley rats HepG2 cells HT-29 cells SH-SY5Y cells Caco-2 cells HaCaT cells DU145 cells U2OS cells C2C12 cells	Hor et al. (2019)
Lactobacillus fermentum	Mitigate stress-related senescence	mTOR PI3K Akt iNOS NF-κB	Preclinic 3 T3-L1 cells	Kumar et al. (2020)
Lactobacillus rhamnosus LV108, *Lactobacillus casei* grx12, and *Lactobacillus fermentum* grx08	Improve lipid metabolism Lower lipid accumulation	SCD1 PPARα LXRα LXRβ ABCA1 ABCA5 HMGR SREBP-1c ChREBP ACC FAS	Preclinic Sprague-Dawley rats feed HFD	Wa et al. (2019)

(continued)

Table 9.1 (continued)

Strain	Effect/activity	AMPK molecular target	Assay (clinic or preclinic)	Reference
		FXR CYP7A1		
Lactobacillus sakei OK67, tOK67, and PK16	Reduce body and liver weights and blood triglyceride, cholesterol, corticosterone and LPS levels Reduce fecal Proteobacteria	SIRT-1 and SERBP-1c	Preclinic Male C57BL/6 mice fed HFD Caco-2 cells stimulated with LPS	Jang et al. (2019)
Lactobacillus plantarum LC27 and *Bifidobacterium longum* LC67	Reduce inflammation Reduce ALT, AST, TG, TC, and LPS	NF-κB SREBP-1c/PGC-1α	Preclinic Caco-2 cells stimulated with fecal lysate or LPS Male C57BL/6 mice fed HFD	Kim et al. (2019)

AMPK AMP-activated protein kinase, *SREBP-1* sterol regulatory element-binding protein, *PPAR* peroxisome proliferator-activated receptor, *PGC-1α* peroxisome proliferator-activated receptor γ coactivator protein-1α, *CPT1* carnitine palmitoyltransferase-1, *SCD1* stearoyl-CoA desaturase-1, *SIRT-1* sirtuin-1, *Nrf-2* nuclear factor erythroid 2-related factor 2, *LPS* lipopolysaccharides, *GLUT4* glucose transporter 4, *NF-κB* nuclear factor-kappa B, *TG* triglycerides, *TC* total cholesterol, *AST* aspartate transaminase, *ALT* alanine transaminase, *PEPCK* phosphoenol pyruvate carboxykinase, *G6Pase* glucose-6-phosphatase, *ACOX* peroxisomal acyl-coenzyme A oxidase, *Akt* protein kinase B, *TNF-α* tumor necrosis factor α, *HMGCR* 3-hydroxy-3-methylglutaryl-coenzyme A reductase, *LXR* liver X receptor, *FXR* farnesoid X receptor, *CYP7A1* cytochrome P450 7A1, *IGFBP-3* insulin-like growth factor binding proteins-3, *LPL* lipoprotein lipase, *CRP* C-reactive protein, *CD36* cluster of differentiation 36

appeared in the last years. Some reports demonstrate the existence and function of a GM-brain communication axis (Berer and Krishnamoorthy 2012; Kraimi et al. 2019) and published works that demonstrated a GM-liver. These communication channels have been the primary source of scientific evidence of the effect of GM over the metabolic control of the energetic homeostasis by the activation of AMPK. Although this research issue has a crescent impetus, the available literature is still scarce. As we can note from the information summarized before, most of the GM-AMPK effects impact lipid metabolism (lowering cholesterol, increasing bile acids synthesis, etc.), modulating the activity of well-defined molecular targets of AMPK like SREBP-1c, PPARα, and HMGR. Besides this, other molecules, like NF-kB or TNF-a, that are involved in different metabolic processes as inflammation or response to stress, are regulated along with AMPK activation. We can found that this behavior is not a direct action to AMPK, but is triggered by the lowering of LPS induced by the kinase (Kim et al. 2019). These kinds of findings demonstrate that more studies regarding all the molecular mechanisms implied in the effects of AMPK by GM activation are needed. It is important to note that all the information has been obtained from preclinical in vitro (cell lines) and in vivo (experimental animals) models, leaving unexplored the necessity of count with evidence from human-based studies due to the possibility of species-specific regulations.

References

Aguilera CM et al (2008) Alterations in plasma and tissue lipids associated with obesity and metabolic syndrome. Clin Sci 114(3–4):183–193. https://doi.org/10.1042/CS20070115

Ali M et al (2016) Telomerase reverse transcriptase induces basal and amino acid starvation-induced autophagy through mTORC1. Biochem Biophys Res Commun 478(3):1198–1204. https://doi.org/10.1016/j.bbrc.2016.08.094

Ananthakrishnan AN et al (2014) Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. Gut 63:776–784. https://doi.org/10.1136/gutjnl-2013-305304

Ansari AZ et al (2018) A controlled fermented Samjunghwan herbal formula ameliorates non-alcoholic hepatosteatosis in HepG2 cells and OLETF rats. Front Pharmacol 9:1–19. https://doi.org/10.3389/fphar.2018.00596

Arena MP et al (2014) Probiotic abilities of riboflavin-overproducing Lactobacillus strains: a novel promising application of probiotics. Appl Microbiol Biotechnol 98(17):7596–7581. https://doi.org/10.1007/s00253-014-5837-x

Berer K, Krishnamoorthy G (2012) Commensal gut flora and brain autoimmunity: a love or hate affair? Acta Neuropathol 123(5):639–651. https://doi.org/10.1007/s00401-012-0949-9

Boone-Villa D et al (2019) The effect of adenosine monophosphate-activated protein kinase on lipolysis in adipose tissue: an historical and comprehensive review. Arch Physiol Biochem 1:17. https://doi.org/10.1080/13813455.2019.1661495

Brun P et al (2007) Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol 292:G518–G525. https://doi.org/10.1152/ajpgi.00024.2006

Chen YT et al (2018) Sugary Kefir strain Lactobacillus mali APS1 ameliorated hepatic steatosis by regulation of SIRT-1/Nrf-2 and gut microbiota in rats. Mol Nutr Food Res 62(8):1–24. https://doi.org/10.1002/mnfr.201700903

Chung EJ et al (2017) Combination of metformin and VSL#3 additively suppresses western-style diet induced colon cancer in mice. Eur J Pharmacol 794:1–7. https://doi.org/10.1016/j.ejphar.2016.11.012

Das UN (2010) Obesity: genes, brain, gut, and environment. Nutrition 26(5):459–473. https://doi.org/10.1016/j.nut.2009.09.020

de Oliveira GLV et al (2017) Intestinal dysbiosis and probiotic applications in autoimmune diseases. Immunology 152:1–12. https://doi.org/10.1111/imm.12765

Diman A et al (2016) Nuclear respiratory factor 1 and endurance exercise promote human telomere transcription. Sci Adv 2:1–10. https://doi.org/10.1126/sciadv.1600031

Egert M et al (2006) Beyond diversity: functional microbiomics of the human colon. Trends Microbiol 14(2):86–91. https://doi.org/10.1016/j.tim.2005.12.007

Endo H et al (2013) Butyrate-producing probiotics reduce nonalcoholic fatty liver disease progression in rats: new Insight into the probiotics for the gut-liver axis. PLoS One 8(5):e63388. https://doi.org/10.1371/journal.pone.0063388

Fava F et al (2006) The gut microbiota and lipid metabolism: Implications for human health and coronary heart disease. Curr Med Chem 13(25):3005–3021. https://doi.org/10.2174/092986706778521814

Finley J (2018) Transposable elements, placental development, and oocyte activation: cellular stress and AMPK links jumping genes with the creation of human life. Med Hypotheses 118:44–54. https://doi.org/10.1016/j.mehy.2018.05.015

Fujii N, Jessen N, Goodyear LJ (2006) AMP-activated protein kinase and the regulation of glucose transport. Am J Physiol Endocrinol Metab 291(5):E867–E877. https://doi.org/10.1152/ajpendo.00207.2006

González Hernández MA et al (2019) The short-chain fatty acid acetate in body weight control and insulin sensitivity. Nutrients 11(8):1943. https://doi.org/10.3390/nu11081943

Hardie DG (2008) AMPK: a key regulator of energy balance in the single cell and the whole organism. Int J Obes 32(4):7–12. https://doi.org/10.1038/ijo.2008.116

Hardie DG, Ross FA, Hawley SA (2012) AMP-activated protein kinase: a target for drugs both ancient and modern. Chem Biol 19(10):1222–1236. https://doi.org/10.1016/j.chembiol.2012.08.019

Hill C et al (2014) Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514. https://doi.org/10.1038/nrgastro.2014.66

Hor YY et al (2019) Lactobacillus strains alleviated aging symptoms and aging-induced metabolic disorders in aged rats. J Med Food 22(1):1–13. https://doi.org/10.1089/jmf.2018.4229

Jang HM et al (2019) Lactobacillus sakei alleviates high-fat-diet-induced obesity and anxiety in mice by Inducing AMPK activation and SIRT1 expression and inhibiting gut microbiota-mediated NF-κB activation. Mol Nutr Food Res 63(6):1–38. https://doi.org/10.1002/mnfr.201800978

Jo D et al (2018) AMP-activated protein kinase regulates the expression of human telomerase reverse transcriptase. PLoS One 13(11):e0207864. https://doi.org/10.1371/journal.pone.0207864

Kim SW et al (2013) Lactobacillus rhamnosus GG improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production. Biochem Biophys Res Commun 431(2):258–263. https://doi.org/10.1016/j.bbrc.2012.12.121

Kim WG et al (2018) Lactobacillus plantarum LC27 and Bifidobacterium longum LC67 mitigate alcoholic steatosis in mice by inhibiting LPS-mediated NF-κB activation through restoration of the disturbed gut microbiota. Food Funct 9(8):4255–4265. https://doi.org/10.1039/c8fo00252e

Kim HI et al (2019) Lactobacillus plantarum LC27 and Bifidobacterium longum LC67 simultaneously alleviate high-fat diet-induced colitis, endotoxemia, liver steatosis, and obesity in mice. Nutr Res 67:78–89. https://doi.org/10.1016/j.nutres.2019.03.008

Koo S-H (2013) Nonalcoholic fatty liver disease: molecular mechanisms for the hepatic steatosis. Clin Mol Hepatol 19(3):210–215. https://doi.org/10.3350/cmh.2013.19.3.210

Kraimi N et al (2019) Influence of the microbiota-gut-brain axis on behavior and welfare in farm animals: a review. Physiol Behav 210:112658. https://doi.org/10.1016/j.physbeh.2019.112658

Kumar A et al (2009) The bacterial fermentation product butyrate influences epithelial signaling via reactive oxygen species-mediated changes in cullin-1 neddylation. J Immunol 182:538–546. https://doi.org/10.4049/jimmunol.182.1.538

Kumar R et al (2020) Cell-free culture supernatant of probiotic lactobacillus fermentum protects against H2O2-induced premature senescence by suppressing ROS-Akt-mTOR axis in murine preadipocytes. Probiotics Antimicrob Proteins 2(2):563–576. https://doi.org/10.1007/s12602-019-09576-z

Kwon EK et al (2017) Lactobacillus plantarum LC27 and Bifidobacterium longum LC67 simultaneously alleviate ethanol-induced gastritis and hepatic injury in mice. J Funct Foods 38:389–398. https://doi.org/10.1016/j.jff.2017.09.036

Lee E et al (2018) Lactobacillus plantarum strain ln4 attenuates diet-induced obesity, insulin resistance, and changes in hepatic mRNA levels associated with glucose and lipid metabolism. Nutrients 10(5):643. https://doi.org/10.3390/nu10050643

Lew LC et al (2018) Lactobacillus plantarum DR7 reduces cholesterol via phosphorylation of AMPK that down-regulated the mRNA expression of HMG-CoA reductase. Korean J Food Sci Anim Resour 38(2):350–361. https://doi.org/10.5851/kosfa.2018.38.2.350

Ley RE et al (2006) Microbial ecology: Human gut microbes associated with obesity. Nature 444:1022–1023. https://doi.org/10.1038/4441022a

Li Z et al (2016) Anti-obese effects of two Lactobacilli and two Bifidobacteria on ICR mice fed on a high fat diet. Biochem Biophys Res Commun 480(2):222–227. https://doi.org/10.1016/j.bbrc.2016.10.031

López-Otín C et al (2013) The hallmarks of aging. Cell 153(6):1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Low KC, Tergaonkar V (2013) Telomerase: central regulator of all of the hallmarks of cancer. Trends Biochem Sci 38:426–434. https://doi.org/10.1016/j.tibs.2013.07.001

Lu J et al (2006) Quercetin reverses D-galactose induced neurotoxicity in mouse brain. Behav Brain Res 171(2):251–260. https://doi.org/10.1016/j.bbr.2006.03.043

Mishra S et al (2019) Probiotics and prebiotics for the amelioration of type 1 diabetes: present and future perspectives. Microorganisms 7(3):67. https://doi.org/10.3390/microorganisms7030067

Mou L, Xie N (2017) Male infertility-related molecules involved in sperm-oocyte fusion. J Reprod Dev 63(1):1–7. https://doi.org/10.1262/jrd.2016-108

Moya-Perez A, Neef A, Sanz Y (2015) Bifidobacterium pseudocatenulatum CECT 7765 reduces obesity-associated inflammation by restoring the lymphocyte-macrophage balance and gut microbiota structure in high-fat diet-fed mice. PLoS One 10(7):e0126976. https://doi.org/10.1371/journal.pone.0126976

Mutlu E et al (2009) Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. Alcohol Clin Exp Res 33(10):1836–1846. https://doi.org/10.1111/j.1530-0277.2009.01022.x

Park SS et al (2018) Lactobacillus acidophilus NS1 attenuates diet-induced obesity and fatty liver. J Endocrinol 237(2):87–100. https://doi.org/10.1530/JOE-17-0592

Precup G, Vodnar D-C (2019) 'Gut Prevotella as a possible biomarker of diet and its eubiotic versus dysbiotic roles: a comprehensive literature review. British J Nutr 122(2):131–140. https://doi.org/10.1017/S0007114519000680

Rourke JL, Hu Q, Screaton RA (2017) 'AMPK and friends: central regulators of β cell biology. Trends Endocrinol Metab 29(2):1–12. https://doi.org/10.1016/j.tem.2017.11.007

Sahin K et al (2013) Anti-diabetic activity of chromium picolinate and biotin in rats with type 2 diabetes induced by high-fat diet and streptozotocin. Br J Nutr 110(02):197–205. https://doi.org/10.1017/S0007114512004850

Sanderlin AH, Todem D, Bozoki AC (2017) Obesity and co-morbid conditions are associated with specific neuropsychiatric symptoms in mild cognitive impairment. Front Aging Neurosci 9:164. https://doi.org/10.3389/fnagi.2017.00164

Spruss A et al (2009) Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. Hepatology 90:1094–1104. https://doi.org/10.1002/hep.23122

Szabo G (2015) Gut-liver axis in alcoholic liver disease. Gastroenterology 148(1):30–36. https://doi.org/10.1053/j.gastro.2014.10.042

Victorelli S, Passos JF (2017) Telomeres and cell senescence—size matters not. EBioMedicine 21:14–20. https://doi.org/10.1016/j.ebiom.2017.03.027

Vijay-Kumar M et al (2010) Metabolie syndrome and altered gut microbiota in mice lacking toll-like receptor 5. Science 328:258–263. https://doi.org/10.1126/science.1179721

Wa Y et al (2019) Effects of single probiotic- and combined probiotic-fermented milk on lipid metabolism in hyperlipidemic rats. Front Microbiol 10:1–9. https://doi.org/10.3389/fmicb.2019.01312

Xie Z et al (2018) Epigenetic regulation of Toll-like receptors and its roles in type 1 diabetes. J Mol Med 96(8):741–751. https://doi.org/10.1007/s00109-018-1660-7

Zhang M et al (2015) Enhanced AMPK phosphorylation contributes to the beneficial effects of Lactobacillus rhamnosus GG supernatant on chronic-alcohol-induced fatty liver disease. J Nutr Biochem 26(4):337–344. https://doi.org/10.1016/j.jnutbio.2014.10.016

Zheng P, Li Z, Zhou Z (2018) Gut microbiome in type 1 diabetes: a comprehensive review. Diabetes Metab Res Rev 34:3043. https://doi.org/10.1002/dmrr.3043

Chapter 10
Metabolic Engineering Approaches for Improvement of Probiotics Functionality

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Abstract Microbiota has an effective health promoting role which is ingested through fermented functional foods, specifically probiotic microorganisms. They are now being administered orally which in turn serves the purpose of potent alternative medication for intestinal disorders by replacing antibiotics. Most frequently used probiotics are *Lactobacilli* and *Bifidobacteria*. For much other therapeutic usage new probiotic strains are being modified to understand their mechanism of action and to strengthen them further through bioengineering. Metabolic engineering shows pathway for modulation of the organism's metabolism so that the maximum amount of desired metabolites can be extracted through genetic manipulations. This chapter concentrates mainly on modifying the genes of probiotic bacteria and their importance in health and wellness.

Keywords Metabolic engineering · Genes · Probiotics · Therapeutic usage

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10.1 Introduction

The World Health Organization elucidated probiotics as "living microorganisms" which on introduction into host body in ample amounts, bestow health benefits. In other words, probiotics are categorized in the bacterial group that keep sound the stomach and intestinal health of people. The bacteria present in fermented products (e.g., yogurt, kefir and sauerkraut) do not persist usually in the intestine of human body because of the hostile GI environment that keeps these bacteria away from the body (McFarland and Elmer 1995). Probiotics confer several perquisites that help in the regulation of intestinal health, including immune system improvement and to prevent diseases that are chronic in nature which includes example like cancer, high blood pressure, obesity, and diabetes; and its efficacy is also reflected on cognitive health.

Normally, Lactic acid bacteria and bifidobacterial strains are being used as probiotics. There is a common assumption about the effects of most probiotics about their strain specific activity. The possible method of probiotic action include: retaining intestinal mucosal resistance to diseases caused by infections; prevention of vaginitis; fabrication of antimicrobial metabolites and nutritional output; immunological modulation; helps in constipation problems and withstanding antibiotic-associated diarrhea; metal detoxification; serum cholesterol and diminishing blood pressure; prevention and relief of allergic diseases; management of atopic dermatitis; prevention of surgical wound infection and dental caries; tumor regression and lowering the production of carcinogens and mutagens (Houard et al. 2002; Kruger et al. 2002; Steidler et al. 2000; Brudnak 2002; Van Loo and Jonkers 2001; Naruszewicz et al. 2002; Rosenfeldt et al. 2003; Isolauri et al. 2002).

LABs are generally defined as a cluster of genetically diverse bacteria which includes species of the genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus,* and *Weissella*. The heterogeneous characteristic is found in the genus Lactobacillus, of which around 60 species contains guanosine and cytosine (GC) content between 34% and 53%, out of which around one third of the species are compulsorily heterofermentative (Stiles and Holzapfel 1997). The analysis of phylogenecity confirmed the existence of 54 species of the genus lactobacilli, among which 18 can be considered as probiotic. 31 species of *Bifidobacterium* were reported among which 11 were detected in human feces. Food and Drug Administration (FDA) has recognized Lactic acid bacteria and bifidobacteria in the generally recognized as safe (GRAS) category. The implication of genetic engineering technology in the field of probiotics has a trending impact in the global community of research as they are being used widely to improve characterized strains or in the development of new strains.

Some scientific reports confirm a number of ways that have been devised during the past 20 years for the introduction of specific genes of heterologous types into LAB strains (Bermúdez-Humarán et al. 2013; Foligné et al. 2013; Rondanelli et al. 2015). There are many other useful techniques for introducing selected

Lactobacillus and *Bifidobacterium* genes for improvement of probiotic activities. Reported studies confer traditional usage of antibiotic resistance genes as resistance markers, however, the usage of transferable genes providing antibiotic resistance is not applicable on food and human beings due to legal and ethical reasons. Therefore, alternative strategies have been devised for selection markers which assisted exploration of probiotic bacteria that have been derived for improvements in the field of food technology and health (Bermúdez-Humarán et al. 2013; LeBlanc et al. 2013; de Vos 1999). This chapter focuses mainly on modifying the genes of probiotic bacteria and their economic importance in food and health as they bear a long pastime record of usage in fabrication and preservation of food.

10.2 Probiotics: Current Perspectives

The probiotics have captured the market and its consumer interest specially lies in dairy products and fermented non-dairy products including other fermented foods and beverages. The selling of probiotic products has been on a rising trend between 2010 and 2014 which impacted upon the global sale that got hiked by 35% from $ 23.1 billion to $ 31.3 billion. As per reports, the statistical data of probiotics consumption were mainly obtained from Western Europe ($ 8.3 billion) as on 2014 (Bravo and Landete 2017). A rising interest in probiotic study (activity, functionality and health benefits) has been highlighted since last decade. Though there are speculations regarding its usage in health improvement of animals and human but most scientific evidences are in the favor of the benefitting side of probiotics. There is a clear picture of the benefitting effects of the probiotic microorganisms that have been proved, and further improvement can be imparted. In addition, the interesting fact is that these probiotic strains could adapt some new properties, which may increase their efficacy. Focuses on the improvement of their characteristics are getting attention and some of them are: bowel adhesion; survival in gastrointestinal conditions, and increased ability to stimulate the immune system.

10.3 Genetically Modified Organisms: A View Point

The regulations which cover GMOs are quite complex in nature and hereby, the acceptance rate of consumer is vital. A clear lack has been seen in the acceptance rate of the GMOs among the consumers that ultimately force food ingredient companies in the usage of enzymes free of GMO substances (Derkx et al. 2014). The present EU legislation consider strains evolved by natural gene transfer methods (conjugation, transduction) to be non-GMO (Sybesma et al. 2006; Johansen 2017). Microorganisms produced by “autocloning” come under the consideration of non-GMO for limited usage. The involvement of engineered vectors has also been reported, provided they consist of DNA from the same strain or closely related strains (Meacher 2000; Verstrepen et al. 2006; Landete 2017). With respect to advanced genome editing tools (CRISPR-Cas), if tool vectors are originated from target strain-related species, they can be considered as "autocloning," which is an added advantage. Though the EU court recently banished the application of new genome editing methods (including CRISPR-Cas) that would not be considered as "non-GMO" anymore, but the US has allowed the Cas9 Plants (Callaway 2018; Bron et al. 2019). More public awareness is still required by dissemination of information and thus further investigation has to be made to monitor the possible long-term effects of GMOs (Sybesma et al. 2006; Fears and Ter Meulen 2017; Johansen 2017; Csutak and Sarbu 2018).

10.4 Recombinant Probiotics

Genetic engineering can aid in improvement, introduction or elimination of bacterial phenotypes. However, the application of genetic engineered microorganisms on humans is subject to high monitoring and bounded by strict laws. The efficacy of genetic engineering can be applied in human trial, if antibiotic resistance elimination is done and probiotic bacteria retain their own modified DNA or DNA of GRAS organisms. The usage of food quality vectors originated from lactic acid bacteria or cryptic bifidobacterial plasmids can aid in the process. Their antibiotic resistance has to be replaced by mechanisms of resistance to a bacteriocin immunity (resistance to nisin or lacticin F) (Landete 2016). The cohesion or complete removal of genes from the chromosome of probiotic strain is done in site-specific recombination using the complex attP/integrase, or based on homologous recombination that depends on a suicide vector (Lin et al. 2013; Song et al. 2014). The discovery of CRISPR-cas9 system in combination with recombinant ssDNA would allow edited genome of probiotic bacteria to acquire advanced properties without antibiotic resistance (Sander and Joung 2014). There have been reports of strains with enhanced proteolytic properties that were organized by gene transfer encoding PepN, PepC, PepX, and PepI peptidases from a highly proteolytic *L. helveticus* strain (Joutsjoki et al. 2002), or *L. delbrueckii* PepI, PepL, PepW, and PepG (Wegmann 1999) into *L. lactis* using a food grade cloning system (O'Sullivan et al. 1993).

The GRAS recombinant product industries are gaining popularity and thus to meet the demand, development of food grade vectors and gene integration system is being made within the chromosome. Site-specific recombination using *attP*/ integrase system or homologous recombination can aid in the process (Lu et al. 2013). The concept of self-cloning has been eliminated from the regulations of the European Union (de Vos 1999). The development of cloning vectors derived from lactic acid bacteria and cryptic bifidobacterial plasmids would help to develop new food quality genetic engineering tools. A technique has been devised in construction of vectors which uses small cryptic plasmid replicons and incorporate selectable markers. Isolation and characterization of many cryptic plasmids have been done which have been originated from lactic acid bacterial species (Shareck et al. 2004). The CRISPR system has been used in modification of a few nucleotides in lactic acid bacteria (Oh and van Pijkeren 2014). However, the CRISPR system modified organisms do not come under the category of GMOs, but their outcomes are considered for safe usage in food manufacturing and human health (Stefanovic et al. 2017).

10.5 Recombinant Lactic Acid Bacteria

Chemically modified LAB has been utilized in induction of mutations which is single gene restricted, and includes lactococcal variants used in metabolism of lactose (McKay et al. 1972), absorption of citrate (Kempler and McKay 1979), and proteolytic activity. The screening method of color selection utilizing X-gal can be used to screen those strains which lack galactosidase. This strategy has been implemented in selection of *Lactobacillus bulgaris* strains that cannot ferment lactose in an efficient way and acidification of yogurt is also not possible post fermentation. However, additional mutations may be observed that require careful testing of other important characteristics. The technique of Recombinant DNA technology was implied to engineer gene and to overcome the problems associated with random mutagenesis, which includes example like improvement of flavor and stability of whey through the process of metabolic engineering by *L. lactis* subsp. *diacetyllactis* (Mollet 1999). Diacetylis is an example of such chemical that imparts the taste of butter in many freshly prepared dairy products. A tasteless compound named acetoin is observed in *L. lactis* which is obtained by decarboxylation of α-acetolactate by α-acetolactate decarboxylase. The gene deciphering (Inactivating the gene encoding) α-acetolactate decarboxylase, aldB aggravates the availability of α-acetolactate for chemical oxidation by inactivation (Monnet et al. 1997). An experimental procedure was conceived to segregate spontaneous aldB mutants in *L. lactis* prototype strains to acquire advantage of the imbalance in acetolactate flow between valine synthesis and acetoin catabolism in the presence of leucine (Goupil et al. 1996). This strain accumulates a large amount of α-acetolactate which help in the genesis of diacetyl that remain stable even after storage for prolonged period. The usage of this strain is viable in Denmark; but the rest of Europe consider it as a genetically modified organism (GMO) and its usage is arrested there (Henriksen et al. 1999). The genetic technology allowing the fabrication of similar B mutants by the method of direct allelic substitution was performed using an appropriate heat-sensitive vector. This approach has been made viable to obtain phage-resistant *L. lactis* that contaminates food products by activating the phage infection protein (pip) implied in phage adsorption and DNA injection.

There have been reports of different types of genetic systems which initiate the analysis and modification process in probiotic bacteria, and special emphasis have been put on LAB and other microbes of industrial interest.

10.5.1 Plasmid Vectors

Interactive plasmid, integrative vector, and a number of selectable markers have been used which pertains selection and perpetuation in the host. Plasmid vectors including pWV01, pVS40, pCI305, and pFR18 are used by combining natural plasmids with the selection markers of food grade quality. TP901-1 is used as interactive plasmid in *L. lactis* (Brondsted and Hammer 1999). It depends on homologous combination and by crossing non-replicative plasmids (integrative vector) eliminating some portion of their replication function (Emond et al. 2001). Two different kind of markers were utilized viz. selectable markers that bestow new phenotypes or metals resistance gene and other that restore a deteriorated function required for cell viability that may be inactivated on conditional terms to produce mutation of auxotrophic type.

10.5.2 Chromosomal Modification Systems

The process of the replacement of alleles on the chromosome is a natural fact because it allows genetic modifications without leaving unnecessary foreign DNA and is better than simple cross-integrating vectors. This procedure can occur as allelic substitution with the help of double crossing among two homology regions surrounding the modification and the close proximate region on the chromosome observed in *Streptococcus pneumoniae* (Campbell et al. 1998) and *Bacillus subtilis* (Dubnau and Lovett 2002). Hence, a plasmid-based thermosensitive system has been developed that permits gene substitution by following a method that includes two steps: identification of mutation in the gene encoding plasmid replication protein pWV01 that permits the plasmid to sustain at 308 °C but not at 378 °C (Maguin et al. 1992) and growth of the modified strain at 378 °C for several generations to permit segregation of plasmid. It follows replication blockage that may secure the thermosensitive plasmid vector which contains markers of selective types. This plasmid can be utilized for selection purpose of mutants of food grade quality that include a single insertion element as a new DNA fragment in the genome (Maguin et al. 1996).

10.5.3 Expression Systems

The expression systems of gene allow regulated expression of homologous or heterologous genes, and were noted to develop in *Lactococcus lactis*; based on sugar-controlled promoters (lactose operon promoter) (Payne et al. 1996), salt (gadC promoter) (Sanders et al. 1997), change temperature (techphago promoter) (Nauta et al. 1996), pH decrease (P170) (Madsen et al. 1999) or phage infection (phi31 promoter) (O'Sullivan et al. 1996). An induction system based on dosage and an expression system which depends upon sugar have also been created in various LABs (Boumerdassi et al. 1997; Swindell et al. 1996; Monteville et al. 1994). Inducible systems need proper handling, especially if production level need to remain constant which is required for metabolic control.

10.6 Metabolically Engineered Lactic Acid Bacteria

Implementation of recombinant DNA techniques in LAB metabolic engineering is useful to alter the outcome of primary and secondary metabolites. Insertion of genes or operons can be made into the chromosome by plasmids to instigate metabolic pathways of interest. Activation of other genes can be made possible by introducing DNA which impact in disrupting metabolic pathways that is of no more use. Inserted genes with homologous characteristics are desirable for primer preparation that imparts favorable resources in food fermentation. Heterologous characterized genes of LABs are viable as useful as cellular complexes for the production of worthy chemicals like, vitamins. Additionally, with recombinant DNA techniques, implementation of other methods, like chemical mutagenesis and lineage selection is vital in metabolic engineering of LAB as it acquires definite characteristics. Their growth rate is rapid, contains small genomes, has simple metabolism and is quite impactful in industries (de Vos and Hugenholtz 2004). Genome sequencing have been done from numerous LABs that are quite significant in elucidating metabolic pathways. The central metabolic pathway in LAB is glycolysis whose end product is pyruvate, which is later converted into lactate by lactate dehydrogenase, with simultaneous recycling of NAD^+ from NADH. Different kind of sugars can be a suitable source of carbon. Though glucose has got much preference, but the utilization of lactose is considerably large because of its abundant presence in milk. Pyruvate metabolism can be redirected from LAB heterofermentative compounds to flavor compounds such as diacetyl (buttermilk flavor), acetaldehyde (yogurt flavor) or alanine (sweet), which is quite significant in the dairy industry. This can be attained with the help of increasing concentration of producing enzymes or by blocking the degrading enzymes. LAB can be a useful tool in glucose production from lactose and for the production of UDP-glucose, which is a precursor of exopolysaccharides (EPS) that is quite eminent for texturing dairy products (de Vos and Hugenholtz 2004). Other products proposed include mannitol, sorbitol, tagatose, folic acid, B12, riboflavin, and some antioxidants (Hugenholtz and Smid 2002; Hugenholtz et al. 2002; Kleerebezem and Hugenholtz 2003). Metabolically engineered LABs can be roughly categorized into food fermentation initiators and cell factories for chemicals, vitamins, and secondary metabolites.

There are a lot of important characteristics which is being featured in the probiotic *Lactobacillus johnsonii* that includes its survival in the gastrointestinal tract for a long period of time, greater adhesion to intestinal cells, and probable improvement of phagocytosis. Hence, the strain modification can be achieved by deactivated lactate dehydrogenase gene which helps in producing only L (+)—lactate instead of D (−)—lactate and L (+)—lactate racemic mixture. One of the problems which mainly occur in children is acidosis which occurs due to the probable presence of D (−)-lactate and hence it should not be included in dietary habits of children and should be avoided in foods before they occur (Delley et al. 2000). There are some noted examples which cite description regarding various techniques for changing metabolism from homolactic to heterolactic, producing prudent products like diacetyl, acetoin, 2,3-butanediol, acetic acid, acetaldehyde, ethanol, and formate (Nielsen et al. 2006). There is a strategy to block pyruvate degradation and hike levels of heterolactic products by reforming pyruvate formate lyase (pfl) and acetaldehyde dehydrogenase (AdhE). The catalyzed conversion of pyruvate into acetyl coenzyme A (AcCoA) and formate occurs due to Pfl under anaerobic conditions, which might work like a shield as an antimicrobial in dairy products. Alternatively, inactivation of pfl can lead to increased pyruvate group and ensuing production of acetoin and diacetyl. A bifunctional enzyme named AdhE catalyzes the alteration of AcCoA to acetaldehyde and more to ethanol. An increased level of acetaldehyde (compound that imparts flavor to yogurt) occurs due to diminishing activity of ethanol dehydrogenase. Decreased level of activity of acetaldehyde leads to a rise in acetate that play a crucial role for food preservation. Occlusion of botanical activities would grant escalated pyruvate concentration and direct metabolic flow to acetoin and diacetyl. Aggravated AdhE activity would favor ethanol production. AdhE also has pfl deactivation activity, which provides another setpoint. Therefore, two specific engineering techniques are viable through pfl and AdhE mitigation: which will help towards greater microbial stability or greater taste, depending on the behest of the food product (Arnau et al. 2003).

10.7 Metabolically Engineered Probiotics

The applications of probiotics are increasing in food industry which shows a positive outcome of this field. Metabolic engineering strategies can be possibly deployed to design desired probiotic strains (Fig. 10.1). The field of metabolic engineering is multidisciplinary that has shown its efficacy in the production of fermented products and their increasing rate of metabolic yield. Some examples of metabolic engineering implication in industrial probiotic strains are the transcriptomic studies conducted on *lactobacilli* strains have shown efficacy in increasing production of bioactive metabolites, whereas overexpression of NADH oxidase have ameliorated the process of EPS biosynthesis in *Lactobacillus casei* LC2W. The increasing rate of activity of NADH oxidase leads to decrease NADH availability that ultimately culminate in higher yield of EPS (Li et al. 2015). Existence of residual galactose and lactose in fermented food products can generate various industrial and health problems. Comparative genomic studies of the *Lactobacillus casei* strain have proved that manifestation of the tagatose-6-phosphate pathway in the organism is a major benefactor to galactose or lactose catabolism. Therefore, the study showed

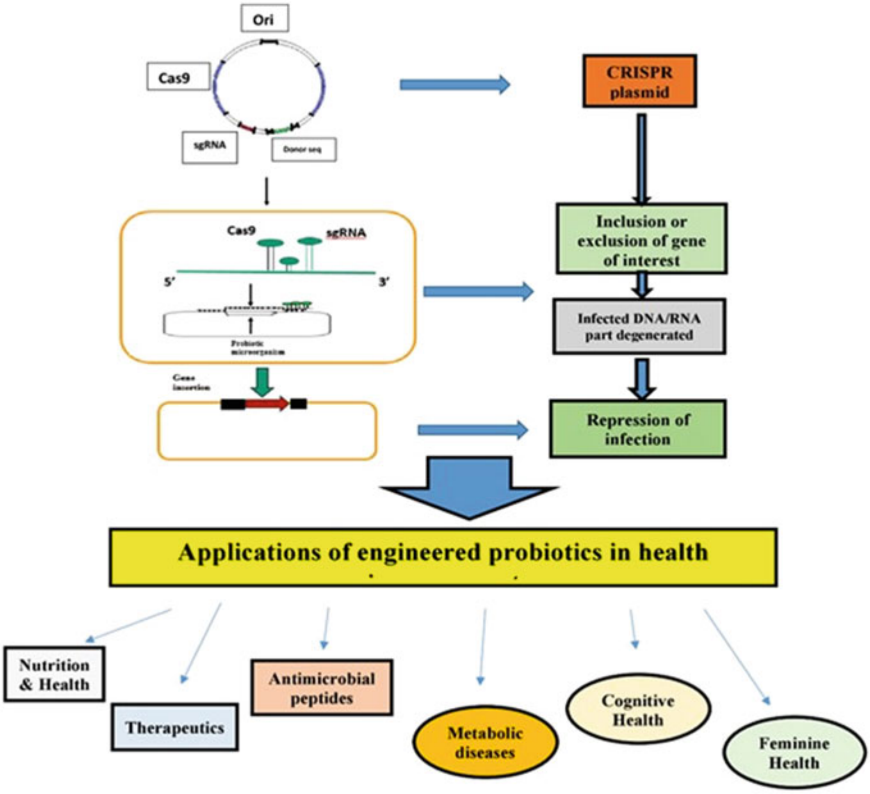

Fig. 10.1 Engineering of probiotics using CRISPR Cas technology for its applications in health

that the due presence of tagatose-6-phosphate pathway, *L. casei* could cut down galactose aggregation in fermented food products.

10.8 Designer Probiotics: Therapeutic Applications

Engineered lactic acid bacteria has found its usage not only in the dairy industry but also in the additional role in the production of proteins and also in food fermentation industries which produce vitamin B, diacetyl, acetaldehyde, and folic acid (Sybesma et al. 2003). Recombinant metabolites and proteins manufactured with the help of engineered organisms do not require vectors of food quality in production process but they are of utmost usage when co-administered to humans or animals. The concept of oral vector evolved using lactic acid bacteria and bifidobacteria as they offer resistance to acid and remain adhered to the epithelium of the mucosa (Guo et al. 2015; Hoang et al. 2015; Landete 2016). These bacteria also nurture genes that express therapeutic molecules, like interleukins which can be administered orally (Steidler et al. 1998, 2000). A potential probiotic strain named *Lactobacillus acidophilus* NCK56 has been assembled with phosphoglycerol transferase deletion after integration by double crossing and excision that eliminate antibiotic resistance (Mohamadzadeh et al. 2011). Khazaie et al. (2013) reported that *acidophilus* helps in regulation of inflammation and provide safeguard against colonic polyposis exhibited in mouse model. Integration of food quality vectors into the chromosome produce more stable vectors. Example of increasing application of food quality vectors includes an integrated chromosomal expression system in *Lactobacillus paracasei* as described by Martín et al. (2011) which was based upon aggregation promoter gene (apf) of *Lactobacillus crispatus* (Marcotte et al. 2004).

Metabolic disorders treatment is quite expensive and require strict adherence that affects patients' lifestyle and survival rates. On the contrast, gastrointestinal tract of patients with full of engineered microbes helps in treating various diseases and provide long-lasting benefits. It has been estimated that by 2025, over a billion people around the globe will live with hypertension, hence some urgent redressal is required against the problem. Angiotensin converting enzyme inhibitor peptides (ACEIP) may help in relaxation of blood vessels and decrease water reabsorption by the kidney that ultimately help in lowering of blood pressure.

The ACEIP is administered with the help of an engineered strain NC8 of *Lactobacillus plantarum*, where the coding sequences of tuna structure protein (TFP) and albacore structure protein (YFP) are integrated by an arginine ligand that impacted in reduction of systolic blood pressure, endothelin, and angiotensin II in spontaneously hypertensive rats (Yang et al. 2015). Altered *L. plantarum* exhibited antihypertensive properties in vivo that lasted for an additional period of 10 days as compared to the traditional ACEIPs and no side effects were observed. The fabrication of *Lactococcus lactis NZ9000*, which expresses the HSP65-6P277 fusion protein, prevented the occurrence of Diabetes Mellitus 1 (DM1) in non-obese diabetic (NOD) mice and exhibited significantly ameliorated glucose tolerance and reduced insulinitis (Ma et al. 2014). Engineered bacteria can help in checking type 2 diabetes mellitus (DM2). Glucagon-like peptide 1 (GLP-1) is an incretin-derived hormone which shows advantageous effects on pancreatic function but it has a short half-life. However, their potential therapeutic application can be achieved when commensal bacteria such as *Bifidobacterium longum* are designed to express and secrete biologically active GLP-1 directly into the colon as a penetrating fusion protein of GLP-1 (Wei et al. 2015).

It has been observed that the count of multiresistant pathogens are on the rise, whereas the antibiotic options are getting reduced. Hence, an emergence of finding alternative methods has been raised to combat deadly microorganisms (Theuretzbacher 2012; Spellberg et al. 2015). The usage of engineered probiotics is showing the pathway as an alternative option as it has several advantages which includes greater specificity, regulated release of antimicrobial agents, and a narrow risk of developing antibiotic resistance (Hwang et al. 2014). Multidrug resistant *Enterococcus sp.* was targeted by using genetically engineered *L. lactis*. The major function of *L. lactis* is to identify multidrug-resistant *E. faecalis* (Geldart et al. 2015).

It was designed to produce bacteriocins under the control of a chloride-induced promoter that gets triggered in the intestinal tract to attack and to sense *E. faecium* cCF10 pheromone (Geldart et al. 2015). *Clostridium difficile* is a kind of pathogen that is responsible for antibiotic-associated diarrhea in the developed countries. *C. difficile* infection can be treated by fecal microbiota transplantation. The virulence factors of engineered *L. lactis* express non-toxic TcdA and TcdB fragments of *C. difficile* which develop an immunity against this pathogen in vivo (Guo et al. 2015). In a similar study, permuted *Bacillus subtilis* spores were outlined to express *H. pylori* urease B protein on the surface (Zhou et al. 2015). Recombinant spores produced were observed to generate a humoral response in mice that notably reduce *H. pylori* burden. *B. subtilis* spores were also designed to express *Mycobacterium tuberculosis* antigens for the development of a tuberculosis vaccine (Das et al. 2016). Designed probiotics has also found its application as vaccines that stand against virus such as HIV. Kuczkowska et al. (2015) and Chamcha et al. (2015) expressed an HIV Gag antigen on the facet of a lactic acid bacterium that exhibited an initiation of immune response that established its potential usage as vaccine.

A project was undertaken by World Health Organization (WHO) on Global Cancer Observatory in 2012, which estimated 14 million new incidents of cancer that is expected to be increased by 70% over the next 20 years. Usage of bacteria in treatment of cancer was evolved in the nineteenth century (Hoffman and Zhao 2014). Reports of the microbiologists have suggested a number of diversified anaerobes over the years which preferentially proliferate in solid tumors (Taniguchi et al. 2010) that encourage application of microbes as conveyors of anticancer agents. Assorted anaerobes that were analyzed included the designed strains like *Clostridium*, *Bifidobacterium*, *Salmonella,* and *Escherichia.* A recent study reported the success of engineered *B. longum* expressing herpes simplex virus thymidine kinase (HSV-TK), that was used in fusion with ganciclovir (GCV), has proved to be a well-studied gene therapy of cancer (Zhou et al. 2016; Wang et al. 2016). Genetically modified microbes co-administered with GCV exhibited shielding effects against colon, liver, stomach, and breast tumor xenografts in mice. Another facultative anaerobe that was commonly designed in cancer treatment is *Salmonella spp*.

10.9 Conclusions and Future Prospects

The usage of LABs has not only been confined as food starter microorganisms, but has also proven its efficacy in improvement of health. The probiotics are gaining popularity in the recent years and is getting public attention as scientific evidences are finding out strong roots. Consumer role is crucial as they acquire the deciding phase regarding intake of GM foods. For the decision to be made precisely, their easy accession of updated information on GMF and GMO technologies is vital. The inception of heterologous genes is paving the pathway in improvement of their functionality. Though there are some ethical problems associated to GMOs that should be treated with wariness. Noted scientific progress has been observed in the usage of food grade selection markers, chromosomal integration, and in implementation of LAB genes. Independent strains have been developed which is not transferable to the environment and has paved the way for clinical applications. The current research of interest is primarily focusing on various species of *Lactobacillus*, though application of other genera like *Bifidobacterium* may be hiked in the future. One of the first applications that was proposed for usage of recombinant LAB was oral vaccine administration. For conducting human clinical trials, a large number of ongoing studies and substantial data accumulation has to be done for effective transferring of the technology. Many new approaches have been introduced which may include selection of specific immune cells by co-expression of affinity molecules or by increasing the immune response by co-expression of molecular adjuvants. The delivery of biologically active proteins for the treatment of chronic diseases has been a notable example of the application of bioengineered LAB. LABs, especially *L. lactis*, have also proven to be potent cell factories with considerable metabolic engineering competency. Expecting level for further exploration of the genus *Lactobacillus* will increase in the future. However, with the increasing number of new applications and considerable advantages of LAB, it is expected that their therapeutic and industrial importance will be significantly hiked.

References

Arnau J, Israelsen H, Vrang A, Joergensen F, Madsen SM (2003) Metabolically engineered lactic acid bacteria and means for providing same. US20030199035A167A2

Bermúdez-Humarán LG, Aubry C, Motta JP, Deraison C, Steidler L, Vergnolle N (2013) Engineering *lactococci* and *lactobacilli* for human health. Curr Opin Microbiol 16:278–283

Boumerdassi H et al (1997) Isolation and properties of *Lactococcus lactis* subsp. lactis biovar diacetylactis CNRZ483 mutants producing diacetyl and acetoin from glucose. Appl Environ Microbiol 63:2293–2299

Bravo D, Landete JM (2017) Genetic engineering as a powerful tool to improve probiotic strains. Biotechnol Genet Eng Rev 33(2):173–189

Bron PA, Marcelli B, Mulder J et al (2019) Renaissance of traditional DNA transfer strategies for improvement of industrial lactic acid bacteria. Curr Opin Biotechnol 56:61–68

Brondsted L, Hammer K (1999) Use of the integration elements encoded by the temperate lactococcal bacteriophage TP901-1 to obtain chromosomal single-copy transcriptional fusion in *Lactococcus lactis*. Appl Environ Microbiol 65:752–758

Brudnak MA (2002) Probiotics as an adjuvant to detoxification protocols. Med Hypotheses 58:382–385

Callaway E (2018) CRISPR plants now subject to tough GM laws in European Union. Nature 560:16–16

Campbell EA et al (1998) A competence regulon in *Streptococcus pneumoniae* revealed by genomic analysis. Mol Microbiol 27:929–939

Chamcha V, Jones A, Quigley BR, Scott JR, Amara RR (2015) Oral immunization with a recombinant *Lactococcus lactis*- expressing HIV-1 antigen on Group A Streptococcus pilus induces strong mucosal immunity in the gut. J Immunol 195:5025–5034

Csutak O, Sarbu I (2018) Chapter 6. Genetically modified microorganisms: harmful or helpful? Elsevier, Cambridge

Das K, Thomas T, Garnica O, Dhandayuthapani S (2016) Recombinant *Bacillus subtilis* spores for the delivery of *Mycobacterium tuberculosis* Ag85B-CFP 10 secretory antigens. Tuberculosis (Edinb) S101:S18–S27

de Vos WM (1999) Safe and sustainable systems for food-grade fermentations by genetically modified lactic acid bacteria. Int Dairy J 9:3–10

de Vos WM, Hugenholtz J (2004) Engineering metabolic highways in lactococci and other lactic acid bacteria. Trends Biotechnol 22:72–79

Delley M, Germond JE, Lapierre L, Mollet B, Pridmore RD (2000) Recombinant sequence-modified *L. johnsonii* bacteria which produce only + L()-lactate. US6110725A

Derkx PM, Janzen T, Sørensen KI et al (2014) The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology. Microb Cell Factories 13:S5

Dubnau D, Lovett CM (2002) Transformation and recombination. In: Sonenehein AL et al (eds) *Bacillus subtilis* and its closest relatives: from gene to cells. ASM Press, Washington, pp 453–471

Emond E et al (2001) Molecular characterization of a theta replication plasmid and its use for development of a two-component food-grade cloning system for *Lactococcus lactis*. Appl Environ Microbiol 67:1700–1709

Fears R, Ter Meulen V (2017) How should the applications of genome editing be assessed and regulated? eLife 6:e26295. https://doi.org/10.7554/eLife.26295

Foligné B, Daniel C, Pot B (2013) Probiotics from research to market: the possibilities, risks and challenges. Curr Opin Microbiol 16:284–292

Geldart K, Borrero J, Kaznessis YN (2015) Chloride-inducible expression vector for delivery of antimicrobial peptides targeting antibiotic-resistant *Enterococcus faecium*. Appl Environ Microbiol 81:3889–3897

Goupil N et al (1996) Imbalance of leucine flux in *Lactococcus lactis* and its use for the isolation of diacetyl-overproducing strains. Appl Environ Microbiol 62:2636–2640

Guo S, Yan W, McDonough SP, Lin N, Wu KJ, He H, Xiang H, Yang M, Moreira MA, Chang YF (2015) The recombinant *Lactococcus lactis* oral vaccine induces protection against *C. difficile* spore challenge in a mouse model. Vaccine 33:1586–1595

Henriksen CM et al (1999) Application of genetically modified microorganisms: gene technology at Chr Hansen A/S. Int Dairy J 9:17–24

Hoang PM, Cho S, Kim KE, Byun SJ, Lee TK, Lee S (2015) Development of *Lactobacillus paracasei* harboring nucleic acid-hydrolyzing 3D8 scFv as a preventive probiotic against murine norovirus infection. Appl Microbiol Biotechnol 99:2793–2803

Hoffman RM, Zhao M (2014) Methods for the development of tumor-targeting bacteria. Expert Opin Drug Discovery 9:741–750

Houard S et al (2002) Engineering of non-conventional yeasts for efficient synthesis of macromolecules: the methylotrophic genera. Biochimie 84:1089–1093

Hugenholtz J, Smid EJ (2002) Nutraceutical production with food-grade microorganisms. Curr Opin Biotechnol 13:497–507

Hugenholtz J, Sybesma W, Groot MN et al (2002) Metabolic engineering of lactic acid bacteria for the production of nutraceuticals. Anton Leeuw 82:217–235

Hwang IY, Tan MH, Koh E, Ho CL, Poh CL, Chang MW (2014) Reprogramming microbes to be pathogen-seeking killers. ACS Synth Biol 3:228–237

Isolauri E et al (2002) Functional foods and probiotics: working group report of the first world congress of pediatric gastroenterology and nutrition. J Pediatr Gastroenterol Nutr 35:S106–S109

Johansen E (2017) Future access and improvement of industrial lactic acid bacteria cultures. Microb Cell Factories 16:1–5

Joutsjoki V et al (2002) Recombinant Lactococcus starters as a potential source of additional peptidolytic activity in cheese ripening. J Appl Microbiol 92:1159–1166

Kempler GM, McKay LL (1979) Characterization of plasmid deoxyribonucleic acid in *Streptococcus lactis subsp. diacetylactis*: evidence for plasmid-linked citrate utilization. Appl Environ Microbiol 37:316–323

Khazaie K, Zadeh M, Khan MW, Bere P, Gounari F, Dennis K, Mohamadzadeh M (2013) Abating colon cancer polyposis by *Lactobacillus acidophilus* deficient in lipoteichoic acid. Proc Natl Acad Sci 109:10462–10467
Kleerebezem M, Hugenholtz J (2003) Metabolic pathway engineering in lactic acid bacteria. Curr Opin Biotechnol 14:232–237
Kruger C et al (2002) In situ delivery of passive immunity by lactobacilli producing single-chain antibodies. Nat Biotechnol 20:702–706
Kuczkowska K, Mathiesen G, Eijsink VG, Oynebraten I (2015) *Lactobacillus plantarum* displaying CCL3 chemokine in fusion with HIV-1 Gag derived antigen causes increased recruitment of T cells. Microb Cell Factories 14:169
Landete JM (2016) Effector molecules and regulatory proteins: applications. Trends Biotechnol 34 (10):777–780
Landete JM (2017) A review of food-grade vectors in lactic acid bacteria: from the laboratory to their application. Crit Rev Biotechnol 37:296–308
LeBlanc JG, Aubry C, Cortes-Perez NG, de Moreno de LeBlanc A, Vergnolle N, Langella P, Bermúdez-Humarán LG (2013) Mucosal targeting of therapeutic molecules using genetically modified lactic acid bacteria: an update. FEMS Microbiol Lett 344:1–9
Li C, Guan X, Du T et al (2015) Inhibition of HIV-1 infection of primary CD4þ T-cells by gene editing of CCR5 using adenovirus-delivered CRISPR/Cas9. J Gen Virol 96:2381–2393
Lin CF, Lo TC, Kuo YC, Lin TH (2013) Stable integration and expression of heterologous genes in several lactobacilli using an integration vector constructed from the integrase and attP sequences of phage ΦAT3 isolated from *Lactobacillus casei* ATCC 393. Appl Microbiol Biotechnol 97:3499–3507
Lu W, Kong J, Kong W (2013) Construction and application of a food-grade expression modified lactic acid bacteria. Int Dairy J 9:3–10
Ma Y, Liu J, Hou J, Dong Y, Lu Y, Jin L, Cao R, Li T, Wu J (2014) Oral administration of recombinant *Lactococcus lactis* expressing HSP65 and tandemly repeated P277 reduces the incidence of type I diabetes in non-obese diabetic mice. PLoS One 9:e105701
Madsen SM et al (1999) Molecular characterization of the pH-inducible and growth phase-dependent promoter P170 of *Lactococcus lactis*. Mol Microbiol 32:75–87
Maguin E et al (1992) New thermosensitive plasmid for gram-positive bacteria. J Bacteriol 174:5633–5638
Maguin E et al (1996) Efficient insertional mutagenesis in lactococci and other gram-positive bacteria. J Bacteriol 178:931–935
Marcotte H, Ferrari S, Cesena C, Hammarström L, Morelli L, Pozzi G, Oggioni MR (2004) The aggregation-promoting factor of *Lactobacillus crispatus* M247 and its genetic locus. J Appl Microbiol 97:749–756
Martín MC, Pant N, Ladero V, Günaydın G, Andersen KK, Álvarez B, Marcotte H (2011) Integrative expression system for delivery of antibody fragments by lactobacilli. Appl Environ Microbiol 77:2174–2179
McFarland LV, Elmer GW (1995) Biotherapeutic agents: past, present and future. Microecol Ther 23:46–73
McKay LL et al (1972) Loss of lactose metabolism in lactic streptococci. Appl Microbiol 23:1090–1096
Meacher M (2000) The genetically modified organisms (contained use) regulations 2000. http://www.legislation.gov.uk/uksi
Mohamadzadeh M, Pfeiler EA, Brown JB, Zadeh M, Gramarossa M, Managlia E, Klaenhammer TR (2011) Regulation of induced colonic inflammation by *Lactobacillus acidophilus* deficient in lipoteichoic acid. Proc Natl Acad Sci 108:4623–4630
Mollet B (1999) Genetically improved starter strains: opportunities for the dairy industry. Int Dairy J 9:11–15

Monnet C et al (1997) Development and use of a screening procedure for production of alpha-acetolactate by *Lactococcus lactis* subsp. lactis biovar diacetylactis strains. Appl Environ Microbiol 63:793–795

Monteville MR et al (1994) Lactococcal bacteriophages require a host cell wall carbohydrate and a plasma membrane protein for adsorption and ejection of DNA. Appl Environ Microbiol 60:3204–3211

Naruszewicz M et al (2002) Effect of *Lactobacillus plantarum* 299 von cardiovascular disease risk factors in smokers. Am J Clin Nutr 76:1249–1255

Nauta A et al (1996) Inducible gene expression mediated by a repressor-operator system isolated from *Lactococcus lactis* bacteriophage r1t. Mol Microbiol 19:1331–1341

Nielsen J, Nissen TL, Kielland-Brandt MC (2006) Metabolically engineered microbial cell with an altered metabolite production. US20067018829

O'Sullivan DJ et al (1993) Effect of increasing the copy number of bacteriophage origins of replication, in trans, on incoming-phage proliferation. Appl Environ Microbiol 59:2449–2456

O'Sullivan DJ et al (1996) Development of an expression strategy using a lytic phage to trigger explosive plasmid amplification and gene expression. Biotechnology (N Y) 14:82–87

Oh JH, van Pijkeren JP (2014) CRISPR–Cas9-assisted recombineering in *Lactobacillus reuteri*. Nucleic Acids Res 42(17):e131. https://doi.org/10.1093/nar/gku623

Payne J et al (1996) Exploitation of a chromosomally integrated lactose operon for controlled gene expression in *Lactococcus lactis*. FEMS Microbiol Lett 136:19–24

Rondanelli M, Giacosa A, Faliva MA, Perna S, Allieri F, Castellazzi AM (2015) Review on microbiota and effectiveness of probiotics use in older. World J Clin Cases 3:156–162

Rosenfeldt V et al (2003) Effect of probiotic Lactobacillus strains in children with atopic dermatitis. J Allergy Clin Immunol 111:389–395

Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32:347–355

Sanders JW et al (1997) A chloride-inducible gene expression cassette and its use in induced lysis of *Lactococcus lactis*. Appl Environ Microbiol 63:4877–4882

Shareck J, Choi Y, Lee B, Miguez CB (2004) Cloning vectors based on cryptic plasmids isolated from lactic acid bacteria: their characteristics and potential applications in biotechnology. Crit Rev Biotechnol 24:155–208

Song L, Cui H, Tang L, Qiao X, Liu M, Jiang Y, Li Y (2014) Construction of upp deletion mutant strains of *Lactobacillus casei* and *Lactococcus lactis* based on counter selective system using temperature-sensitive plasmid. J Microbiol Methods 102:37–44

Spellberg B, Bartlett J, Wunderink R, Gilbert DN (2015) Novel approaches are needed to develop tomorrow's antibacterial therapies. Am J Respir Crit Care Med 191:135–140

Stefanovic E, Fitzgerald G, McAuliffe O (2017) Advances in the genomics and metabolomics of dairy lactobacilli: a review. Food Microbiol 61:33–49

Steidler L, Robinson K, Chamberlain L, Schofield KM, Remaut E, Le Page RW, Wells JM (1998) Mucosal delivery of murine interleukin-2 (IL-2) and IL-6 by recombinant strains of *Lactococcus lactis* coexpressing antigen and cytokine. Infect Immun 66:3183–3189

Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, Remaut E (2000) Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. Science 289:1352–1355

Stiles ME, Holzapfel WH (1997) Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol 36:1–29

Swindell SR et al (1996) Genetic manipulation of the pathway for diacetyl metabolism in *Lactococcus lactis*. Appl Environ Microbiol 62:2641–2643

Sybesma W, Starrenburg M, Kleerebezem M et al (2003) Increased production of folate by metabolic engineering of *Lactococcus lactis*. Appl Environ Microbiol 69:3069–3076

Sybesma W, Hugenholtz J, De Vos WM et al (2006) Safe use of genetically modified lactic acid bacteria in food. Bridging the gap between consumers, green groups, and industry. Electron J Biotechnol 9:424

Taniguchi S, Fujimori M, Sasaki T, Tsutsui H, Shimatani Y, Seki K, Amano J (2010) Targeting solid tumors with non-pathogenic obligate anaerobic bacteria. Cancer Sci 101:1925–1932

Theuretzbacher U (2012) Accelerating resistance, inadequate antibacterial drug pipelines and international responses. Int J Antimicrob Agents 39:295–299

Van Loo J, Jonkers N (2001) Evaluation in human volunteers of the potential anticarcinogenic activities of novel nutritional concepts: prebiotics, probiotics and synbiotics (the SYNCAN project QLK1-1999-00346). Nutr Metab Cardiovasc Dis 11(Suppl. 4):87–93

Verstrepen KJ, Chambers PJ, Pretorius IS (2006) The development of superior yeast strains for the food and beverage industries: challenges, opportunities and potential benefits. In: Querol A, Fleet G (eds) Yeasts in food and beverages. Springer, Berlin, pp 399–444

Wang C, Ma Y, Hu Q, Xie T, Wu J, Zeng F, Song F (2016) Bifidobacterial recombinant thymidine kinase-ganciclovir gene therapy system induces FasL and TNFR2 mediated antitumor apoptosis in solid tumors. BMC Cancer 16:545

Wegmann U (1999) Introduction of peptidase genes from *Lactococcus delbrueckii subsp. lactis* into *Lactococcus lactis* and controlled expression. Appl Environ Microbiol 65:4729–4733

Wei P, Yang Y, Li T, Ding Q, Sun H (2015) A engineered *Bifidobacterium longum* secreting a bioactive penetratin-glucagon-like peptide 1 fusion protein enhances glucagon-like peptide 1 absorption in the intestine. J Microbiol Biotechnol. https://doi.org/10.4014/jmb.1412.12030

Yang G, Jiang Y, Yang W, Du F, Yao Y, Shi C, Wang C (2015) Effective treatment of hypertension by recombinant *Lactobacillus plantarum* expressing angiotensin converting enzyme inhibitory peptide. Microb Cell Factories 14:202

Zhou Z, Gong S, Li XM, Yang Y, Guan R, Zhou S, Yao S, Xie Y, Ou Z, Zhao J et al (2015) Expression of *Helicobacter pylori* urease B on the surface of *Bacillus subtilis* spores. J Med Microbiol 64:104–110

Zhou H, He Z, Wang C, Xie T, Liu L, Liu C, Song F, Ma Y (2016) Intravenous administration is an effective and safe route for cancer gene therapy using the bifidobacterium-mediated recombinant HSV-1 thymidine kinase and ganciclovir. Int J Mol Sci 17:891