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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

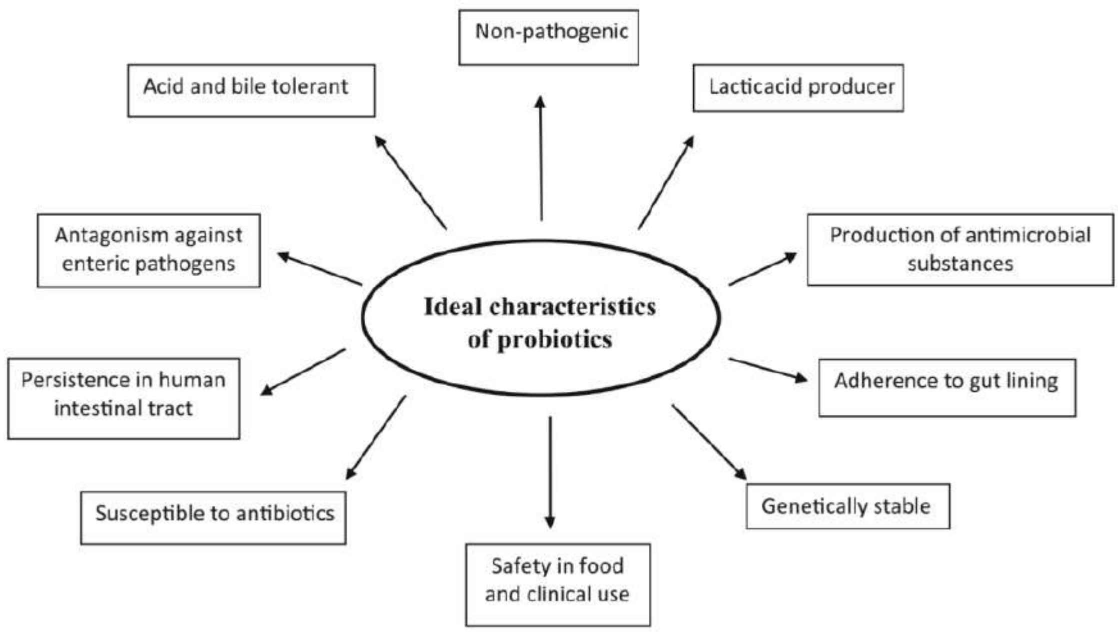

Fig. 1.1 Prerequisites of an ideal probiotic organism

(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 microencapusle 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