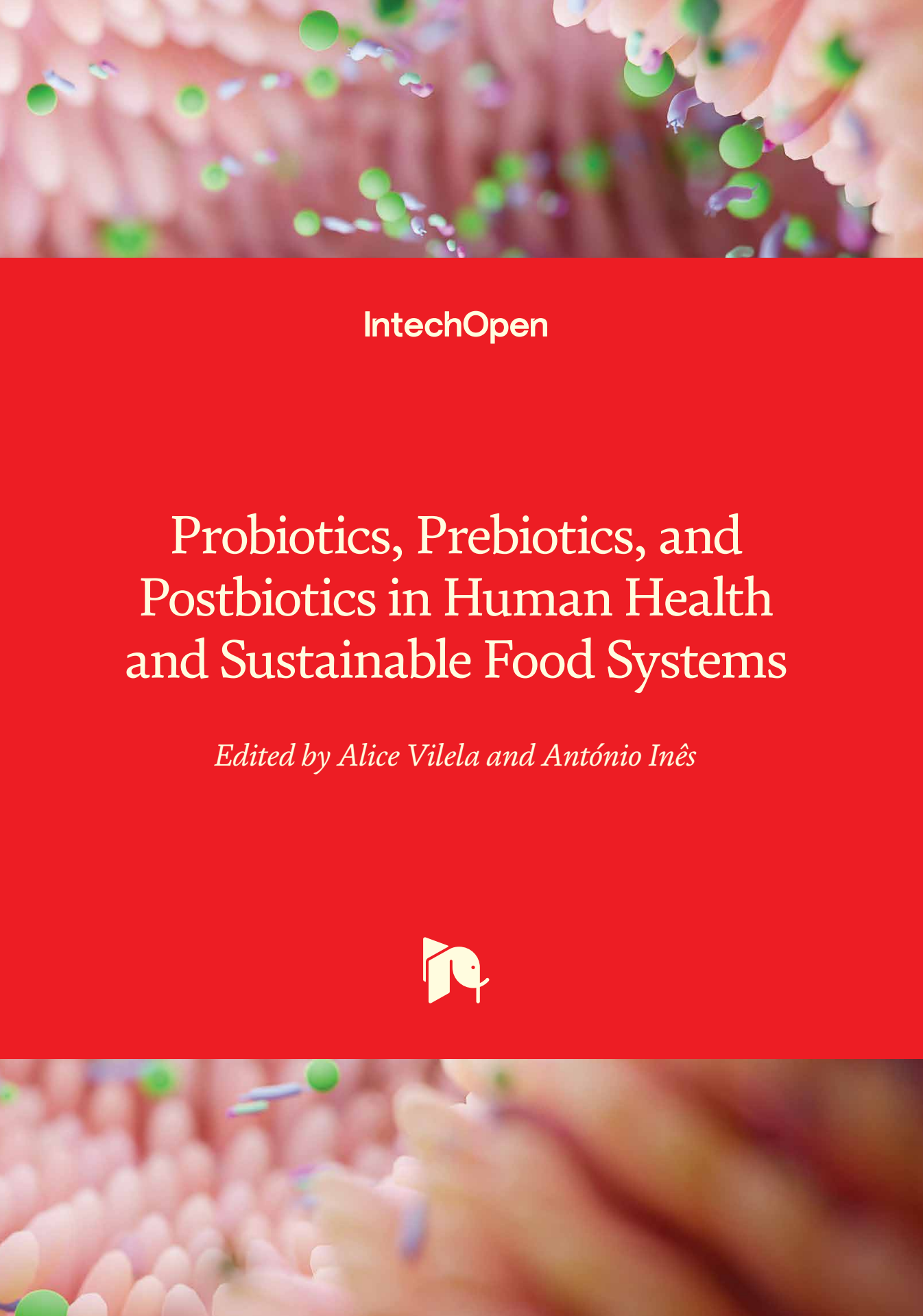




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# Probiotics, Prebiotics, and Postbiotics in Human Health and Sustainable Food Systems

*Edited by Alice Vilela and António Inês*





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Edited by Alice Vilela and António Inês

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# Meet the editors



Alice Vilela received her BSc in enology, a master's in genetics, and a Ph.D. in microbiology from the University of Trás-os-Montes and Alto Douro (UTAD). She is an assistant professor at UTAD and a Chemistry Research Center (CQ-VR) member at Vila Real. She published 88 articles in international scientific journals, 14 technical papers, 24 book chapters, and more than 100 publications in conference proceedings. She has supervised Ph.D. and masters' theses besides having supervised completion of work for bachelors degrees in the field of biological sciences. She has received two awards and is currently involved in three research projects. Her research lines are linked with studies on volatile acidity bioreduction in wines, wine bio-dealcoholization, wine microbiology, and food and wine sensory evaluation and development of sensory tests. She is an active reviewer for *Food Research International*, *Journal of the Science of Food and Agriculture*, *Food Chemistry International*, *Journal of Gastronomy and Food Science*, and MDPI journals (*Foods*, *Fermentation*, *Beverages*, *Molecules*, *International Journal of Molecular Sciences and Materials*); academic editor of *Asian Research Journal of Agriculture*, *International Journal of Environment and Climate Change*, and *Insights in Organic & Inorganic Chemistry Journal* and an editorial board member of *Journal of Agriculture and Crops* and *Fermentation* (MDPI).



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# Preface

Probiotics, prebiotics, and postbiotics have garnered significant attention for their potential health benefits and roles in sustainable food systems. These substances interact with the gut microbiota to promote health, prevent diseases, and enhance the nutritional value of foods. In the comprehensive book *Probiotics, Prebiotics, and Postbiotics in Human Health and Sustainable Food Systems*, we explore the world of biotics and their advantages for health.

Probiotics are live microorganisms that confer health benefits to the host when administered adequately. They are commonly found in fermented foods and dietary supplements. Probiotics such as *Lactobacillus* and *Bifidobacterium* species are known to improve gut health by enhancing intestinal microbial balance, improving digestion, enhancing immune function, and reducing the risk of gastrointestinal diseases. Additionally, probiotics have been associated with preventing cardiovascular diseases and tumorigenesis through their influence on gut microflora and immune modulation.

Prebiotics are nondigestible food ingredients that selectively stimulate the growth and activity of beneficial bacteria in the gut. Common prebiotics include fructooligosaccharides and inulin, found in various plant-based foods. Prebiotics help maintain a healthy gut microbiota, which is crucial for overall health, by promoting the development of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*. They also play a role in lipid metabolism and can help reduce inflammation and oxidative stress, thereby preventing chronic diseases.

Postbiotics are metabolic byproducts or components of probiotics that benefit the host. Unlike live probiotics, postbiotics are stable and can withstand harsh conditions during processing and storage. They include short-chain fatty acids, vitamins, and peptides, which have been shown to have anti-inflammatory, immunomodulatory, and antioxidant properties. Postbiotics can enhance the epithelial barrier, modulate immune responses, and potentially serve as a safer alternative to live probiotics in food and pharmaceutical applications.

Integrating probiotics, prebiotics, and postbiotics into food systems can contribute to sustainability by utilizing locally available raw materials and promoting the consumption of functional foods. Rich in probiotics, fermented foods are often made from seasonal and locally sourced ingredients, making them cost-effective and environmentally friendly. Additionally, developing functional foods enriched with prebiotics and postbiotics can enhance the nutritional value of diets and support sustainable agricultural practices.

Probiotics, prebiotics, and postbiotics offer promising health benefits by modulating the gut microbiota and enhancing immune function. Their incorporation into sustainable food systems can improve public health and support environmental

sustainability. Further research and development in this field can lead to innovative functional foods and therapeutic applications that promote overall well-being.

This book provides a comprehensive overview of an important and contemporary topic. It covers the role of probiotics in the intestinal ecosystem and the immune system and their presence in the global market. It also explores the study of new species with probiotic potential, emphasizing sustainability within the global food system.

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# Perspective Chapter: Role of Probiotics in the Intestinal Ecosystem and the Immune System

*Ivanna Novotny Núñez, Eva Vélez, Silvia I. Cazorla  
and Carolina Maldonado Galdeano*

## Abstract

The microbial colonizers of the intestine, collectively known as microbiota, are an essential and irreplaceable component of the human organism. They provide valuable functions and genetic resources (the microbiome) and play a critical role in various physiological processes, including somatic development, nutrition, and immunity. Maintaining intestinal homeostasis requires a delicate balance between the diverse array of microorganisms within the intestinal microbiota and the host cells, all within an environment constantly influenced by numerous stimuli from both external and internal sources. Disruption of this balance, known as dysbiosis, is characterized by reduced microbial diversity and deviation from the ancestral microbial environment. Dysbiosis is linked to several chronic non-communicable diseases prevalent in developed societies, such as atopic conditions, metabolic syndrome, inflammatory diseases, cancer, and certain behavioral disorders. The increasing focus on the intestinal microbiota and its interaction with the immune system has spurred the development of new strategies to maintain intestinal balance and enhance overall health. Among these approaches, dietary interventions involving probiotics, prebiotics, synbiotics, and postbiotics hold significant promise for providing substantial health benefits to consumers.

**Keywords:** probiotics, prebiotics, synbiotics, posbiotics, intestinal immune system, epithelial cells (IECs), immune cells

## 1. Introduction

It is known that diet plays a fundamental role in the health of the intestinal microbiota. Its diversity depends largely on the variety and quality of the foods consumed. In addition to diet, probiotics and prebiotics are widely used to maintain a healthy microbiome.

There are numerous studies demonstrating the health benefits attributed to the consumption of probiotics. Probiotics are defined by the Food and Agriculture

Organization of the United Nations (FAO) and the World Health Organization (WHO) as “live microorganisms which, when administered in adequate amounts, confer health benefits on the host” [1]. Currently, there is a growing interest in their use, as a strategy to improve the intestinal barrier and the health of the consumer. Probiotics are known to have numerous benefits such as modulation of the immune system, reinforcement of the intestinal barrier, improvement of the intestinal microbiome, reduction of serum cholesterol, alleviation of allergic processes and symptoms of inflammatory bowel disease (IBD), as well as anticancer activity [2–7]. Lactic acid bacteria, including species of lactobacilli, bifidobacteria, streptococci, lactococci and some strains of yeast (*Saccharomyces*) are the most widely used microorganisms for the development of probiotic products [8].

Prebiotics have been defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon” [9]. Prebiotics are substrates that are selectively utilized by host microorganisms, conferring a health benefit. Selectivity plays a key role in the prebiotic concept. Prebiotics such as fructooligosaccharides (FOS) and galactooligosaccharides (GOS) primarily stimulate the proliferation of Lactobacilli and Bifidobacteria, unlike fibers (cellulose, pectins and xylans), which promote the growth of many microorganisms in the intestine. There are different prebiotic effects, such as defense against pathogens, immune modulation, mineral absorption, intestinal function, metabolic effects and satiety [10].

Synbiotics refer to the combination of a probiotic with a prebiotic with the aim of improving human or animal health [11]. They are used to amplify the benefits of the probiotic as well as to stimulate the growth of indigenous beneficial microorganisms [12]. In synbiotic products, probiotic bacteria selectively use prebiotics as a substrate for their growth [13]. Synbiotics can be of two types: (1) complementary synbiotics: which consist of a probiotic and a prebiotic together, which confer one or more health benefits but do not require codependent functions; (2) synergistic synbiotics: they contain a substrate, which is selectively used by the co-administered microorganisms, according to the International Scientific Association of Probiotics and Prebiotics [14].

Postbiotic refers to a term derived from the Greek “post,” meaning after, and “bios,” meaning life. Therefore, the term postbiotic refers to substances derived

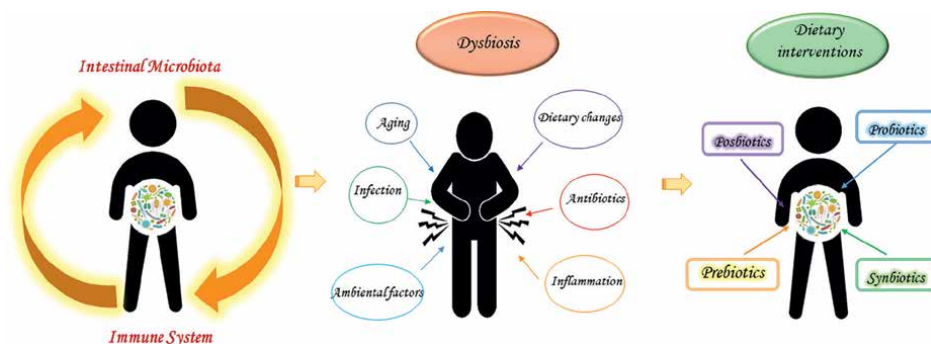


**Figure 1.**

A stable gut microbiome is essential for defending the host against harmful microorganisms and maintaining homeostasis, while also playing a critical role in balancing the gut microbiota with immune system. Disruptions to this balance can occur due to dietary changes, antibiotic use, aging, or infections, leading to alterations in the gut microbiota. These disturbances are often linked to inflammatory, pathogenic, and metabolic conditions. In this context, dietary interventions-such as the use of probiotics, prebiotics, synbiotics, or postbiotics-are crucial for restoring microbiota balance.

after the microorganisms are no longer alive, that is, they are dead or inactivated and confer health benefits. A postbiotic was defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as “a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host”. Postbiotics may be composed of intact inanimate cells or may be structural fragments of microorganisms, such as cell walls. Many postbiotic preparations may contain substances produced by microorganisms (metabolites, proteins or peptides), which although not essential for a postbiotic may contribute to the health effect elicited by the postbiotic itself (**Figure 1**) [15].

## 2. Probiotics

The human intestine is known to be a complex ecosystem, where microbiota, nutrients and host cells interact to maintain intestinal homeostasis and host development. The intestinal microbiota is composed of different bacteria, which carry out certain functions, such as metabolic functions and barrier effect, among others [16].

The intestinal microbiome can be altered by diet, lifestyle, exposure to toxins and the use of antibiotics. This is why the microbiota can play a fundamental role in gastrointestinal disorders such as inflammatory bowel disease, celiac disease, functional and motility disorders, metabolic and immune functions, among others [12].

These close relationships between the intestinal microbiota, health and disease have generated great interest in the use of probiotics. In recent years, various mechanisms have been described on probiotics maintenance of intestinal homeostasis and immune system modulation [8].

The imbalance of the structure and function of the intestinal microbiota is known as microbial dysbiosis. Recently, it has been shown that intestinal microbiota dysbiosis is associated with intestinal disorders such as IBD, irritable bowel syndrome (IBS) and celiac disease, being probiotics an alternative to restore intestinal microbial balance and prevent infections in patients after treatment with antibiotics, which induces intestinal microbiota dysbiosis [17].

Intestinal microbiota is also modified in metabolic disorders as obesity [18]. There are studies in animal and human models where it has been shown that the intestinal microbiota is different in lean and obese hosts. Therefore, the modification of the intestinal microbiota could have a beneficial effect and be used as a tool for future treatments [19].

Obesity is a chronic and multifactorial disease. It is strongly associated with an inflammatory process in which adipose tissue plays an important role, as well as hepatic steatosis and insulin resistance [20–22]. In a mouse model of obesity induced by a high-fat diet, the probiotic administration improved the intestinal microbiota and mucosal immunity, downregulated some blood biochemical parameters associated with metabolic syndrome, and decreased hepatic steatosis. Administration of probiotics, as fermented milk, was associated with a decrease in proinflammatory cytokines, in the small intestine and liver of diet-induced obese mice. The authors demonstrated the potential of probiotics in obese individuals to reduce body weight and improve biochemical and immunological parameters that are altered in obesity. Therefore, due to the anti-inflammatory effects of probiotic microorganisms, they could be used as dietary supplements to combat obesity and its related disorders [23, 24]. There are studies in which the administration of probiotics to mice was evaluated in two experimental models: obesity and senescence, including mice of

different ages (21, 28, 45, 90 and 180 days). Authors observed that probiotic consumption improved the cortical/medullary ratio, cytokine production and regulation, as well as the recovery of mature T cell populations in thymus, in both obese and aged mice. Authors concluded that probiotics incorporation into diet could modulate the immune system and lead to the recovery of thymus function, thus improving the quality of life [25].

It is known that malnutrition, both by excess and deficiency, can negatively affect the number and activity of the immune cells [26, 27]. Malnutrition is a systemic disorder caused by an imbalance between nutrient intake and energy requirements, which causes a significant deterioration of the immune system. The architecture of the intestinal mucosa and the thymus is one of the most affected organs. Malnutrition can be reversed with adequate renutrition. In this sense, the administration of probiotics as a renutrition supplement could be useful to restore gastrointestinal and immune functions, regenerate the intestinal mucosa and improve its microbiota.

In a study carried out on malnourished mice, the administration of probiotics, such as probiotic fermented milk, restored the number of immune cells in the lamina propria of the small intestine, its functionality, reconstituted the architecture of the intestinal mucosa and stimulated local and systemic immunity. The importance of probiotic supplementation in the histological and functional recovery of the thymus has also been demonstrated in a model of non-severe protein-energy malnutrition in mice [28, 29].

The beneficial effect of probiotics in allergic processes has also been described. One of the most important signs that can be distinguished in this process is the increase of immunoglobulin E (IgE) [30]. It has been shown that probiotics could be effective in decreasing this immunoglobulin and in alleviating symptoms. In an experimental model of respiratory allergy, it was shown that probiotics induced a clear balance of T helper 1 (Th1) cells, favoring the production of IgG immunoglobulin instead of IgE and increased the levels of anti-inflammatory cytokines. The consumption of probiotics could be a strategy to improve respiratory tract allergies, mediating by the production of IgG [31].

It is known that probiotics have an effect on the functioning of the immune system. Probiotics are able to interact with intestinal epithelial cells (IECs) through their surface molecules; these cells establish a dialog between the host and the external environment. Immune cells associated with the intestinal epithelial can be activated after the internalization of the probiotic bacteria fragments in IECs, thus prolonging the survival of the gastrointestinal immune system and promoting the activation of immune cells in other distant organs (**Figure 2**) [32].

Lemme Dumit et al. [33] evaluated the effect of oral administration of probiotic bacteria cell walls (PBCW) in healthy mice, mainly on intestinal epithelial cells (IECs). Probiotic bacteria and their cell walls stimulate IECs, exhibiting significant activation and release of cytokines. Probiotic bacteria and their cell walls have an important immunoregulatory effect on IECs without altering the homeostatic environment. These findings on the ability of probiotic bacteria cell walls to stimulate IECs and macrophages, as well as improve the functioning of the immune system, suggest that such structures could be applied as a new oral adjuvant [33].

Other important cells that play a fundamental role in the epithelial barrier are Paneth cells, which produce antimicrobial peptides (AMPs) that act in innate immunity. A study by Cazorla et al. [34] evaluated whether the antimicrobial activity against the pathogens *Staphylococcus aureus* and *Salmonella Typhimurium*, and Paneth cells are influenced by the probiotics administration, in a mouse model. The authors

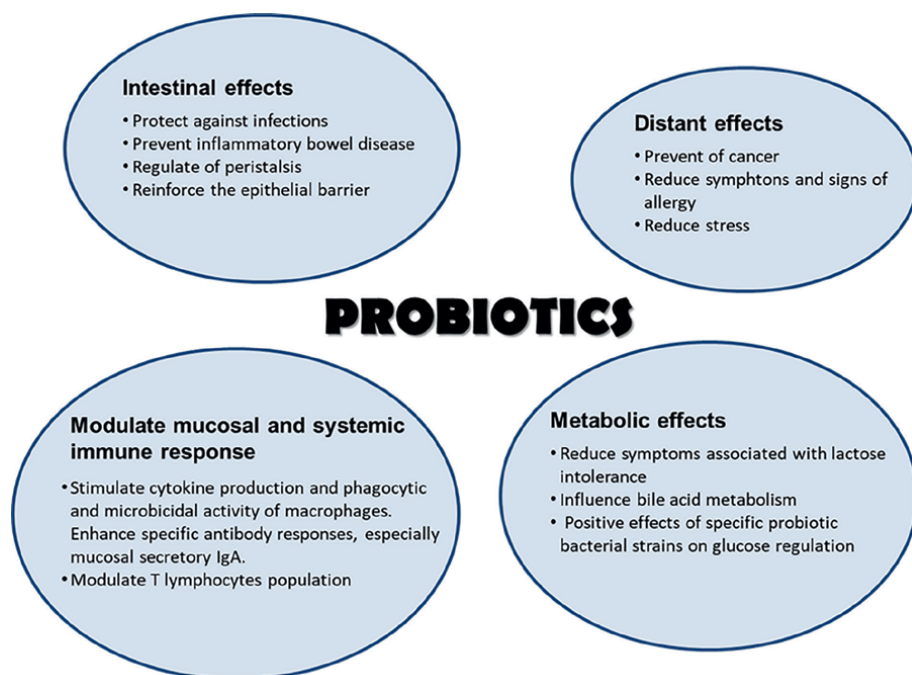


**Figure 2.**  
 Principal effects of probiotics.

demonstrated that oral administration of probiotics can increase intestinal antimicrobial activity and Paneth cells, thereby strengthening the epithelial barrier against pathogens. They concluded that this could be another mechanism by which probiotics protect the host against infectious diseases [34].

However, mechanisms by which probiotics can modulate the immune system are not fully known.

### 3. Prebiotics

Prebiotics were recognized over 20 years ago as a class of compounds capable of manipulating the host microbiota for the benefit of the host [9]. At that time, fructans, fructooligosaccharides, inulin and galactooligosaccharides fit that category, by an enrichment of *Lactobacillus* and/or *Bifidobacterium spp* intestinal microbiota. This definition has been reviewed several times until 2016, when the International Scientific Association for Probiotics and Prebiotics set prebiotics as a substrate that is selectively utilized by host microorganisms, conferring a health benefit [35].

While probiotics are live microorganisms, prebiotics are non-viable substrates that will boost the growth of beneficial microorganisms present in the host. This latest definition enlarges the benefit action of prebiotics to other body sites and not exclusively to the gastrointestinal tract [36]. Even more important, this last definition points to other microorganisms like *Roseburia*, *Eubacterium* or *Faecalibacterium spp.* as the drivers of the health-beneficial effects [37]. It was not until molecular-based methods replaced culture ones that employment of prebiotic substrates by these other bacteria genera was determined.

Prebiotics are early in life introduced to the human through breast milk. Currently, prebiotics include galactooligosaccharides (GOSs), mannan oligosaccharide (MOS), xylooligosaccharide (XOS), fructooligosaccharides (FOSs), inulin, as well as human milk oligosaccharides (HMO) [38, 39]. Plant polyphenols and other phytochemicals, pyrodextrins, conjugated linolenic acid (CLA), and PUFA (polyunsaturated fatty acids) constitute a class of compounds that can also meet the criteria of prebiotics [40–42]. Since oligosaccharides exhibit resistance to digestion, over 95% of HMOs reach the intestine, where they play their qualities.

Prebiotics elicit their effects through multiple mechanisms:

1. Promote the proliferation of beneficial microorganisms and inhibit the growth of detrimental microorganisms.

HMOs, through a competitive mechanism, inhibit the adhesion of pathogens to the neonate intestinal epithelium and avoid infections [43]. GOSs, also limit the growth of pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [44] and the adherence of *Escherichia coli* E2348/69 [45]. Additionally, GOS, by selectivity, increases the abundance of *Bifidobacterium*, leading to large concentrations of colonic short-chain fatty acids (SCFAs), particularly butyrate, which promotes the intestinal homeostasis through effects on the integrity of the colonic epithelium, metabolic and immunological actions [12]. Recent studies demonstrated that the production of biofilms, a mechanism displayed by the bacteria to adhere to various surfaces, was significantly decreased in the presence of prebiotics [46].

Studies in mice have shown that oligofructose (a fructan) reduced inflammation by mechanisms linked with changes in specific gut microorganisms [35].

2. Contribute to gut maturation and strengthen the intestinal barrier.

In vitro, Kuntz et al. reported the growth inhibition of intestinal cells by MOS [47]. Similarly, Roselli et al., described that GOS inhibited an inflammatory response induced by dextran sulfate sodium through the NF- $\kappa$ B pathway in Caco-2 cells [48]. GOS has also modulated the function of intestinal Goblet cells by the regulation of the following genes: muc2, tff3, and retnlb [49].

3. Play a role in shaping the responses and development of the immune system.

Eiwegger et al. [50] revealed that sialylated MOS diminishes postnatal allergen-specific immune responses by shifting the Th-2 phenotype toward a more balanced Th-1/Th-2 response. Additionally, MOS suppressed the production of IL-6 and IL-1 $\beta$ , and decreased the overexpression of Th17 [50]. Zhou P has also reported that prebiotics stimulate mucosal immunity by increasing IgA antibody production [51].

Prebiotics are used to treat a wide variety of disease, such as obesity, chronic enteritis, skin disease, autism spectrum disorders, and ulcerative colitis [52–54]. The mechanism of prebiotics in alleviating UC in mice seems to be through the inhibition of the TLR4/NF- $\kappa$ B signaling pathway, the JAK2/STAT3 signaling pathway, the inhibition of T cells naive conversion into Th17 cells and their conversion into Treg cells [55].

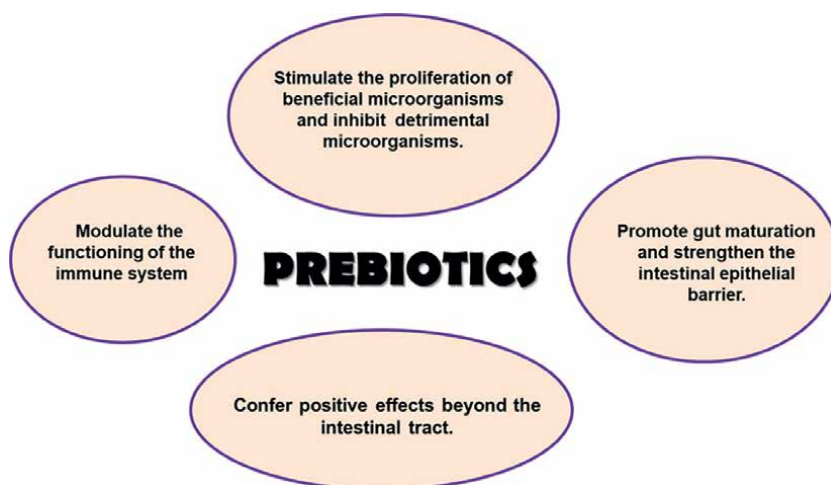


**Figure 3.**  
 Summary of the main effects that highlight the importance of including foods rich in prebiotics in the diet for general health and well-being.

#### 4. Confer positive effects beyond the intestinal tract (**Figure 3**).

Prebiotics elicited beneficial properties at different levels: mental, cardiovascular, the brain, the bones, and the bile acid metabolism [41, 56].

Compared to infants, studies of prebiotics' effects in older are relatively underexplored. Recently, Hu et al. reviewed the current knowledge on the immunomodulatory mechanisms elicited by GOS to maintain immune homeostasis in elders [57]. Prebiotics have also been probed to reduce the age-associated compromised intestinal permeability by increasing muc2 expression and mucus thickness [49, 58, 59] in aged mice. Additionally, GOS modulates the intestinal microbiota by increasing *Bifidobacterium*, *Lactobacillus-Enterococcus* spp., and *Clostridium coccoides*–*Eubacterium rectale* in older individuals [60, 61]. The increases of *Bifidobacterium* protected against irritable bowel syndrome, pouchitis and *Clostridium difficile* infection [62, 63]. In older individuals, GOS also increases phagocytosis, NK cell activity and IL-10 production and decreases proinflammatory cytokines [64].

## 4. Synbiotics

A stable gut community plays a crucial role in protecting the host against invading microorganisms, maintaining homeostasis, and regulating immune function [64]. However, disruptions can occur due to dietary changes, antibiotic use, aging, or infection, resulting in an altered gut microbiota linked to inflammatory, pathogenic, and metabolic conditions such as inflammatory bowel diseases, colorectal cancer, metabolic syndrome, and atopic disorders [65]. Various strategies have been proposed to modulate the composition and function of the gut microbiota. These include fecal microbiota transplants, probiotics, other live microorganisms, and non-digestible dietary substrates such as prebiotics. More recently, the use of synbiotic has also

emerged as a promising approach. The concept of synbiotic, introduced 25 years ago, involves combining selectively fermentable non-digestible food ingredients (prebiotics) with probiotics. Recently, a panel of experts from the International Scientific Association for Probiotics and Prebiotics (ISAPP) updated the definition of synbiotic, describing them as: “A mixture comprising live microorganisms and substrates selectively utilized by host microorganisms that confer a health benefit on the host.” Host microorganisms are understood to include both those naturally occurring in the host microbiota and those introduced as probiotics.

According to this definition, synbiotic are divided into two categories: complementary and synergistic. A synergistic synbiotic is characterized by a substrate designed to counteract potential difficulties in the survival of co-administered probiotics in the gastrointestinal tract. In contrast, a complementary synbiotic combines a probiotic and a prebiotic to jointly confer one or more health benefits without the need for interdependent functions [66]. However, these definitions still present some ambiguity. For a microorganism to be considered a probiotic, it must be able to withstand passage through the gastrointestinal tract, meaning it does not necessarily require the co-administration of a prebiotic to reach the intestine intact. In contrast, a synbiotic is expected to be more effective than the use of prebiotics or probiotics alone.

Central to formulating a synbiotic is the careful selection of the appropriate probiotic microorganism and prebiotic. The prebiotic must not only protect the probiotic in the harsh gastrointestinal environment but also promote the growth of microorganisms that confer beneficial health effects. Furthermore, an essential characteristic of a synbiotic is that its live microbial components must include strains with verified identity, potency, and safety deposited in an internationally recognized collection. Additionally, the structure and purity of the prebiotic substrate used are crucial factors [67].

One of the most commonly used combinations in synbiotic includes bacteria from the *Bifidobacterium*, *Streptococcus*, *Lactobacillus* or *Propionibacterium* genus along with fructooligosaccharides.

Early reports indicated that probiotics or synbiotic have a beneficial effect on gut microflora, intestinal structure, function, and local immune response. However, the mechanisms through which synbiotic exert these effects are not fully understood. It is hypothesized that these benefits are mediated by the combined contributions of both probiotic and prebiotic components [68].

The literature describes various beneficial effects of synbiotics on human health. These include balancing the intestinal microbiota by increasing bifidobacteria and lactobacillus species, improving liver function in patients with cirrhosis, and enhancing immune system status [69, 70].

Several authors have reported on the effects of synbiotics in specifically modulating the immune system. For instance, in an experimental rat model, the administration of a synbiotic blend consisting of two probiotic bacteria (*Lactobacillus rhamnosus* and *Bifidobacterium lactis*), along with two prebiotics (inulin and oligofructose), resulted in increased levels of intestinal IgA, as well as reductions in blood cholesterol levels and blood pressure [71].

In a randomized controlled trial involving 120 patients with remission or active ulcerative colitis (UC), participants were divided into three groups receiving either a probiotic, a prebiotic, or a combination of both (synbiotic). At the end of the treatment period, various parameters were evaluated to assess the inflammatory status. The authors concluded that the administration of synbiotics was more effective than administering probiotics or prebiotics alone in maintaining UC remission [72].

In another study involving a synbiotic mixture of *Bifidobacterium longum* and fructo-oligosaccharide/inulin administered to patients with active ulcerative colitis (UC), a significant decrease in TNF- $\alpha$  and IL-1 $\alpha$  was observed after 4 weeks compared to the placebo group. Additionally, there was a notable increase in mucosal bifidobacteria in the synbiotic group [73].

Recent studies conducted on a large cohort of patients with IBD have demonstrated the effectiveness of synbiotic administration in reducing inflammatory parameters [74]. Other studies have also shown that synbiotics can induce changes in TCD4+ cells and increase intestinal concentrations of short-chain fatty acids [75].

Danq et al. [76] conducted an investigation into several published studies on the efficacy of bacterial strains and synbiotics in altering the allergy status of children. Their analysis revealed that probiotics or synbiotics may reduce the incidence of eczema in infants aged less than 2 years [76].

Synbiotics have shown beneficial effects in the treatment of individuals with depression and in dental caries prevention [77, 78]. Studies in animal models and among patients with IBD are promising; however, data are still limited, and only a few placebo-controlled trials have been conducted.

Future studies are likely to explore combinations of probiotics and prebiotics, further advancing the development of synbiotics and elucidating the mechanisms of action of these components. This research may uncover their beneficial effects on human health.

## 5. Postbiotics

### 5.1 Definition

A new category has emerged in the past years that join the already well-described probiotics, prebiotics, and synbiotics, named “postbiotics”. The current definition proposed in 2021 by de ISAPP establishes postbiotics as a: “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” [8, 12, 15–78].

Reports of postbiotics use in human health are varied and increasing; they can be found in literature mentioned as parabiotics, ghostbiotics and tyndalized probiotics, among other names. Here, the discussion of the beneficial effects will consider only those that strictly apply to the postbiotics definition proposed by de ISAPP [8, 12, 15–78].

### 5.2 Postbiotics beneficial effects in the intestinal ecosystem and the immune system

Postbiotics are not able to colonize the host intestine, however they can still induce changes in the microbiota composition or its function or directly stimulate the immune system, the lack of living cells makes it easier to interact with the intestinal surface, for example, it does not need to compete with the local microbiota to reach and penetrate the mucosal barrier and interact with the epithelial cells or to be previously processed to induce signaling [79, 80]. The possible changes may affect the local immune response as well as the homeostasis, and in distant sites such as the respiratory tract or the brain (gut-lung axis or gut-brain axis, etc).

A study published in 2020 by Zhang et al. reported that mice with depleted microbiota using a mix of antibiotics and treated with heat-killed *Lactobacillus*

*paracasei* N1115 maintained similar values of IL-6 and corticosterone to those observed in normal mice which were completely altered in mice only administered with the antibiotic mix. Treatment with postbiotic led to an improvement in the intestinal crypts depth and the expression of  $\gamma$ -aminobutyric acid type A receptor  $\alpha 1$  (GABAA $\alpha 1$ ),  $\gamma$ -aminobutyric acid type B receptor 1 (GABAb1) in the brain (gut-brain axis) [81].

As said before, not only the inactivated microbial cell can act as a postbiotic but also parts of the cell. The component HM0539 from *Lactobacillus rhamnosus* GG (a soluble protein) was reported to prevent infection mediated by *E. coli* O157: H7 in mice. This postbiotic can induce the expression of tight junction proteins such as ZO-1 (zonula occludens-1) and intestinal mucin MUC2 *in vitro*, blocking *E. coli* O157: H7 adhesion and invasion process; *in vivo*, it prevented body weight loss after pathogen challenge and jejunal damage [82].

Another work showed that this same postbiotic HM0539 had inhibitory effects over the inflammatory response induced by LSP on RAW264.7 cells (*in vitro*) and DSS-induced murine colitis (*in vivo*); *in vitro* HM0539 inhibited the expression of COX-2 and iNOS (cyclooxygenase-2 and inducible nitric oxide synthase enzymes) and in consequence reducing the release of prostaglandin E2 and nitric oxide. *In vivo* it reduced the expression of TLR4 (TLR4/MyD88/NF- $\kappa$ B Axis) and could be a promising therapy treatment for IBD [83].

In humans a clinical trial was published in 2020 and evaluated the use of heat-inactivated *Bifidobacterium bifidum* MIMBb75 for IBD treatment. The most important finding was that the group receiving MIMBb75 had a good tolerance to treatment (no adverse effects) and reduced the symptoms in all the subtypes of IBS. This is important because heat-inactivated *Bifidobacterium bifidum* MIMBb75 was originally a probiotic, with a positive effect on IBS in its live and inactivated form [84].

At the metabolic level, probiotics and prebiotics have a proven effect. In the case of postbiotics, a human proof-of-concept exploratory study was carried out with obese and overweight patients using *Akkermansia muciniphila*, a resident commensal bacteria in the intestine. This bacterial strain was used in its live form and pasteurized for comparison. Both forms were proven to be safe; however, the pasteurized supplement showed better performance, improving insulin sensitivity and reducing insulinemia and total plasma cholesterol, among other parameters. Over time, it also reduced inflammation and liver dysfunction [85].

Regarding the microbiota, it was observed that healthy mice administered with ADR-159 (a heat-killed fermented product containing two *Lactobacillus* strains) had a subtle variation in the microbiota, mainly affecting the less abundant taxa; in addition, ADR-159-fed mice were more social and had lower corticosterone level [86].

There are several works published about the use of postbiotics that present immunomodulatory effects whether it directly affects the innate and adaptive immune response, or indirectly improves the mucosal barrier. For example, in children, a double-blind trial studied heat-killed *Pediococcus acidilactici* K15 in respiratory infections. Children from pre-scholar were given the K15 formula and showed increased levels of secretory IgA in saliva, and fever events duration was shortened [87].

In a pilot trial with adult volunteers who were intranasally challenged with human rhinovirus (HRV A39) that previously consumed juice enriched with live or heat-inactivated *L. rhamnosus* GG showed a tendency to have a lower HRV load from intranasal lavage, however, these results were not significantly higher than the placebo group. However, these results were not conclusive, and the possibility of a clinical trial should be evaluated [88].

Ou et al. [89] showed in research using 11 lactic acid bacteria (LAB) killed with four different heat treatments that two of the selected strains *E. faecalis* YM-73 and *L. salivarius* AP-32 had good immunomodulatory activity *in vitro*, after heat treatment. Th1 response was favored over Th2 response by increasing the release of IFN- $\gamma$  and IL12p70 [89]. It is important to mention that the immunomodulatory activity can change when different killing techniques are applied.

When using a mice infection model with *Salmonella*, feeding mice with heat-killed multispecies combination of LAB (HMLAB) and the cell walls from HMLAB resulted in the reduction of proinflammatory cytokines (TNF- $\alpha$  and IL-6) and enhanced level of IL-10; also, HMLAB significantly inhibited *Salmonella* invasion [90].

*Salmonella* infection model has been widely used. Dunand et al. demonstrated that postbiotic cell-free supernatant produced at laboratory and industrial scale increases the release of secretory IgA in feces of adult BALB/c mice and prevents *Salmonella* infection [91].

Finally, at the cellular level, the secreted metabolite (MET) fraction of *Ganeden Bacillus coagulans* 30 (GBC30) bacterial cultures were proven to induce maturation of peripheral blood mononuclear cells (PBMC) such as mononuclear phagocytes (to macrophage stage) and dendritic cells from healthy human donors *in vitro* [92].

### 5.3 Discussion

The term postbiotic has brought controversies into a discussion as it must contain inactivated microbial cells or parts of it (like cell wall, cytoplasmic content, etc), and it may or may not contain products of their metabolism that are released into the culture media (whether it comes from pure culture or as a fermented product), as these products may enhance their effect but are not essential to achieve them [15, 78]. The controversy arises as most of the probiotics preparations, for example, fermented products, contain live and dead cells and their metabolism products. To induce a beneficial effect, a probiotic must be administered in a certain amount of live cells, which is often higher in the product (whether it is on a functional food or a pharmaceutical preparation), only to ensure that the amount of live cells that reach the intestines is the minimum required [78]. In this scenario, microbial cells die within time; however, the preparation remains as a probiotic product and does not turn into a postbiotic just because there are inactivated cells. Another limitation is the origin; a postbiotic does not necessarily need to be a probiotic in its live form but needs to have a confirmed beneficial effect when administered to the host and most importantly derived from a “well-defined microorganism or combined microorganisms.” Further investigations should focus on these particular points [15, 78].

It is important to mention some differences between postbiotics and probiotics in terms of use and production. Probiotics must have living cells in the preparation and an appropriate legal regulation regarding the safety of the product, the route of administration, and the concentration, whereas postbiotics are composed of inactivated cells or parts of it having an intrinsic safety in comparison to probiotics (postbiotics do not colonize the intestine), however, the manufacturing process must be specifically described and reproducible [15, 78, 79].

Currently, reports on the effects of postbiotics and health claims are increasing. The complete underlying mechanisms are still unknown, and we cannot assume they work in the same way as probiotics do, even if the origin of the postbiotic is a probiotic microorganism.

More research is needed in this field, and should focus on critical points: the origin of the probiotic (nature of the microorganism), processing (growing, obtaining, the specific killing or inactivating process), and clinical trials.

## **6. Conclusions**

The growing interest in the intestinal microbiota and its interaction with the intestinal immune system has contributed to the attention paid to the use of probiotics, prebiotics, synbiotics and postbiotics, due to their potential benefits for host health. The clue by which prebiotics elicited their beneficial effects on the host is the shift of the microbiota toward beneficial microorganisms that diminishes inflammatory environments and increases the levels of SCFA. One limitation of prebiotics is that they can only promote the growth of bacteria present in the ecosystem. Interesting, this drawback has already been overcome by the administration of probiotics on food matrices. Growing evidences indicate that the beneficial effects of probiotics, prebiotics, synbiotics and postbiotics go beyond the intestinal tract, reaching several organs and system of the host. Postbiotics can be an interesting alternative for live probiotics for immunocompromised people.

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## **Conflict of interest**

The authors declare that the chapter of the book was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Probiotics as Feed Additives for Improved Animal Health and Nutrition: The Current Perspectives

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## Abstract

The world currently requires more food (particularly, animal products) than it can produce, but the food has to be produced in a safe and sustainable manner. For a long time, antibiotics have been used to promote growth and prevent diseases in livestock production, but their prolonged usage can lead to antibiotic resistance in the gut microbiota. Probiotics are postulated as a safer alternative to antibiotics in boosting animal performance and productivity. Basically, probiotics are live microorganisms that can confer health benefits to the host when administered in appropriate quantities. Once ingested, probiotic microorganisms can modulate the balance and activities of the gastrointestinal microbiota. In livestock production, probiotics have since been linked to improved performance and productivity, as well as good meat quality. This chapter, therefore, serves to highlight the beneficial effects of probiotics either as feed additives or supplements, and their effect on animal health, nutrition, growth and productivity, and the animal's product quality. The chapter also explores the mode of action of probiotics in an *in vitro* animal feeding model.

**Keywords:** probiotics, gut microbiota, antibiotic resistance, livestock, meat quality, feed additives

## 1. Introduction

The global population is projected to surpass 9 billion by the year 2050, creating food security challenges, especially in developing nations. Furthermore, the upsurge in economic growth has led to a higher need for livestock products, resulting in the livestock sector facing the challenge of producing more with inadequate resources. Still, the livestock industry is rapidly expanding in the agriculture sector and accounts for approximately 40% of the total value of agricultural production worldwide, providing sustenance and food security for nearly 1.3 billion individuals [1], with the average daily animal protein consumption in developed countries on average above 65% of the total protein intake [2].

The expansion of population growth poses issues regarding optimizing resource use to produce food for humans efficiently; effects of land conversion and more intensified use on conservation of environmental services and biodiversity; effects of ruminant methane emissions on climate change; and impacts of rising temperatures due to climate change on animal production. Livestock offer a significant source of disposable income for underprivileged and marginalized communities in developing countries, and they serve as a key tool in combating poverty in rural areas [3–5]. Livestock also offer draught power and manure, which can be used as fuel and fertilizer, in addition to serving as a valuable source of income and nutrition. Livestock businesses can also provide animal assets that are immune to inflation for insurance and financing purposes [6, 7].

In order to meet the growing demand of population, livestock sectors of different types resorted to various techniques to increase their production [2]. These techniques are set to establish intensive and semi-intensive farming systems aiming at accelerating animal growth, preventing diseases, enhancing feed conversion efficiency, and optimizing reproduction rates. However, this leads to a greater need to ensure that animal welfare concerns are properly taken into account [8]. Significant advancements have been made in this area over the last nine decades, primarily due to enhancements in genetic selection, health status, nutrition, and the utilization of antibiotics and growth promoters. This advancement led to the benefits of increased livestock resulting in an approximately 18% increase in the overall growth performance [9]. Nevertheless, the use of antibiotics and growth promotants has raised concerns regarding antibiotic-resistant bacteria, an increase in foodborne allergies, and harmful effects on the environment like agricultural runoff [10–12]. Additionally, there is a growing concern about the impact of antibiotics and growth promotants on human health [7]. To that end, researchers have been exploring different ways to improve the quantity and quality of farm animals and their products. One such potential option is the supplementation of probiotics, as single or mixed strains, to the feeding regime of livestock.

Probiotics are defined as living microbial supplements that beneficially impact the host by enhancing its intestinal microbial composition [13]. In order for a microorganism to be considered probiotic, it should be nonpathogenic, able to give a viable cell count, positively impact the host's health, and improve intestinal function. The most commonly used probiotics includes; *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus salivarius*, *Bifidobacterium spp.*, and other probiotic fungi such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* [14, 15]. Copious topical research has demonstrated that supplementing probiotics in animal feed can positively alter the gut microbiota, leading to reductions in pathogen shedding and disease symptoms, as well as enhancements in gut immunity, disease resistance, and overall health [16, 17]. Furthermore, probiotics can reduce foodborne pathogens like *Campylobacter*, *Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, and *Staphylococcus aureus* through their antagonistic effect [18–21]. This chapter, therefore, serves to highlight the beneficial effect of probiotics, either as feed additives or supplements, and their effect on animal health, nutrition, growth and productivity, and product quality. The chapter also explores the mode of action of probiotics in an *in-vitro* animal feeding model.

## 2. The gut microbiota

The gastrointestinal tract (GIT) of animals encompasses a diverse microbial population [22], with maximal populations in regions where the pH is close to neutral

[22, 23]. Such regions encompass the pre-gastric rumen and the post-gastric caecum of ruminants, including horses, pigs and chickens. Depending on whether the animal is a ruminant or a monogastric animal, the GIT can harbor up to numerous 1000 distinctive microbial species, including bacteria, archaea, fungi, and protozoa [24]. Most gut bacteria belong to two main groups, *Firmicutes* and *Bacteroidetes*, which account for approximately 99% of the total microbiota in the bovine rumen [25]. However, species from the phyla *Actinobacteria* and *Proteobacteria* are also present [26, 27], constituting 96% microbial species in the rumen of sheep [24]. The indigenous gut microbiota plays an important role in animals' overall health, growth and development, and productivity by enhancing immune system responses and development and facilitating nutrient extraction from the diet [28]. The latter is demonstrated in ruminants, where the gut microbiome provides approximately 70% of their daily energy needs [29]. This percentage is mostly derived from the fermentation of carbohydrates by microorganisms, which releases volatile fatty acids that are absorbed and used as a source of energy. Additionally, after leaving the rumen and digesting in the small intestine, the microbial populations themselves can be utilized as a source of protein [30].

There is indirect contact between immunomodulatory cells located in the lamina propria and intestinal epithelial cells located in the lumen [31, 32]. The microbiota and immune cells are separated by an intestinal epithelium that has two necessary functions. The primary function is to physically segregate any foreign substances or microbes from the host immune cells, and the second function is triggering an immunological response by signaling immune cells, which, in turn, invokes an immune response [33]. As a result, the relationship between the host's health and the gut microbiota is complex, involving interactions between the innate immune system, epithelium, and resident commensal bacteria. Sustaining a diverse microbiota is valuable to the animal's welfare [34]. An imbalanced microbiota in which the proportion of pathogenic to beneficial commensal bacteria is higher impairs gut health and, in turn, an animal's growth, behavior (including social interaction, feeding, and stress response), and general health [35]. Diet, host genetics, and environmental factors are a few of the elements known to affect the microbiota's richness and diversification [36]. The connection between these three determinants is complex, contingent on the circumstances, as one element may be more prominent than the other. For example, the GIT of a neonatal animal is initially sterile but quickly becomes colonized by microbial populations from the mother and environment [37, 38].

### **3. Probiotics in animal feeding**

Modern breeding practices, which include artificial conditions and feeding, induce stress and can alter the microflora, which compromises the animal's ability to fight off infection [39]. Therefore, the purpose of the probiotic approach is to repair the deficiencies in the microflora and enhance the animal's immunity against disease. Such a supplement does not run the risk of introducing any foreign substances into the animal's internal environment, nor does it contaminate the carcass and introduce dangerous chemicals into the food chain. Currently, farmers give probiotic feed supplements to both ruminants and poultry. Most probiotics consist of gram-positive bacteria, although gram-negative bacteria, yeast, and fungi are also consumed as feed additives and have beneficial effects on animal health. Probiotics also assist in enhancing healthy microflora growth [40]. Most probiotic products utilize one or

a combination of several strains of bacteria. The most common probiotics utilized in livestock productions are: *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, *Streptococcus*, and *Saccharomyces* (Table 1; [40, 59]).

| Genus                           | Species                                  | References |
|---------------------------------|------------------------------------------|------------|
| <b>Gram - positive bacteria</b> |                                          |            |
| <i>Bacillus</i>                 | <i>B. subtilis</i>                       | [41]       |
|                                 | <i>B. coagulans</i>                      | [42]       |
|                                 | <i>B. amyloliquefaciens</i>              | [43]       |
|                                 | <i>B. toyonensis</i>                     | [44]       |
| <i>Bifidobacterium</i>          | <i>B. longum</i>                         | [45]       |
|                                 | <i>B. bifidum</i>                        | [46]       |
|                                 | <i>B. lactis</i>                         | [45]       |
|                                 | <i>B. thermophilus</i>                   | [47]       |
| <i>Lactobacillus</i>            | <i>B. animalis</i>                       | [48]       |
|                                 | <i>L. delbrueckii subsp. bulgaricus</i>  | [49]       |
|                                 | <i>L. acidophilus</i>                    | [50]       |
| <i>Limosilactobacillus</i>      | <i>L. gallinarum</i>                     | [51]       |
|                                 | <i>L. reuteri</i>                        | [52]       |
|                                 | <i>L. plantarum</i>                      | [52]       |
| <i>Levilactobacillus</i>        | <i>L. brevis</i>                         | [52]       |
| <i>Lactocaseibacillus</i>       | <i>L. casei</i>                          | [52]       |
| <i>Lactococcus</i>              | <i>L. lactis</i>                         | [53]       |
| <i>Streptococcus</i>            | <i>S. salivarius subsp. thermophilus</i> | [49]       |
|                                 | <i>S. faecium</i>                        | [50]       |
| <i>Pedicoccus</i>               | <i>P. acidilactici</i>                   | [48]       |
| <i>Leuconostoc</i>              | <i>L. mesenteroides</i>                  | [54]       |
| <i>Clostridium</i>              | <i>C. butyricum</i>                      | [55]       |
| <i>Enterococcus</i>             | <i>E. faecium</i>                        | [48]       |
| <b>Gram - negative bacteria</b> |                                          |            |
| <i>Megasphaera</i>              | <i>M. elsdenii</i>                       | [45]       |
| <i>Prevotella</i>               | <i>P. bryantii</i>                       | [45]       |
| <i>Escherichia</i>              | <i>E. coli Nissle 1917</i>               | [56]       |
| <b>Yeast and fungi</b>          |                                          |            |
| <i>Aspergillus</i>              | <i>A. niger</i>                          | [45]       |
|                                 | <i>A. oryzae</i>                         | [49]       |
| <i>Saccharomyces</i>            | <i>S. cerevisiae</i>                     | [57]       |
|                                 | <i>S. boulardii</i>                      | [58]       |

**Table 1.**  
Different microorganisms commonly used as probiotics in livestock production.

#### 4. Importance of probiotics in animal health and nutrition

Probiotics have been shown to improve animal performance, as evidenced by increased production of volatile fatty acids, increased nutrient digestibility, increased feed conversion efficiency, and stimulation of lactic acid-dependent protozoa [60]. They have been utilized to increase the efficiency of the feed usage, decreasing diarrhea in pigs and cattle and enhancing milk production. They have also been used to prevent *Salmonella* colonization of the intestinal tract of chickens [61]. In addition to its improved ability to regulate the immune system, Lvipan, a commercial in-feed probiotic, significantly lowers the abundance of *Campylobacter* spp. invading the gastrointestinal tract, thus enhancing hygiene in the poultry environment by suppressing pathogenic pollutants [62].

Roselli and colleagues [63] observed that feeding probiotics to weaned piglets and sows improves gut health through a balanced microbiota, enhancing immunological and physiological processes and preventing gastrointestinal disorders. The main responses were observed as immediate changes in the microbial ecosystem of the gastrointestinal tract, antagonizing the survival of neighboring pathogens while producing favorable fermentation products. This was confirmed by a related study by Hanczakowska et al. [64], who found that *Enterococcus faecium*, when fed as a feed additive to piglets, showed an inhibitory effect against *Clostridium perfringens*. Based on its richness, the microflora in animals' gastrointestinal tracts can be thought of as an active metabolic organ. Therefore, in order to prevent disease invasion in animals with large population densities, it is crucial to maintain a functional gut flora [65]. In general, lactic acid bacteria with probiotic potential excrete organic acids that increase the acidity of the gastrointestinal tract environment, thus reducing the risk of pathogen infestation while regulating the microbial ecosystem within the gut habitat [66, 67].

A combination of probiotic supplements containing *Lactobacillus* strains drastically reduced *Salmonella* and *Shigella* in goat fecal samples [68]. Similarly, it has been observed that a complex mixture of lactobacilli isolated from the intestines of piglets improved the density of beneficial microorganisms in the gastrointestinal tract while condensing that of enteric pathogens such as *E.coli* [69]. Weaned pigs fed with lactic acid bacterial supplements in their basal diet showed significant improvements in digestion rate, growth, fecal microbial count, intestinal morphology, maintenance of gastrointestinal pH, and control of diarrhea [70, 71]. Feeding turkeys with dietary inclusion of lactobacilli increased their daily body weight and improved the hen's ability to lay eggs [72]. In addition, bifidobacteria are an important component of the intestinal flora of chickens and have been shown to have a positive effect when administered to piglets and other mammals. These bacteria are an essential part of the intestinal flora of chickens. A commercial strain of *Bifidobacterium bifidum* was effective in treating broiler chickens infected with cellulitis. Furthermore, *B. longum* PCB 133, when administered to chickens, decreased the abundance of *Campylobacter jejuni* in poultry feces [73, 74]. *B. adolescentis* Z25 also showed significant potential in the treatment of disorders of blood glucose levels, lipid metabolism, tissue damage, and dysbiosis of the gut microbiota of cattle [75]. Numerous species of *Bifidobacterium* have demonstrated the ability to reduce lactose intolerance by increasing  $\beta$ -galactosidase [76].

Conventionally, a number of probiotics have been employed as bioprotectors in meat products [76, 77]. They have been observed to release exopolysaccharides

that have the ability to stop pathogenic pollutants from forming biofilms [78]. *Lactobacillus* strains have also attained commendable outcomes in raw chicken meat for safety against *Listeria monocytogenes* and *Salmonella Enteritidis* (*Salmonella enterica* subsp. *enterica* Serovar *Enteritidis*) [79]. Furthermore, mycotoxins are commonly detected in animal feed, posing a major health concern to livestock and having a tendency to make their way into the human food chain through meat and other dairy products [80]. Probiotics combat pathogens by producing different inhibitory substances, such as hydrogen peroxide, organic acids, and bacteriocins [81]. Additionally, they release metabolites including; acidophilin, aciolin, lactolin, and lactobacillin, which have inhibitory activities against different pathogenic microorganisms, including *Shigella*, *Salmonella*, *Proteus*, *Pseudomonas*, *E.coli*, *Staphylococcus*, *Proteus*, and *Klebsiella* [82]. Furthermore, probiotics can modulate the anti- and pro-inflammatory cytokine production to control inflammation in the host [83]. The administration of a lactobacilli-based probiotic preparation has somehow resulted in a better degree of detoxification in chickens against nephrotoxic, carcinogenic, and immunotoxic mycotoxin [84].

#### 4.1 Probiotics increase digestibility

Probiotics accelerate an animal's rate of digestion. In broilers, probiotics can improve the composition of cecal microorganisms and the way nutrients are absorbed [40]. According to Zhang and Kim [85], probiotics increase the ileal digestibility of key amino acids, increasing chicken body weight by 5%. Maas et al. [86] conducted experiments combining probiotics comprising of *Bacillus amyloliquefaciens* and the enzymes phytase and xylanase. They observed the impact of *Bacillus amyloliquefaciens* on digestion and metabolism, enhancing microbial interaction in the gut and increased absorption of calcium. Probiotics improve the host's ability to digest food by boosting the activity of digestive enzymes in the GIT [86]. For instance, research in buffalo calves revealed that probiotic feed containing *Lactobacillus acidophilus* could guarantee higher dry matter intake, daily feed conversion, and specious digestibility of nutrients in comparison to the control group [87].

#### 4.2 Probiotics improve the immune system

Probiotics can enhance immunity in the host in many ways. In several studies, probiotics have been verified to exhibit immune-stimulatory properties [88]. Probiotics encompassing *Saccharomyces cerevisiae* and *Lactobacillus fermentum* stimulated the gut T-cell immunity, as evidenced by the increased yield of CD3+, CD4+, and CD8+ T lymphocytes in the chickens GIT [89]. When *Lactobacillus gasseri* TL2919 and *Lactobacillus jensenii* TL2937 probiotics were fed to neonatal chicks, their small intestines expressed more CD3+, IL-2, and IFN- $\gamma$  genes than the control group [90].

In neonatal chicks of three-day- and seven-day-old expression of CD3+, IL-2, and IFN- $\gamma$ -genes were higher in the small intestine when provided with food with the probiotics *Lactobacillus gasseri* TL2919 and *Lactobacillus jensenii* TL2937 than in the absence of probiotics [90]. Chicken serum immunoglobulin levels can also be increased by probiotics. IgA and IgM serum levels were raised in chickens using a probiotic feed supplement comprising of *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Clostridium butyricum* [87]. Probiotics can also boost serum immunoglobulin levels in chickens. A probiotic feed supplement containing *Bacillus subtilis*, *Clostridium butyricum*, and *Lactobacillus acidophilus* enhanced IgA and IgM serum levels in chickens [85].

Lactic Acid Bacteria (LAB) are capable of producing a wide variety of antimicrobial compounds that decrease pathogenic invasions. These comprise antimicrobial peptides (AMPs), such as organic acids, defensins, bacteriocins, diacetyl, and carbon dioxide [91]. It has been demonstrated that organic acids such as lactic acid, short-chain fatty acids, and formic acids can reduce potentially dangerous microorganisms that are significant to farm animals. According to Russo et al. [92], the primary outcome of glucose metabolism by *Lactobacillus* bacteria is the production of lactic acid. Organic acids target the bacterial cell wall, cytoplasmic membrane, and specific metabolic processes like replication and protein synthesis, causing the elimination and demise of harmful microorganisms [93].

Probiotics have the ability to impact the immune response of the host, including both innate and adaptive immunity. Various types of immune cells, including dendritic cells, granulocytes, macrophages, B lymphocytes, and T lymphocytes, participate in inflammation processes, which are controlled by cytokines such as IL-8, TNF $\alpha$ , IL-1 $\beta$ , IL-6, and IL-15 interleukins. Innate immunity offers natural physical and chemical defenses against pathogens for the host organism. For instance, intestinal epithelial cells (IECs) stop harmful microbes from spreading to cause infections [59].

Studies have shown that certain types of *Lactobacillus* bacteria, including *Lactobacillus salivarius*, *Lactobacillus crispatus*, *Lactobacillus fermentum*, and *Lactobacillus gasseri*, can positively influence the production of pro- and anti-inflammatory interleukins IL-6, IL-8, and IL-10 in animals to control inflammation and restore balance [94–97]. *Lactobacillus delbrueckii* strain has the ability to promote anti-inflammatory qualities and reduce the levels of pro-inflammatory cytokines IL-8. *Aeromonas hydrophila*-contaminated carp (*Cyprinus carpio* Huanghe var.) exhibited inflammatory reactions when given  $1 \times 10^7$  CFU g/L of *Lactobacillus delbrueckii* probiotics, according to Zhang et al. [98]. The immune response that is adaptive relies on B cells and T cells, which trigger a targeted response to antigens. Adding  $1 \times 10^9$  CFU/kg of *Lactobacillus acidophilus* LA5 to poultry feed increased the levels of CD8+, CD4+, and TCR1+ T cells in both the gastrointestinal tract and peripheral blood system of the birds [99].

## 5. Probiotic use in animal production

A number of variables, including sex, nutrition, age, and genetics, can influence animal growth [100]. Among these, giving an animal a sufficient nutritional platform is crucial for its growth and development [101]. Enhancing the digestibility of feed is essential for optimizing growth in addition to providing the right amount of feed to the animal. According to a study by Meng et al. [101], probiotic supplements of *Clostridium butyricum* and *Bacillus subtilis* have been shown to improve the productivity of pigs. The improved nutritional digestibility in the probiotic-supplemented pigs as compared to the control group was ascribed to the improvement in growth performance. Likewise, pigs that were supplemented with a type of *Bacillus* culture showed increased nutritional absorption. In this instance, pigs supplemented with a probiotic demonstrated a 10% improvement in protein utilization after 4–5 months of feeding as compared to none supplemented fed pigs.

Probiotic supplements have also been shown to improve performance and weight gain in calves [102, 103] and broiler chicks [104–107]. Turkeys supplemented with *Bacillus amyloquelaciens* exhibited higher feeding frequency and duration [108]. The precise process remains unknown, but more recent studies on the relationship

between gut-brain axis and gut microbiome have suggested that there may be neurological alterations that cause the microbiota to influence farm animals' eating habits [34]. According to Jørgensen et al. [109], probiotic supplementation is more effective for the pigs throughout the weaning and growth stages and less sensitive during the finishing stages [110]. It has also been demonstrated that adding dietary probiotics enhances the quantity and quality of milk in dairy cows and eggs produced by hens [111]. During feeding, the inclusion of *Bacillus subtilis* and *Bacillus licheniformis* greatly increased the milk's protein and fat content in sheep [112]. In a similar vein, *Aspergillus oryzae* culture supplements raised the percentage of protein and dry fat-free solids in milk produced by dairy cows [113]. Xu et al. [114] observed an increase in milk yield of 37% in dairy cows supplemented with *Lactocaseibacillus casei* Zhang and *Lactiplantibacillus plantarum* P-8 [52]. Addition of *Bacillus licheniformis* and *Bacillus subtilis* to the diet of laying hens resulted in a 3% increase in egg output and a 35% decrease in cholesterol levels in the yolk [115]. Furthermore, inclusion of  $10^7$  CFU/g of probiotic to laying hens' diet reduced the negative impacts of heat stress through increased egg production, increased feed intake, and improved immunological response [116].

## 6. Impact of probiotics on meat quality

Meat quality is a predominant term describing characters persuading consumer purchasing decisions and eating experiences. Such characters include meat texture, juiciness, flavor, color, and water holding capacity (WHC) [117]. These attributes can be influenced by diet, sex, breed, and factors such as pre- and post-slaughter, climate change packaging, and also the storage of final meat products. Over the years, quite a number of studies have investigated the use of feed additives or supplements, such as probiotics, to improve meat quality. As a dietary supplement, probiotics exhibited positive effects on carcass weight and meat quality of lambs and goats (**Table 2**; [127, 128]). Such effects on meat quality include improved shelf life, color, tenderness, and healthy fatty acid profiles [119, 120, 122, 125, 126]. Sensory characteristics such as color are regarded as significant characteristics that influence consumer choice. This is because customers infer freshness and wholesomeness from meat based on color [129]. Meat's color is related to the quantity of myoglobin pigment, pH level, oxidation of lipids, and pre- and post-mortem handling. Meat's color is typically affected by myoglobin oxidation or reduction, which can lessen the meat's color intensity.

According to a study by Nie et al. [127], giving lambs a combination of probiotics (i.e., *B. subtilis*, *B. licheniformis*, and *L. plantarum*) for 60 days enhanced the color of the meat by lowering myoglobin oxidation. *Lactobacillus* sp., *Lactobacillus plantarum*, and *Lactobacillus casei* are probiotics that have been demonstrated to improve both the color and flavor of meat [128]. Supplements including *Lactobacillus* and yeast (i.e., *Saccharomyces cerevisiae*) also enhanced the color of the meat [124]. The flavor and odor of meat are often caused by volatile chemicals; probiotic supplementation boosts antioxidant activity, which may lessen lipid oxidation and enhance the flavor of lamb meat [128].

Researchers have also looked into pH as a significant factor in meat color and its impact on lactic acid production and glycolysis throughout the post-mortem stages. pH typically decreases steadily from an initial value of roughly 7.2 to a final pH of roughly 5.6. However, pale, mushy, and exudative flesh develops when the pH level in carcasses drops quickly. In this instance, water that exudes from the flesh and

| Probiotics                                                                                                                        | Administration                                                         | Host                           | Duration | Effects on carcass/Meat quality                                                                                                                                                                                                                       | References |
|-----------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <i>Saccharomyces cerevisiae</i> + <i>elenium</i><br><i>Saccharomyces cerevisiae</i>                                               | 3 g/d/head ( $5 \times 10^6$ CFU/g)                                    | 24 male Texel lambs            | 120 d    | ↑ fat% in meat<br>~ moisture%, ash%, protein%,<br>~ meat quality (cooking loss, color,<br>shear force)<br>~ sensory traits (flavor, juiciness and<br>tenderness)                                                                                      | [118]      |
| <i>Lactiplantibacillus plantarum</i> P-8 and<br><i>Lactiacetibacillus casei</i> Zhang                                             | 1% level in diet $1.5 \times 10^9$ CFU/g                               | 12 Sunit lamb                  | 90 d     | ↑ intramuscular fat deposition<br>↑ muscle production<br>(myogenesis) and meat quality<br>↓ MYOD1 gene expression<br>in muscle<br>↓ cooking loss and shear force<br>↑ MAPK signaling pathway activity in<br>muscle<br>↑ body length, LT area          | [119]      |
| Mixture of <i>B. subtilis</i> , <i>Bacillus licheniformis</i> ,<br>and <i>Lactiplantibacillus plantarum</i> (ratio of<br>1:1:0.5) | 10 g/kg feed                                                           | 40 lambs<br>(Chuanzhong black) | 70 d     | ↑ carcass yield<br>↑ abdominal fat%<br>↑ moisture and intramuscular fat in LT<br>muscle<br>↑ mRNA expression of MyHC<br>↓ lightness, yellowness,<br>shear force value and<br>heneicosanoic acid in LT muscle<br>↑ meat quality (color and tenderness) | [120]      |
| <i>Saccharomyces cerevisiae</i>                                                                                                   | 0.5, 1.0 and 1.5 mL/kg body<br>weight<br>( $3.6 \times 10^9$ cells/mL) | 16 Malpura lambs               | 180 d    | ~ meat quality (cooking loss, water<br>holding capacity and chilling loss)<br>~ carcass trait (weight and<br>composition)                                                                                                                             | [121]      |

| Probiotics                                                                                               | Administration                                                                                                                | Host                                          | Duration | Effects on carcass/Meat quality                                                                                                                                                                                                                                                                                                                                                                                                        | References |
|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <i>Lactiplantibacillus plantarum</i> HM-10 and <i>Lactiacetibacillus casei</i> HM-09 and of              | <i>L. plantarum</i> ( $1.5 \times 10^9$ CFU/g) and <i>L. casei</i> ( $1.5 \times 10^9$ CFU/g)                                 | 24 Sunit lambs                                | 90 d     | ↑total antioxidative capacity (T-AOC) and catalase (CAT) activity of LT muscle<br>↓superoxide dismutase (SOD) activity<br>↓shear force values and lightness in LT) muscle<br>↑meat tenderness and flavor<br>~ pH, meat color (lightness, yellowness, redness)<br>~ cooking loss<br>Alter the composition of meat volatile flavor compounds (nonanal, undecanal, 1-pentanol, 1-hexanol, and 2,3-octanediol) (observed by electric nose) | [122]      |
| <i>Bacillus licheniformis</i> and <i>Bacillus subtilis</i>                                               | 600 mg/kg<br>$1 \times 10^{11}$ CFU/g                                                                                         | 39 male goats (Yantse River Delta White)      | 80 d     | ↑ rib tissue thickness (GR)<br>↑ pre-slaughter weight                                                                                                                                                                                                                                                                                                                                                                                  | [123]      |
| <i>Lactobacillus reuteri</i> E81<br><i>Saccharomyces cerevisiae</i><br><i>Lactobacillus rhamnosus</i> GG | 300/600 ppm of <i>Lactobacillus</i> /head<br>( $4 \times 10^{10}$ CFU/g)                                                      | 90 Anaoian Merino weanling lambs              | 70 d     | ↑daily weight gain, body weight gain, and feed conversion ratio<br>↑lightness in meat ~ redness and yellowness in meat<br>↑ meat pH value<br>↑fattening performance of lamb                                                                                                                                                                                                                                                            | [124]      |
| <i>Saccharomyces cerevisiae</i><br><i>Lactobacillus acidophilus</i>                                      | 2.5 and 5.0 g/h/d<br><i>S. cerevisiae</i> $5.0 \times 10^{11}$ CFU/g and <i>L. acidophilus</i> $2.0 \times 10^{12}$ CFU/g and | 30 growing goats (Thai native × Anglo-Nubian) | 56 d     | ↑ rumen metabolism and growth performance<br>↑ conjugate linoleic acid, total n-6, and total polyunsaturated fatty acids in plasma                                                                                                                                                                                                                                                                                                     | [125]      |

| Probiotics                                                                                                    | Administration                                                                                                                                                                    | Host                     | Duration | Effects on carcass/Meat quality                                                                                                        | References |
|---------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|----------|----------------------------------------------------------------------------------------------------------------------------------------|------------|
| <i>Streptococcus faecalis</i> T-110<br><i>Clostridium butyricum</i> TO-A<br><i>Bacillus mesentericus</i> TO-A | 5 g/d/kid<br><i>S.faecalis</i> T-110 ( $2 \times 10^8$ CFU/g);<br><i>C. butyricum</i> TO-A<br>( $2 \times 10^6$ CFU/g)<br><i>B. mesentericus</i> TO-A<br>( $2 \times 10^6$ CFU/g) | 30 male native goat kids | 180 d    | ↑ pre-slaughter weight<br>↑ carcass weight<br>↑ dressing percentage and dressed weight<br>↑ head and stomach weight<br>↑ carcass yield | [126]      |

Abbreviations: CFU, colony-forming unit; ↑, increased; ↓, decreased; ~, not changed, LT, *Longissimus thoracis*; MyHC, myosin heavy chain; MAPK, mitogen-activated protein kinase; DM, dry matter; LAB, lactic acid bacteria.

**Table 2.**  
 Effect of probiotics on carcass and meat quality of small ruminants.

myoglobin, a protein that is soluble in water, are lost, giving the meat a pale, bland appearance. The oxidation of muscle fiber affects the color and lightness of meat in addition to pH [128]. Meat loses color due to myoglobin oxidation [127]. Amidst the aforementioned details, scientists have lately investigated the possible application of probiotics to enhance the color and consistency of meat. For instance, Tian et al. [130] found that adding *Limosilactobacillus reuteri* to the diet changed the properties of muscle fibers and may even improve the color of the meat. Additionally, feeding *L. reuteri* increases the muscle's ability to hold water, which greatly improves the softness of the meat. Meat's ability to retain water is what gives it its softness and juiciness [130]. Probiotic-supplemented diets help maintain meat's high pH, which increases meat's WHC.

According to Lambe et al. [131], probiotic supplementation has a beneficial effect on intramuscular fat deposition and is positively correlated with marbling, softness, and meat flavor. The theory is that taking probiotic supplements stimulates the growth of adipocytes, which help store fat in the skeletal muscle. Probiotic supplements have been shown by Liu et al. [122] to improve muscle fiber density, which contributes to meat's tenderness and suppleness. In a number of prior sensory studies involving a group of panelists, probiotic-supplemented meat was preferred by panelists over meat without probiotic supplements since probiotics were shown to improve meat's color, flavor, and softness [118, 122]. Furthermore, the composition of volatile chemicals, which is connected to the quality characteristics of meat, may also change as a result of dietary probiotic supplementation [120, 122]. Probiotics also help to improve the polyunsaturated fatty acid profile of meat [132] and have antioxidant qualities [133]. Certain probiotic strains have the ability to create bacteriocins, which preserve meat's beneficial organoleptic qualities and function as preventive measures against lipid oxidation. It is evident that using probiotics as a feed supplement enhances the quality of meat.

## 7. Mode of action of probiotics

Probiotics exert their effectiveness in a variety of ways. Probiotics enhance animal health and productivity by inhibiting and managing intestinal infections. The basic mechanisms of action of probiotics includes (i) inhibition of pathogen adhesion; (ii) production of antimicrobial components; (iii) competitive exclusion of pathogenic microorganisms; (iv) enhancement of barrier function; (v) reduction of luminal pH; and, (vi) modulation of the immune system (**Figure 1**).

Probiotics stimulate health conditions by inhibiting pathogenic bacteria. For example, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* can prevent *Escherichia coli* from adhering to the intestinal tract of pigs [134]. Bacteria frequently interact with host cells subsequent to the release of chemical signals that influence bacterial organisms' approaches through the techniques called quorum sensing [135, 136]. Probiotics inhibit the adhesion and translocation of bacteria by producing antimicrobial compounds. *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Bifidobacteria*, *Leuconostoc*, *Streptococcus*, and *Lactococcus* can produce bacteriocins (proteins) that inhibit the growth of closely related bacterial organisms. These probiotic strains decrease the number of harmful microbes in GIT [137, 138]. The ribosome of many bacteria produces bacteriocins, which are bioactive antimicrobial peptides that adhere to pathogenic microbial cells and pierce their phospholipid membranes. According to van Zyl et al. [139], the fundamental pattern of the bacteriocin-mediated pathogen

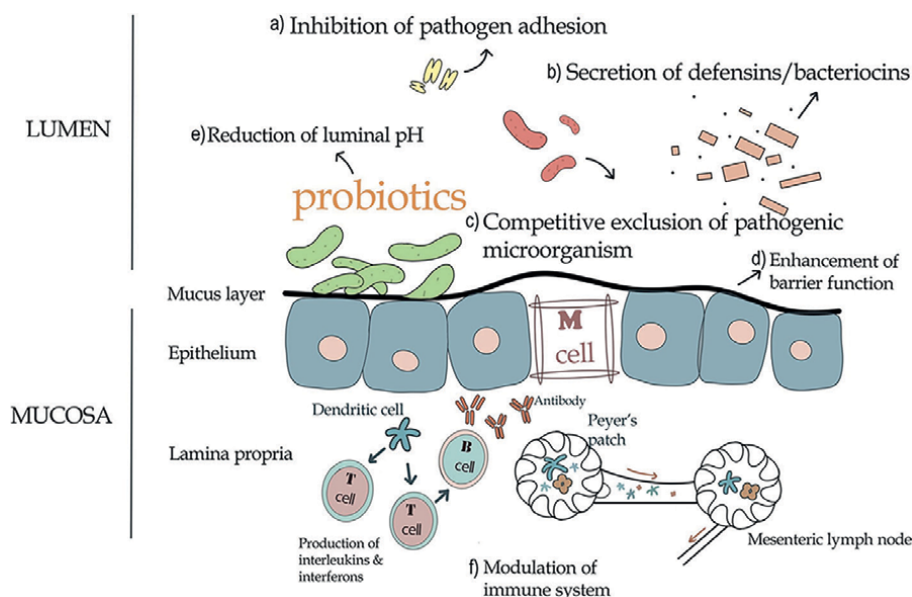


**Figure 1.**  
 Probiotic mode of action. These comprise six proposed mechanisms: (a) Inhibition of pathogen adhesion; (b) secretion of bacteriocins; (c) competitive exclusion of pathogenic microorganisms; (d) enhancement of barrier function; (e) reduction of luminal pH; and (f) modulation of the immune system.

reaction encloses the cytoplasmic membrane penetration of pathogenic bacteria that results in the suppression of RNA and DNA synthesis and cell leakages.

Probiotics have the capacity to improve the intestinal communication system, which allows them to competitively exclude harmful microbes from the host intestine [50]. According to van Zyl et al. [139], this anti-pathogenic process, known as competitive exclusion, demonstrates that bacterial species tenaciously compete to connect to receptors at specific binding sites in the GIT. It may also combine the release of antimicrobial compounds with competing for available nutrients. Probiotics can shape the cell-to-cell communication of bacteria and host and maintain cellular uniformity by institution of the intestinal barrier function. Such consistency is accomplished through the modulation of cytoskeletal and tight junctional protein phosphorylation [50].

By reducing luminal pH, probiotic microbes can resist harmful pathogens. *Bifidobacterium breve*, a probiotic, can reduce luminal pH. In a lethal Shiga toxin-producing *Escherichia coli* O157:H7 animal experimental model, *B. breve* can cause acetic acid generation at greater concentrations [140]. Probiotics have the ability to modify the immune system, which enhances the host's immunity. Probiotics that are eaten are essential for inducing a signal network and activating the mucosal immune system (MIS). Using various experimental techniques, the impact of several probiotic microorganisms on dendritic cells (DC) has been studied. Antigen-presenting dendritic cells perform vital roles in both innate and adaptive immunity. In addition to initiating primary immune responses that directly result in the formation of T- and B-cell responses, dendritic cells have the ability to recognize and respond to components of bacteria. With pathogen recognition patterns (PRPs) on their surface, which let them accurately identify the pathogen-associated molecular patterns (PAMPs) on the bacterial organism, probiotics have the ability to directly control intestinal dendritic

cells. By upregulating co-stimulatory molecular expression, this therapeutic technique promotes DC maturation. Cytokine release stimulates T-cell activation when the immune system becomes active [141, 142]. The type of T-cell responses, such as T helper cell polarization or T regulatory response, are determined by DC-originated signals. This information influences B-cell responses against pathogenicity [143].

## **8. Conclusion**

It is eminent that by 2050 the global population is projected to exceed 9 billion people. The modern consumer consumes a high percentage of meat and animal products; therefore, by 2050, agricultural food production (especially animal production) may need to expand by 110% to meet the growing demand for animal products. In today's conventional animal production system, the public is strongly against subtherapeutic-level antibiotics in food animal supplementation since it has led to the emergence of antibiotic-resistant bacteria. Probiotics commonly applied as direct-fed microbial (DFM) are live microorganisms incorporated in an animal's diet as feed supplements. They usually convey beneficial properties predominantly through action in the GIT of the animal. Probiotic supplementation has been shown to improve animal performance and health by supporting gut health and nutrient utilization, thereby ultimately increasing animal performance and productivity and the quality of animal products such as meat and milk. In the outlook, research premised to: define or depict ideal probiotic microbial strains for specific animal types, determine the ideal dosages and mixes, and comprehend the web of interactions between probiotics and the gut microbiota, may aid in the development of more potent feed formulations for use in animal production.

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# Probiotics, Prebiotics, and Postbiotics Have a Positive Impact on Both Health and the Production of Food

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### Abstract

The *Lactobacillus* group, which includes many species and strains, presents an astoundingly vast spectrum of diversity, which has grabbed the attention of scientists who continue to investigate and assess its enormous potential in a variety of biotechnology fields. The goal of this extensive inquiry is to explore deeper into *Lactobacillus*' numerous applications, particularly in food manufacture, food safety, and the development of supplements targeted at optimizing general well-being. By doing a more thorough investigation of these factors, we may gain a better grasp of the tremendous benefits and bright future that lie ahead for the use of *Lactobacillus* in the vast field of biotechnology.

**Keywords:** biotechnology, *Lactobacillus*, human health, postbiotics, sustainable food systems

### 1. Introduction

Probiotics, considering one of the most eco-healthy solutions for bacteria in life, has gained great importance in recent years [1]. Probiotics are defined as live microorganisms which exert when administered in appropriate amounts, a beneficial effect on the host. Probiotics are widely used in the food industry, especially in fermented dairy products. Fermented milk products containing probiotics become a valuable part of the diet since they improve the functional properties of food and have a positive effect on the health status of the consumer. They can be a preventive and therapeutic tool in many diseases due to the modulation of the composition of intestinal microflora. Postbiotics are inactive bacterial cell components that possess beneficial physiological and health effects on the host. The idea of postbiotics is relatively new and is still being studied to clarify some aspects [2].

Global food demand is projected to increase by 70% by 2050 due to world population growth and increases in global prosperity (**Figure 1**). The current food system is harmful to people's health and the natural environment. Meat and dairy consumption levels are expected to increase by 70–90% by 2050. The industrialized global food

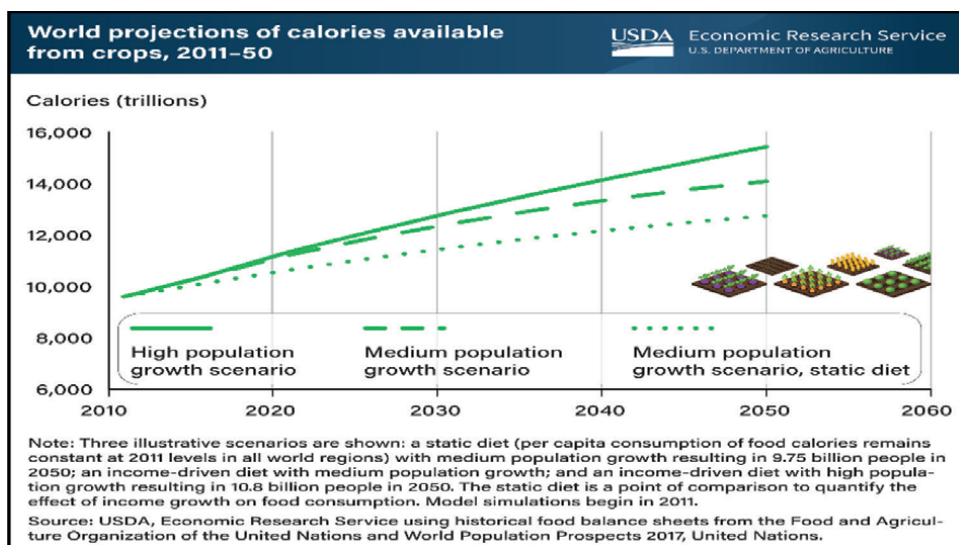


**Figure 1.** Illustration of a static diet scenario with per capita food consumption adjusted for population growth (Source: USDA, Economic Research Service using food balance sheets from the Food and Agriculture Organization of the United Nations).

system is a major contributor to climate change, biodiversity loss, deforestation, and water pollution. Climate change is projected to increase food security challenges in developing countries by increasing the frequency of extreme weather events, pests and diseases, and detrimental effects on soil, as well as water quality and availability. The food system is a major determinant of public health. An unhealthy diet is the leading risk factor explaining the burden of non-communicable diseases worldwide. Evidence suggests a global increase in overnutrition, unhealthy diets, and sedentary lifestyles [3].

## 1.1 Explanation and overview

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. They are commonly found in fermented foods such as yogurt and kefir. These foods contain live bacteria and yeasts that are beneficial for digestion and overall gut health. They are known as probiotic foods and are becoming increasingly popular for their health benefits [4]. This section provides an explanation and overview of the role of probiotics, prebiotics, and postbiotics in human health and sustainable food systems. It examines the benefits and applications of these components in promoting overall well-being and addressing food security challenges [5, 6].

In addition to probiotic foods, there are also prebiotic foods that promote the growth of beneficial bacteria in the gut. Prebiotics are non-digestible fibers that serve as food for probiotics and help them to thrive in the gut environment. Prebiotics are defined as selectively fermented ingredients that result in specific changes in the composition and/or activity of the gut microbiota, which confer benefits upon host health. This definition extends to food ingredients that yield beneficial effects on the gut microbiota, including specific but non-fermentable polyphenols or flavonoids, fermented dairy products, fiber-rich foods containing whole grain, fruit, and

vegetable polysaccharides, and drinks like coffee, green tea, and chocolate. These compounds pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria within the colon [7].

Postbiotics are defined as preparations of inanimate microorganisms and/or their components that confer a health benefit on the host. They are the metabolic byproducts of probiotic microorganisms and have been shown to have various health benefits. Postbiotics are gaining attention in the field of nutrition and health research. They are defined as the bioactive compounds produced by food-grade microorganisms. They are defined as the bioactive compounds produced by food-grade microorganisms, which have been shown to have beneficial effects on human health. Postbiotics include a wide range of compounds such as short-chain fatty acids, antimicrobial peptides, and vitamins [8, 9].

Fermented food and gut microbiota have co-evolved with humankind throughout history. Recently, there has been an increasing interest in the consumption of fermented functional foods and their role in sustaining gut health and the microbiota. Fermented foods are a part of the human culture that has existed throughout history, and fermented food relies on certain microorganisms used during specific processing steps. Fermented foods, and dairy in particular, have been the focus of microbiota intervention studies in children and the elderly, and a growing body of clinical evidence highlights the beneficial effects of dairy food on preventing allergic disease, bone, and cardiovascular health, preventing and recovering from infections, and declining cognition among the elderly population. Fermented foods have been recommended as healthy food alternatives by various institutions. They are known to contain probiotics, which are beneficial bacteria for gut health. Probiotics, prebiotics, and postbiotics play a crucial role in maintaining a healthy gut microbiota. They contribute to improved digestion, nutrient absorption, and overall immune function. These beneficial microorganisms can be found in fermented foods such as yogurt, kimchi, and sauerkraut. They are known for their potential to improve gut health and overall well-being. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [10, 11].

Globally, the population around the world is experiencing unprecedented growth, which in turn has led to an increase in urbanization and food production. However, this rapid growth has put immense pressure on various aspects of our society, such as health, agriculture, and the environment. In order to cope with these challenges, it is crucial for us to develop sustainable food systems that can effectively minimize waste generation and environmental impact, while simultaneously maximizing resource utilization and food security. Furthermore, it is well known that there exists a strong relationship between diet, gut health, and the occurrence of microbial dysbiosis and infectious diseases. As a potential solution to address this issue, a diverse diet that is rich in both pre- and probiotics has been proposed. Such a diet has the potential to boost gut health by modulating the composition of the gut microbiota, which ultimately enhances the body's resilience against various health challenges. In light of these considerations, it is suggested to explore the feasibility of coupling the recovery of food processing side-streams with the growth of specifically selected probiotics, such as lactobacilli. By doing so, we can leverage these tools for the purpose of developing food products that possess gut health-promoting characteristics. This approach holds great promise as it not only contributes to the creation of sustainable food systems but also offers a viable strategy for tackling the issue of microbial dysbiosis on a larger scale within the population. Hence, by embracing the concept of sustainable food systems and leveraging the potential of gut health-promoting diets, we can make

substantial progress in addressing the challenges posed by the ever-growing population, urbanization, and food production. Ultimately, by adopting these approaches, we can strike a balance between meeting our nutritional needs, preserving the environment, and safeguarding the health of current and future generations [12, 13].

## **1.2 Significance in human health and sustainable food systems**

The important role of probiotics, prebiotics, and postbiotics in health, nutrition, and disease prevention has been the topic of intense and growing interest, both from scientific and industrial perspectives. This interest continues to expand dramatically with increasing investigations into new and novel applications of these compounds in the food as well as non-food industries. There are many diverse areas of potential application and exploitation of probiotics, prebiotics, and postbiotics as beneficial human health adjuncts, disease prevention technologies, and even technologies for better fertility and child development. Probiotics, prebiotics, and postbiotics are also being investigated and applied in health-research areas aside from human health, in the fields of veterinary, aquaculture, and agricultural/plant health. There is perhaps also a growing recognition of the potential wider socio-economic importance of probiotics/prebiotics/postbiotics technology in many developing countries [2]. Sustainable food systems will help fight world poverty, hunger, and malnutrition, protect the biosphere, promote renewable resources and cycles, and safeguard non-renewable resources for future generations. Ecosystems, global warming, energy, freshwater, and land-use changes are crucial aspects of sustainability. More than 1 billion people are under-nourished, and more than 2 billion people are overweight in a world where over a billion people are chronically poor.

Development of sustainable food systems will require not only abundant but also nutritious food, for which dietary fibers, probiotics, and fermentation processes are of particular importance. Prebiotics and probiotics are key in creating functional foods for the elderly. Biotechnology can play an important role in developing sustainable food systems by processing food, bio-energy production, reducing environmental pollution, enrichment, supplementation, and detoxification of food, and food safety and security. The development of biotechnological food processes has often focused on the processes themselves. Biotechnology has great potential to contribute to cost-effective approaches to produce food on degraded and marginal soils around the world. Phytoremediation and bio-energy production of waste products can also be useful in developing countries [14].

## **2. Probiotics**

Probiotics refer to living microorganisms that confer health benefits on the host when administered in an adequate amount. The most frequently used probiotic strains belong to the genera *Lactobacillus* and *Bifidobacterium*. Other genera such as *Enterococcus*, *Lactococcus*, *Propionibacterium*, *Streptococcus*, and *Saccharomyces* are also listed in the GRAS (generally recognized as safe) category by the United States Food and Drug Administration (FDA). Probiotics can be defined functionally as living unicellular microorganisms, which, when ingested, are followed by colonization of the gut. Even though several strains show beneficial effects in humans, Genus *Lactobacillus*, *Lactocaseibacillus rhamnosus*, and *Saccharomyces boulardii* have been considered as best characterized probiotic strains and among the most commonly

used. Probiotic products are most commonly found in fermented milk, yogurt, dietary supplements, soy milk, and some juices [14]. In recent years, probiotics have also been included in cereals, chocolates, ice creams, and savory goods.

## 2.1 Mechanisms of probiotic action

Probiotics prevent pathogens from adhering to the epithelial cells. The interactions of *Lactobacilli* and *Bifidobacteria* with mucin, which is one primary component of the mucosal barrier, are crucial for attachment to the epithelial cells. *Lactobacilli* can also inhibit pathogen attachment by co-aggregation with them. *In vitro* studies show that probiotic-associated bacteria reduce the binding of pathogens to human intestinal cells by half [15]. *In vivo* studies also confirm that feeding rats with *L. acidophilus* prevents the colonization of *E. coli*. Probiotics can aggregate with pathogens through their surface charge, hydrophobicity, and mucus-binding properties. It has also been shown that specific cell-surface-associated polysaccharides of probiotics can facilitate aggregate formation with pathogens. Probiotics exert antibacterial activity by producing antimicrobial compounds such as hydrogen peroxide, lactic acid, cyclic dipeptides, bacteriocins, and exotic bacteriocin-like inhibitory substances. They also inhibit pathogens by producing short-chain fatty acids (SCFA), which lowers the pH and inhibits the growth of pathogenic bacteria. Probiotics can provide an alternative approach to antibiotic therapy in pigs, chickens, lambs, and calves by inhibiting salmonella colonization in the intestine [14, 16].

## 2.2 Types of probiotics

Probiotics are categorized into various types based on their genera, species, and the specific health benefits they offer. The most common probiotic bacteria belong to the genera *Lactobacillus* and *Bifidobacterium*, but other bacteria and even some yeasts are also recognized as probiotics. Each type has unique properties and functions that contribute to different aspects of human health.

### 2.2.1 *Lactobacillus*

*Lactobacillus* is one of the most widely studied and used genera of probiotics. These bacteria are known for their ability to convert sugars into lactic acid, which helps create an acidic environment in the gut that inhibits the growth of harmful bacteria. *Lacticaseibacillus acidophilus*: Often found in yogurt and other fermented foods, it supports digestion and enhances the absorption of nutrients. *Lacticaseibacillus rhamnosus*: Known for its role in preventing and treating diarrhea, particularly antibiotic-associated diarrhea. *Limosilactobacillus reuteri*: Contributes to oral health, reduces inflammation, and may help with infant colic. *Lacticaseibacillus casei*: Improves gut health, supports immune function, and is often used in the prevention of gastrointestinal infections [17, 18].

### 2.2.2 *Bifidobacterium*

*Bifidobacterium* is another major group of probiotics, predominantly found in the intestines, particularly in infants. These bacteria help maintain a healthy balance of gut flora and play a critical role in the overall function of the digestive system. *Bifidobacterium bifidum*: Supports the immune system and aids in digestion, particularly

of dairy products. *Bifidobacterium longum*: Known for its ability to break down carbohydrates and produce lactic acid, it supports a healthy gut environment. *Bifidobacterium lactis*: Enhances digestion and may help lower cholesterol levels. *Bifidobacterium breve*: Effective in reducing inflammation and improving skin health [19, 20].

### 2.2.3 *Saccharomyces boulardii*

Unlike the bacterial probiotics, *Saccharomyces boulardii* is a yeast. It is unique in its ability to survive stomach acid and reach the intestines intact, where it exerts its beneficial effects. Effective in treating and preventing diarrhea, including traveler's diarrhea, antibiotic-associated diarrhea, and diarrhea caused by infections. Supports gut health by inhibiting the growth of pathogenic bacteria and yeasts. It may help in managing symptoms of inflammatory bowel diseases (IBD) like Crohn's disease and ulcerative colitis [21, 22].

### 2.2.4 *Streptococcus thermophilus*

*Streptococcus thermophilus* is a lactic acid bacterium commonly used in the production of yogurt and other fermented dairy products. It assists in the digestion of lactose, making it beneficial for individuals with lactose intolerance. Supports gut health by maintaining a balanced gut flora. It may have potential anti-inflammatory effects in the gastrointestinal tract [23].

### 2.2.5 *Enterococcus faecium*

*Enterococcus faecium* is a less common but important probiotic, particularly used in animal probiotics and sometimes in human supplements. Helps restore gut flora balance, particularly after antibiotic use. It may reduce the incidence of gastrointestinal infections. It supports overall gut health by inhibiting pathogenic bacteria [24].

### 2.2.6 *Escherichia coli* Nissle 1917

This specific strain of *Escherichia coli* is a probiotic used particularly for its ability to treat certain gastrointestinal disorders. Effective in treating ulcerative colitis and preventing relapses. It supports gut health by maintaining intestinal barrier function and modulating the immune response [25].

### 2.2.7 Other probiotic species

*Leuconostoc*, which is often used in fermented vegetables, this genus produces lactic acid and contributes to gut health. *Pediococcus*, which is commonly found in fermented foods, helps preserve and enhance food quality. *Propionibacterium* is used in the production of Swiss cheese and can support gut health by producing beneficial compounds like propionic acid [26, 27].

## 2.3 Mechanisms of action

Elucidation of probiotic mechanisms of action is necessary to provide a better understanding of how microorganisms impart their beneficial effects. Probiotics can be thought of as bioreactors for converting biopolymers into oligosaccharides and free

fatty acids, which help boost the immunity of the host [28]. They are also beneficial to the food system as they can make substrates available for growth or elimination of spoilage mycobiota and pathogens. Probiotic bacteria can also play an essential role in ameliorating food-related pathologies caused by harms that well-developed food webs impose upon them. Anti-inflammatory (eicosanoid/thromboxane-related), antioxidant (AH-activating antioxidant-product), cytoprotective (Apc/Ah-related), or passive (mucous layer-thickening) mechanisms of action were identified [2]. The capacity of embryo microflora to accelerate the food transition through the gastrointestinal tract or the acceleration of the luminal phase of nutrient fermentations is especially important in lowering the probability of transient food pathogen development as well as in removing toxic metabolites from ingested food.

Probiotics diminish the probability of toxic metabolite/overt pathogen food development, modify the product spectrum of nutrient fermentation, and enable the transformation of food biopolymers prior to absorption. In addition, they can be beneficial to the host as far as immune system potentiation is concerned. Probiotics applied in proper doses (usually amounts of  $10^6$  cells/g of food) during pre- or post-harvest handling of food can act as biological safety agents, while in excess amounts may provoke spoilage, digesting the nutrients prior to consumers, development of toxins, and production of undesirable odor compounds, eventually resulting in food-related pathologies.

## 2.4 Health benefits

Probiotics are live microbes that confer health benefits when administered in adequate amounts. Most commonly probiotics are lactic acid bacteria and bifidobacteria as per the FDA regulations. The microorganisms including *Lactobacillus*, *Bifidobacterium*, and lactic acid bacteria, produce various bioactive metabolites, which are beneficial for human health and well-being. The various metabolic end products include vitamin D, vitamin B2, vitamin B6, vitamin B9, cholesterol-lowering n-3 fatty acids, antibiotics, and antioxidants, which have several health benefits. Lactic acid bacteria (LAB) in the intestines limit intestinal colonization by pathogenic bacteria. There are several health benefits including improved lactose digestion and absorption, amelioration of lactose maldigestion, reduction of allergenicity of food products, prevention of gastrointestinal infections, modification of gut flora in the prevention of inflammation and diarrhea, lowering of blood cholesterol levels, and alleviation of symptoms of inflammatory bowel diseases and many other future endpoints [29, 30]. These beneficial microbes produce various bioactive compounds, including vitamins, fatty acids, antibiotics, and antioxidants, which contribute to numerous aspects of human health and well-being.

### 2.4.1 Gut health and digestive support

One of the primary health benefits of probiotics is their ability to improve gut health. Lactic acid bacteria, particularly *Lactobacillus* and *Bifidobacterium* species, play a crucial role in maintaining a balanced gut microbiota. They promote the growth of beneficial bacteria while inhibiting the colonization of harmful pathogens such as *Listeria monocytogenes*, *Salmonella Enteritidis*, and *Escherichia coli* [14]. This protective effect helps prevent gastrointestinal infections, reduce the incidence of diarrhea, and alleviate symptoms associated with inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis [29, 30].

Probiotics also enhance the digestion and absorption of nutrients. For example, they produce lactase, an enzyme that breaks down lactose, thereby aiding individuals with lactose intolerance. This improves lactose digestion and reduces symptoms such as bloating, gas, and diarrhea. Additionally, probiotics help in the fermentation of non-digestible carbohydrates, producing short-chain fatty acids (SCFAs) that nourish colon cells and promote gut health.

#### *2.4.2 Immune system modulation*

Probiotics have been shown to modulate the immune system, enhancing the body's natural defenses against infections and inflammation. By interacting with gut-associated lymphoid tissue (GALT), probiotics stimulate the production of immunoglobulins, such as IgA, which play a key role in mucosal immunity. They also help in the maturation of immune cells, such as dendritic cells and macrophages, which are essential for mounting an effective immune response [31]. Probiotic strains, particularly those from the *Lactobacillus* and *Bifidobacterium* genera, have been found to reduce the severity of respiratory infections, decrease the incidence of allergic reactions, and mitigate the inflammatory response in conditions like eczema and atopic dermatitis. Their ability to modulate cytokine production further contributes to the prevention of chronic inflammatory diseases [32, 33].

#### *2.4.3 Cardiovascular health*

Probiotics may also contribute to cardiovascular health by influencing lipid metabolism. Certain strains of *Lactobacillus* and *Bifidobacterium* have been shown to reduce blood cholesterol levels by breaking down bile salts, leading to decreased reabsorption and increased excretion of cholesterol. Additionally, the production of bioactive compounds such as conjugated linoleic acid (CLA) and n-3 fatty acids by these probiotics has been associated with a reduction in blood pressure and a lower risk of cardiovascular diseases [34, 35].

#### *2.4.4 Mental health and the gut-brain axis*

Emerging research has highlighted the role of probiotics in mental health, particularly through their influence on the gut-brain axis. The gut microbiota communicates with the brain via the vagus nerve, immune system, and microbial metabolites such as SCFAs and neurotransmitters. Probiotics can modulate this communication, potentially affecting mood, cognition, and stress response [36, 37].

Several studies have shown that probiotic supplementation can reduce symptoms of anxiety, depression, and stress-related disorders. For instance, *Lactobacillus* and *Bifidobacterium* strains have been linked to the production of gamma-aminobutyric acid (GABA), a neurotransmitter that exerts a calming effect on the nervous system. This suggests that probiotics may have therapeutic potential in managing conditions such as anxiety and depression, contributing to overall mental well-being [14].

#### *2.4.5 Metabolic health*

Probiotics also play a significant role in metabolic health by influencing the gut microbiota composition and function. They have been shown to improve insulin sensitivity and regulate glucose metabolism, which are crucial for the management

of metabolic disorders such as type 2 diabetes and obesity. By modulating the gut microbiota, probiotics can reduce chronic low-grade inflammation—a key factor in the development of metabolic syndrome and related conditions [38, 39].

Moreover, probiotics can aid in weight management by affecting appetite regulation, energy expenditure, and fat storage. Certain probiotic strains have been associated with a reduction in body fat and waist circumference, making them a potential adjunct therapy for obesity management.

#### 2.4.6 Cancer prevention

Probiotics may contribute to cancer prevention by modulating the gut microbiota and enhancing the body's immune response. Some studies suggest that probiotics can inhibit the growth of carcinogenic bacteria, reduce the production of harmful metabolites, and enhance the body's detoxification processes. Additionally, probiotics may induce apoptosis (programmed cell death) in cancer cells and prevent the proliferation of tumor cells, particularly in colorectal cancer [40].

### 3. Prebiotics

Prebiotics are naturally occurring non-digestible carbohydrates, promoting selective growth and/or activity of probiotic bacteria and other beneficial bacterial populations in the gut. Well-known prebiotics, mainly non-digestible oligosaccharides, include fructo-oligosaccharides (FOSs), galacto-oligosaccharides (GOSs), and inulin. Furthermore, resistant starch, arabinoxyloligosaccharides, xylan-oligosaccharides, and glycogen oligosaccharides have also been shown to have prebiotic effects. Prebiotics enhance gut health by improving gut microbiota and stabilizing the colonic pH, which helps in improving calcium absorption [41]. Prebiotics also reduce the risk of infection by enhancing *in situ* immunity in the gut, controlling inflammation, inhibiting pathogen colonization, improving epithelial barrier function, and others.

In comparison to probiotics, prebiotics exert a more selective and indirect impact on gut microbiota. Recently, many studies have brought insights into how specific prebiotics affect gut microbiota and overall human health. Prebiotics act as selective nutrient sources for certain beneficial gut bacterial populations and exert stimulatory or inhibitory effects based on the inherent structural conformational preferences (i.e., sugar patterns) of the oligosaccharides. Notably, GOS is well digested by bifidobacteria but not by lactobacilli, while xylo-oligosaccharides, mannan-oligosaccharides, and glucose-oligosaccharides display stimulatory growth effects only on *Bacteroides* species [15]. Although they share functional similarities and elicited activities, prebiotics vary in terms of their structural and physicochemical characteristics, mode of action, bifidogenic potency, and growth-supporting capabilities.

In a broader sense, prebiotics favorably influences bacterial populations in the colon by selectively stimulating the growth and/or activity of probiotic bacteria like bifidobacteria. The probiotics and prebiotics can be successfully incorporated into products like yogurt, curds, cheese, fermented milk, and soups. Management of food systems is of immense concern to scientists, policymakers, and technologists to address ongoing issues and capture the opportunities arising from food innovations. The probiotics displayed antimicrobial activity against a wide variety of bacteria. Probiotics, prebiotics, and postbiotics have wide applications in the dairy

industry. Postbiotics have potential applications in food, pharmaceutical, and feed industries and several health benefits [15, 42].

### **3.1 Types of prebiotics**

Prebiotics are substances that are not digestible by the human digestive system but selectively stimulate the growth and/or activity of one or a limited number of microorganisms in the colon while improving host health. Prebiotics are generally found as part of the daily diet, in particular, those foods that are either high in carbohydrates or are vegetables. They are of several types. Carbohydrates (oligosaccharides) make up the largest percentage of the total number of prebiotics. A literature-based report on oligosaccharides has been published by several studies; however, other types of prebiotics exist that are not carbohydrates [43, 44]. For several reasons, the two of the most studied types of prebiotics are inulin and fructo-oligosaccharides (oligo-fructose). Fructans comprise inulin and FOS (also known as oligo-fructose). Structurally, fructans consist of fructose chains arranged in a linear form, with  $\beta$  (2, 1) linkage. The existence of FOS has been documented in plants, although the concentrations are relatively low for exerting prebiotic efficacy. Fructans have selective growth-promoting effects on lactic acid bacteria (LAB) [45]. The second type of prebiotic, galacto-oligosaccharides (GOS), has been recognized globally as a prebiotic after several studies [46]. GOS are generated from the extension of lactose, a disaccharide sugar found in milk and other products. GOS can promote the growth of bifidobacteria and lactobacilli. High populations in infants have been traced to the incorporation of GOS in their diet [47]. An isomer of lactose, known as lactulose, can also be a source of some GOS. Lactulose resists digestion in the upper gut, and it is not absorbed by the small intestine; however, it undergoes fermentation to yield SCFAs, carbon dioxide, and hydrogen, which results in reducing the fecal pH. Lactulose-derived GOS are considered to be prebiotics [33].

### **3.2 Role in gut health**

Prebiotics are non-digestible, fermentable food ingredients selectively utilized by certain gut microbes, with beneficial effects on host health. They positively shape gut microbiota composition, increase short-chain fatty acids (SCFAs) production, and enhance anti-inflammatory and gut mucosal barrier protection. These effects collectively reduce the risk of various gut pathologies and systemic diseases. Prebiotics, especially dietary fibers and some polyphenol compounds, successfully modulate gut microbiota and health outcomes, but their efficacy varies among individuals [48].

The prebiotic definition highlights selective fermentation. Alongside probiotics, they constitute the biotics concept, which refers to food components favoring gut bacterial activity. The beneficial functions of biotics are mainly ascribed to the gut microbiota, which converts non-digestible ingredients into different metabolites. There is a consensus on beneficial bacteria, but undiscovered gut microbes may also have a central role. Prebiotic fermentation leads to jejunal fat absorption enhancement, insulin sensitivity increase, glucose tolerance improvement, lipid metabolism modulation in the liver, cholesterol and bile acids regulation, and subsequently, atherosclerosis and kidney injury risk reduction. Studies demonstrated that the gut microbiota is critical in mediating health-promoting effects. Human trials showed that prebiotics positively affected gut microbiota in normal and unhealthy subjects, indicating their impact on health and well-being [49, 50].

### 3.3 Impact on the microbiome

Prebiotics, the dietary non-digestible carbohydrates selectively fermented in the gut, positively modulate the gut microbiota, promoting probiotic bacteria growth, metabolism, and colonization. Oligosaccharides such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and inulin are frequently used in prebiotics. There are three general classification systems for prebiotics: those based on the structure (from oligosaccharides to their polymerization degree), those based on the origin (prebiotics can come from plants, dairy products, and marine food), and those based on the physiological effect on gut microbiota (e.g., *Bifidobacterium* and *Lactobacillus*). However, the most known and used definition in the current scientific literature is “a non-digestible oligosaccharide that benefits host well-being by selectively stimulating the growth and/or activity of probiotic bacteria in the colon” [51].

Currently, among the many functional ingredients promoted and proposed for the food industry, prebiotics have been the best studied. Prebiotics possess multiple biological effects beyond gut microbiota modulation that positively impact human health, including anti-obesity, cardioprotective, cholesterol lowering, anti-inflammatory, antioxidant, antihypertensive, antiallergic, anticancer, and antiviral effects. This broad range of effects suggests prebiotics may positively affect the microbiome and modulate gene expression, metabolism, and signaling pathways [41]. Such a comprehensive understanding is needed to grow the interest in prebiotics among stakeholders such as the food industry, regulatory entities, and consumers.

## 4. Postbiotics

The terms probiotics and prebiotics are firmly established in the scientific community and the general public with robust definitions. In the last decade, the research community began to coalesce around the concept of postbiotics, defined as [4, 52]. Since then, several publications have examined the postbiotic definition in relation to some of the finer points of terminology, the microbial or bioactive components that comprise postbiotics, the mechanisms by which postbiotics act, production issues, and evidence of postbiotic efficacy and safety in health and disease applications. Postbiotics are bioactive compounds produced by probiotic microorganisms during fermentation. These compounds, including short-chain fatty acids (SCFAs) [53], peptides, enzymes, vitamins, and organic acids, exert health benefits by modulating the immune system, reducing inflammation, and interacting with the gut microbiota. Postbiotics are emerging as potential therapeutic agents for treating various health conditions due to their stability and targeted bioactivity. It must comprise a preparation containing bioactive components released or liberated from microbes, or microbial products, with bioactivity [54, 55]. The general concept of what can be considered a postbiotic is elaborated, which also encompasses several examples vital to the development of the postbiotics field and highlights topics for future investigation [56].

### 4.1 Definition and production

Postbiotics can be defined as preparations of previously alive microorganisms which, by inactivation or destruction, have lost the ability to replicate and to form colonies but own biological properties; or compositions obtained from microorganisms in culture or after inactivation or destruction, which preserve functional

or biological properties. They are in fact endotoxins of Gram-negative bacteria or components of cell walls or other cellular components of both Gram-negative and Gram-positive bacteria, with potentially beneficial biological activities for the host [4]. However, a wider definition (comprising the peptides, enzymes, polysaccharides, organic acids, vitamins, etc., of all types of bacteria of potential beneficial activity) is preferable. In fact, considering this definition of postbiotics, it is either possible to encompass a broad range of bioactive compounds from bacteria that can be beneficial for the host or to limit it to bioactive compounds from dead bacteria.

For industrial application, one must consider that production and purification processes that would allow obtaining a given postbiotic with beneficial bioactivity are also of patentable or secret character. They must therefore be defined efficiently, in terms of raw materials used, conditions of production or purification, and characterizations of the postbiotic, for both scientific communications and regulatory applications [57].

## **4.2 Health benefits and applications**

Postbiotics, as bioactive compounds produced by probiotics in their fermented metabolic products, have demonstrated important beneficial effects on host health, medication, and food safety [41]. The term postbiotics describes soluble substances produced by probiotic microorganisms and those released after lysis. Similarly, to probiotics, the postbiotics doctrine was first adopted in fermented dairy products and yogurt drinks. While there was increasing evidence for the health benefits of probiotics, there was also a greater interest in understanding more highly characterizable biogenic substances playing an active role in the preparation of fermented products and beverages that could also benefit health [52].

Postbiotics represent a wide range of crude extracts containing solid particles or a specific and quantifiable set of non-living biogenic compounds such as cell wall components, soluble cellular constituents, bioactive peptides, postbiotic polysaccharides, short-chain fatty acids (SCFA), exopolysaccharides (EPS), vitamins, enzymes, bacteriocins, and other antimicrobial substances, macromolecules, and small organic acids, all containing metabolites and effluents released from bacteria that exert a positive physiological effect after ingestion [15]. Compared to probiotics, they express a more stable functional matrix in terms of source, constancy, predictability, and manufacturability. In particular, they are easily amenable to evaluation in terms of structure and function and toxicity to the host, including bioavailability, metabolism, and pharmacokinetics. Consequently, there is an increasing interest in postbiotics as components for new pharmaceutical formulations, functional foods, and animal feed additives, as well as bio-packaging.

## **5. Synergies and interactions**

Recent interest in gut health has led to an ever-growing number of research papers and product launches highlighting the potential of probiotics and prebiotics as functional food ingredients. Due to the increased interest in probiotic and prebiotic functionality, a new category of ingredients is being investigated: postbiotics. Postbiotics have been traditionally defined as metabolic byproducts of live bacteria, but over the last couple of years, the interest has changed to keep a more general product definition that includes dead cells and products thereof [41].

With the inclusion of postbiotics, there are practically three different ingredients with greater synergies than with the standalone products. It is well-documented that prebiotics serve as a selective medium for probiotic growth [2]. This, in turn, has various implications not only for improving survival and colonization of the intestines during digestion but also for enhancing the general functionality of probiotic products. There are also indications that prebiotics can help probiotics gain a higher tolerance toward oxygenation, pH, and temperature during digestion. However, evidence of similar effects on postbiotics is nonexistent. Nevertheless, it is reasonable to assume that adventitious additional metabolites from prebiotic fermentation and partial probiotic treatment, that are known to impact intestinal functionality, can be beneficial to their robustness during processing, as well as stability, safety, and health implications alike. As such, the combination concept creates a complex product category that merits deeper research into all synergies affecting such an ingredient, not only with probiotic and prebiotic products but also with macronutrient-based food categories.

## **5.1 Combined effects on health**

Probiotics, prebiotics, and postbiotics play crucial roles in enhancing gastrointestinal health and overall well-being. Their synergistic interactions provide health benefits, from dietary components to microbial fermentation products. Recent breakthroughs in studies reveal their joint effects, offering hope for better human health and hence sustainable food systems.

The health-promoting abilities of probiotics, prebiotics, and postbiotics are complemented and interconnected. Probiotics are viable and health-advantageous live microorganisms that can exert effects on the host when supplemented in adequate amounts. Multiple potential mechanisms, including microbial enzymes, signaling molecules, surface proteins, and metabolites, are involved in the mechanisms of action of probiotics. Prebiotics are non-digestible food ingredients that confer health benefits on the host by selectively stimulating the growth and/or activity of one or some limited number of probiotic microbes in the gut. They promote the colonization of gut probiotics, production of nutrients and metabolites, establishment of gut mucosal barriers, regulation of gut microbiota composition, and modulation of mucosal immunity. Postbiotics are bioactive compounds released from fermented probiotics, offering health benefits to the host. They may include lysed probiotic cells, cell-free supernatants or soluble factors, biogenic metabolites, specific fatty acids, proteins or peptides, polysaccharides, enzymes, and cell wall fragments [2].

## **6. Challenges and future directions**

Regulatory considerations relevant to postbiotics in the human health sphere are very diverse. The use of non-viable microorganisms and their products as health-beneficial agents (postbiotics) yields a complex scenario in terms of academic research and industrial development. In the pharmaceutical and medical device fields, Good Manufacturing Practices impose strict quality requirements and testing. For purposes other than medications, beverages, dairy foods, and other items offered to consume are certainly perceived by authorities as foodstuffs and thus require the adherence to food laws. Because of that complexity, market access possibilities and the nanotechnologies for packaging and delivery of probiotic microbes might be at stake [58].

This collection highlights research and other activities focusing on probiotics, prebiotics, and postbiotics that would enhance food systems sustainability. It does not foment activity in human health applications of these same elements. Research on other members of the prokaryota kingdoms, like plant-bacteria interactions, shows enormous potential to sustainably improve agroecosystem resilience to climate change acts efficiency of resource utilization while shrinking the environmental impact of food production systems. Natural selection and agricultural practices recovered plant-specific beneficial prokaryotic communities in soils and/or their surrounding environments, and coevolution resulted from thousands of years of close relationships [59].

## **7. Conclusion**

In conclusion, probiotics, prebiotics, and postbiotics have shown promising potential in enhancing human health and contributing to sustainable food systems. Further research is needed to fully understand their mechanisms of action and potential applications in various fields. In addition, exploring the long-term effects of probiotics, prebiotics, and postbiotics on human health and the environment is essential. Furthermore, researching the potential applications of probiotics, prebiotics, and postbiotics in sustainable food systems is crucial. This research can lead to innovative methods for enhancing food production and reducing food waste.

## **Conflict of interest**

The authors declare no conflict of interest.

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## Chapter 4

# Gut Microbiota: Role in Health and Diseases

*Kanchan Bhardwaj, Bhabatosh Das, Tao Zuo  
and Sudhanshu Vrat*

### Abstract

Microbes colonize human intestine and establish a relationship with the host, which may be mutualistic, commensal or parasitic, soon after birth. Influenced by several inherent and external factors, the gut microbiota undergoes changes, matures and stabilizes by the age of 2–3 years. Due to its pivotal role in human physiology, the gut microbiota is occasionally referred to as an organ in human body. The aim of this chapter is to provide a comprehensive overview of the composition of human gut microbiota, mechanisms by which gut microbes interact with each other and the human host leading to homeostasis, diseases associated with dysbiosis in gut microbiota and approaches to alter gut microbiota. The chapter seeks to highlight the potential of the knowledge about human gut microbiome for harnessing it toward developing sustainable food systems.

**Keywords:** gut microbiota, metabolites, fecal microbial transplantation, probiotics, prebiotics

## 1. Introduction

### 1.1 Introduction to sustainable food systems

A sustainable system must offer economic (profitable), social (beneficial for society) and environmental (harmless to the natural environment) sustainability. The central issue for the development of a sustainable food system is to deal with the global nutrition crisis. Malnutrition, which includes undernutrition and obesity, has lasting effects. The global burden of malnutrition is analyzed by the prevalence of stunting, wasting, anemia, low birth weight and obesity. UNICEF, WHO and World Bank Group Joint Malnutrition estimates of May 2023 show that a significant number of children are affected worldwide due to malnutrition and these numbers have not reduced significantly for almost a decade (**Figure 1**). Stunting affects physical and cognitive development. Child wasting is caused by nutrient deficiency and the affected children are at high risk of mortality. Obesity in children predisposes them to various types of Non-Communicable Diseases (NCD) such as diabetes and cardiovascular diseases and it could have psychological effects. Infants weighing less than 2.5 kg at birth are at around 20 times higher risk of mortality, and the survivors face

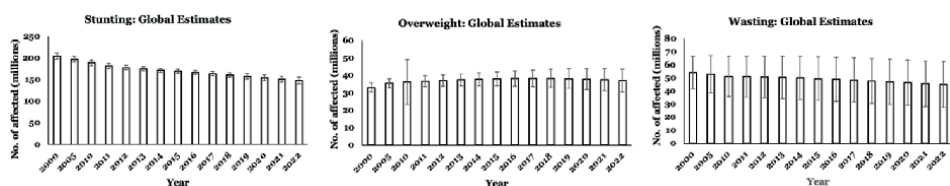


**Figure 1.**

*Global prevalence and the numbers affected by malnutrition, based on the UNICEF, WHO, World Bank Group Joint Malnutrition Estimates, May 2023 Edition.*

health and developmental challenges such as higher risk for NCD and diminished cognitive development than those who are born with adequate weight [1].

To end all forms of malnutrition is among the United Nation's 2030 agenda for sustainable development (SDG 2.2) [2]. Addressing the nutritional needs of children under the age of 5 years, adolescent girls, pregnant and lactating women and elderly could directly or indirectly impact achievement of all of the 17 Sustainable Development Goals. World Health Organization, with its member states aims to achieve the global nutrition targets through sustainable food systems [3]. This chapter presents the scientific understanding of the human gut microbiome, which can be targeted for development of sustainable food systems and manage nutrition crisis. Last part of the chapter summarizes the state of the approaches that are available for modulation of the gut microbiome, which presents innovation opportunities for developing sustainable food systems.

## 1.2 Microbial residents of human gut

Human Microbiome Project (HMP) was initiated to generate resources for a comprehensive understanding of the human microbiome and help researchers gain new insights into the understanding of human health. The project was supported by the National Institutes of Health (NIH) Common Fund, from 2007 to 2016. In the first phase of the project, microbial communities from five major body sites, skin, gut, nasal passages, oral cavity and urogenital tract were examined to estimate the microbial complexity at each site and to address if there is a core microbiome. This initial analysis of 300 healthy adults, by amplicon- and whole genome metagenome—sequencing (mWGS) resulted in accumulation of a large dataset. A total of 3055 microbial genomes were sequenced completely and a reference set of 3000 genomes was developed. The challenge of un-culturability for analyzing microbial communities was overcome by the advances in DNA sequencing technology. Collective efforts of the four sequencing centers, J. Craig Venter Institute, Broad Institute, Baylor College of Medicine, Washington University School of Medicine and Data Analysis and Coordination Center (DACC), along with a number of independent groups, generated the data and developed bioinformatics tools for processing and analysis (<https://www.hmpdacc.org/hmp/membership/>). Ethical regulations and repositories for the data were also established [4]. In the second phase, referred to as the Integrative Human Microbiome Project (iHMP), studies were undertaken to analyze the microbiome dynamics under three physiological conditions: pregnancy and preterm birth; inflammatory bowel disease; and diabetes [5]. More recently, developments in the culturomic approaches, supported by high-throughput cultivation methods and formulation of complex growth media has allowed isolation and cultivation of a number of novel gut microorganisms. Establishment of gut microbiota and its dynamics, as learned through microbe identification by such projects and independent studies, are summarized and presented below.

Biochemical environment, digesta flow rates, substrate availability and physiology across the anatomical regions of the intestine varies and so does the composition of the residing microbiota. The large intestine supports the most dense and largest microbial community, possibly due to slow flow rates and mildly acid/neutral pH. Microbial residents of the human gut include species of prokaryotes (bacteria, archaea), eukaryotes (fungi, protozoa) and viruses (eukaryotic, endogenous retroviruses, bacterial, archaeal). Bacteria in the gut is represented by around 160 species. The dominant phyla are Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia. The phylum Firmicutes is represented mainly by the genera including *Clostridium*, *Enterococcus*, *Lactobacillus*, *Bacillus* and *Ruminococcus*. *Bacteroides* and *Prevotella* are the predominant genera of the phylum, Bacteroidetes. *Bifidobacterium* genus represents the phylum Actinobacteria ([6]; and references therein). Gut virome includes bacteriophages, archaeal viruses, eukaryotic viruses and endogenous retroviruses. Bacteriophages are the most dominant viruses (around 98%) in the gut followed by eukaryotic viruses (around 2%) and archaeal viruses (around 0.1%) [7]. Archaea, the domain of anaerobic prokaryotes, shares traits with bacteria and eukaryotes. Due to technical limitations, such as the culturing and marker-based sequencing, the information about gut archaeome is limited. However, two archaeal species, *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* are found to be associated with humans, specifically. They are characterized by metabolic pathways that are unique to this domain of life, which is their ability to respire  $H_2$  into methane [8]. Although, a low abundance of fungi is the characteristic of healthy human gut microbiome, a diverse collection of more than 200 fungal species has been found in the gut of an adult human. They include the members of Ascomycota, Basidiomycota and Zygomycota phyla; Genera-*Candida*, *Saccharomycetales*, *Paecilomyces*, *Penicillium*, *Fusarium*, *Aspergillus*, *Cladosporium*, *Trichosporon*, *Rhodotorula*, *Aureobasidium* and *Cryptococcus* [9]. Among the gut protists are *Blastocystis*, *Dientamoeba* and *Entamoeba* species [10].

### 1.3 Impact of external factors on gut microbiota

Local biochemical environment determines microbial colonization of the gut [11]. In addition, various external factors also influence microbial colonization of the gut. Gestational age at birth is one of the major determinants. In premature infants, factors such as the organ immaturity, hospital stay and enteral feeding affect microbial colonization. Preterm infants show low microbial diversity, higher colonization with potentially pathogenic bacteria from the phylum Proteobacteria (family *Enterobacteriaceae*) and reduced levels of the strict anaerobes, *Bifidobacterium*, *Atopobium* and *Bacteroides* [12, 13]. Microbial colonization also varies based on the mode of delivery. Microbiota of the vaginally delivered infants is characterized by anaerobic groups such as *Bifidobacterium longum*, *Bifidobacterium catenulatum*, *Escherichia coli*, *Staphylococcus*, *Bacteroides fragilis* and *Streptococcus*. Whereas, infants born by cesarean section, acquire microbes from mother's skin and hospital environment. Their microbiota is less diverse and is represented by members such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp. [14–16]. Colonization is also shaped by components of human milk such as milk oligosaccharides and lactoferrin. Breastfed infants harbor a complex and diverse *Bifidobacterium*-dominated microbiota with lower levels of *Clostridium difficile* and *E. coli*, as compared to formula-fed infants [17–19].

Significant changes in microbiota happen at the termination of milk-feeding, which is characterized by the abundance of *Bifidobacterium*, *Clostridium coccoides* and *Bacteroides*. Weaning and introduction of carbohydrates with high-fiber foods

support an increase in Firmicutes and *Prevotella*. Whereas, increase in Bacteroidetes is associated with introduction of high-fiber and animal protein foods, at weaning [20, 21]. Microbial diversity increases with age and the gut microbiota stabilizes by around 3 years of age. Inter-individual variations are introduced by factors such as body mass index (BMI), lifestyle, exercise levels, ethnicity, dietary habits and cultural habits ([6]; and references therein). Microbial diversity is higher in overweight or normal BMI children than in underweight children [22, 23]. Over the age of 70 years, microbiota can further be affected due to changes in dietary habits, digestion, nutrient absorption and immune activity. A decrease in anaerobic bacteria and an increase in Proteobacteria and *Clostridium* have been noted with old age [24–27].

## 2. Human gut ecosystem in harmony and dysbiosis

A number of studies have established that the gut microbiota plays an important role in human physiology. The underlying mechanisms involve trans-kingdom interactions *via* physical and biochemical means, which lead to local and/or systemic effects. Dysbiosis in the gut microbiota is associated with diseases such as gastrointestinal diseases, hepatic diseases, metabolic diseases, immune system-related diseases, oncologic diseases and diseases of nervous system. This section describes the mechanisms of trans-kingdom interactions in the gut ecosystem and the diseases associated with the dysbiosis of gut microbiota.

### 2.1 Microbiota-derived metabolites

Gut microbes are estimated to encode more than 20 million genes, enabling them to perform several versatile functions. Their activity produces unique metabolites either by (i) digestion of dietary components, (ii) modification of metabolites produced by the host or by (iii) *de novo* production. For instance, gut bacteria can metabolize dietary components such as cellulose, hemicellulose, lignin, resistant starch, pectin, oligosaccharides and protein-derived branched-chain amino acids (BCAA) that are not digestible by human enzymes to produce metabolites such as SCFA (short-chain fatty acids). In the large intestine, SCFAs are absorbed and used as source of energy by colonocytes. Additionally, SCFAs play important physiological roles in regulating the composition of gut microbiota, maintaining integrity of gut barrier and stimulating absorption of water and salts [28–32]. Similarly, gut microbiota also plays an important role in bile acid metabolism and forms the liver-gut axis. Primary bile acids (cholic acid and chenodeoxycholic acid) are synthesized in liver, conjugated with either glycine or taurine to form eight possible bile salts, stored in gall bladder and secreted into the lumen of the intestine. The secreted bile salts are modified by the bacteria present in the gut to form secondary bile acids (deoxycholic acid and lithocholic acid). Around 95% of the primary and secondary bile acids are reabsorbed in the ileum and colon to be transported back to liver *via* portal vein in the process of enterohepatic circulation, whereas around 5% are eliminated in the feces. Secondary bile acids play important physiological roles such as in host immune function, cellular signaling, production of serotonin, prevention of viral infections and *C. difficile* colonization. Notably, excess levels of secondary bile acids are associated with gallstone formation and colon cancer [33–36]. Examples of the *de novo* synthesized metabolites of gut microbes include vitamins B and K, produced by *Bifidobacteria*, which are essential for cellular metabolism [37, 38].

Essential vitamins, SCFA and bile acids are the most studied microbiota-derived metabolites. Others include tryptophan and indole derivatives, neurotransmitters, gases, lipids, organic acids, polyamines, microsin, lantibiotics, triphosadenine, ethanol and oncometabolites. They perform a variety of specific functions such as regulation of gut motility, gut inflammation, epithelial secretion, susceptibility to infections, mucosal blood flow, intestinal barrier functions, gut hormone secretion, microbial spore- and biofilm—formation in gut, virulence, drug resistance, regulate memory and stress responses and immune function of nervous system ([39–52]; and references therein). The metabolites secreted by the gut microbes, mainly by bacteria, and their functions are summarized in **Tables 1** and **2**.

Although, studies so far have established the importance of gut microbiota-derived metabolites, the number of microbial metabolites is likely to be vast and most

| Metabolites                                                                                                                                                                                                                        | Functions                                                                                                                                                                                                                                                                                       |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Short-chain fatty acids</i><br>Acetate, propionate, butyrate, hexanoate, isobutyrate, valerate, isovalerate, 2-methylpropionate                                                                                                 | Regulation of intestinal barrier integrity, energy homeostasis, appetite, gut microbiota composition, gut hormone production, circadian rhythm; inhibition of proinflammatory cytokines; stimulation of water and sodium absorption; modulation of systemic immune response [28–32].            |
| <i>Bile acids</i><br>Cholate, deoxycholate, hyocholate, taurocholate, taurohyocholate, taurodeoxycholate, ursodeoxycholate, lithocholate, glycocholate, tauro- $\alpha$ -muricholate, tauro- $\beta$ -muricholate, hyodeoxycholate | Facilitate the absorption of lipids and vitamins; regulate gut microbiota composition, intestinal hormones, intestinal immunity, electrolyte and fluid balance, intestinal motility, maintain homeostasis of lipid, glucose and amino acids circadian rhythm; affect neurotransmission [33–36]. |
| <i>Tryptophan and indole derivatives</i><br>Indole, indole-3-lactic acid, indole-3-acetamide, indole pyruvic acid, indole acetic acid, indoxyl sulfuric acid, Serotonin                                                            | Regulate microbial spore formation, drug resistance, virulence and biofilm formation; regulate systemic immune response gut barrier function, gut hormone secretion, and gut motility [53, 54].                                                                                                 |
| <i>Vitamins</i><br>Vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B9, vitamin B12, vitamin K                                                                                                                              | Facilitate cellular metabolism; affect immune function and cell proliferation [37, 38].                                                                                                                                                                                                         |
| <i>Choline metabolites</i><br>Trimethylamine (TMA), methylamine, dimethylamine, dimethylglycine                                                                                                                                    | Inhibit bile acid synthesis, exacerbate mitochondrial dysfunction, promote inflammation and thrombosis, affect myocardial hypertrophy and fibrosis [39, 40].                                                                                                                                    |
| <i>Lipids</i><br>Cholesterol, LPS, triglycerides, phosphatidylcholine, conjugated fatty acids                                                                                                                                      | Conjugated fatty acids regulate lipoprotein profiles, hyperinsulinemia and immune system; cholesterol acts as material bases for bile acid synthesis; LPS triggers systemic inflammation [41–43].                                                                                               |
| <i>Neuromodulatory metabolites</i><br>Gamma-aminobutyric acid (GABA), 5-hydroxytryptophan, dopamine, catecholamines                                                                                                                | Regulate memory and stress responses, gut motility and immune function of nervous system [44, 45].                                                                                                                                                                                              |
| <i>Gases</i><br>H <sub>2</sub> , H <sub>2</sub> S, NO, CH <sub>4</sub> , CO <sub>2</sub>                                                                                                                                           | H <sub>2</sub> S regulates gut motility, epithelial secretion, inflammation and susceptibility to infections; NO mediates mucosal protection and regulates mucosal blood flow and CH <sub>4</sub> slows down gut motility [46–48].                                                              |
| <i>Miscellaneous</i><br>Polyamines, triphosadenine, lantibiotics, microcin, organic acids, rthanol                                                                                                                                 | Affect gut barrier, regulate intestinal and systemic immune response, modulates gut microbiota composition, and supplies nutrients that are toxic to host cells [49–51].                                                                                                                        |

**Table 1.**  
*Microbial metabolites and their functions.*

| Metabolites                                                                                                                                                                                                   | Group                             | Host targets                                                   |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------------------------|
| Acetate, propionate, butyrate, hexanoate, isobutyrate, valerate, isovalerate, 2-methylpropionate                                                                                                              | Short-chain fatty acids           | GPR41, GPR43, GPR81, GPR109A, GPR91, HDAC1, HDAC3 [28–32].     |
| Cholate, deoxycholate, hyocholate, taurocholate, taurohyocholate, taurodeoxycholate, ursodeoxycholate, lithocholate, glycocholate, tauro- $\alpha$ -muricholate, tauro- $\beta$ -muricholate, hyodeoxycholate | Bile acids                        | VDR, FXR, PXR/SXR, TGR5, CAR, FPR, S1PR2, mAChR [33–36].       |
| Indole, indole-3-lactic acid, indole-3-acetamide, indole pyruvic acid, indole acetic acid, indoxyl sulfuric acid, serotonin                                                                                   | Tryptophan and indole derivatives | AhR and PXR [53, 54].                                          |
| Vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B9, vitamin B12, vitamin K                                                                                                                            | Vitamins                          | Receptors for vitamins [37, 38].                               |
| Trimethylamine (TMA), methylamine, dimethylamine, dimethylglycine                                                                                                                                             | Choline metabolites               | —                                                              |
| Cholesterol, LPS, triglycerides, phosphatidylcholine, conjugated fatty acids                                                                                                                                  | Lipids                            | TLR2 and TLR4 for LPS [41–43].                                 |
| Gamma-aminobutyric acid (GABA), 5-hydroxytryptophan, dopamine, catecholamines                                                                                                                                 | Neuromodulatory metabolites       | GABA receptors, 5-HT receptors, adrenergic receptors [44, 45]. |
| H <sub>2</sub> , H <sub>2</sub> S, NO, CH <sub>4</sub> , CO <sub>2</sub>                                                                                                                                      | Gases                             | Soluble guanylate cyclase for NO [47].                         |
| Polyamines, triphosadenine, lantibiotics, microcin, organic acids, ethanol                                                                                                                                    | Miscellaneous                     | P2X and P2Y receptors for triphosadenine [49–51].              |

**Table 2.**  
*Microbial metabolites and their targets.*

of them still remain uncharacterized. Approaches such as (meta)genomics, functional genomics and metabolomics are employed for discovery and characterization of microbial metabolites.

## 2.2 Trans-kingdom interactions in the gut

The net functional output of the gut ecosystem depends on the interactions among the co-residing communities of bacterial-, viral-, fungal-, archaeal- and protist taxa, in addition to their interaction with the human host. These interactions control the abundance, functions and colonization of gut residents, through direct or indirect mechanisms. Bacteriophages control the number and activity of the co-residing bacterial communities by predation and lysogeny [55]. Prophages carry genes that can encode for toxins, virulence factors, antibiotic resistance and/or auxiliary metabolic genes [56]. Such genes alter the functional potential and/or regulate gene expression of the bacterial host. Predation of the bacterial host by lytic phages also takes place in the gut which controls the abundance of bacteria. Phage-mediated lysis is also an important modulator of immune response. Pathogen-associated molecular patterns (PAMPs) revealed as a result of bacterial lysis are recognized by the cells of the innate immune system to mount a host response (**Figure 2**) [57]. Bacterial and archaeal communities affect the growth of each other. The majority of the gut-resident archaea are methanogens. They metabolize the byproducts of bacterial fermentation into methane. Excess hydrogen in the gut can

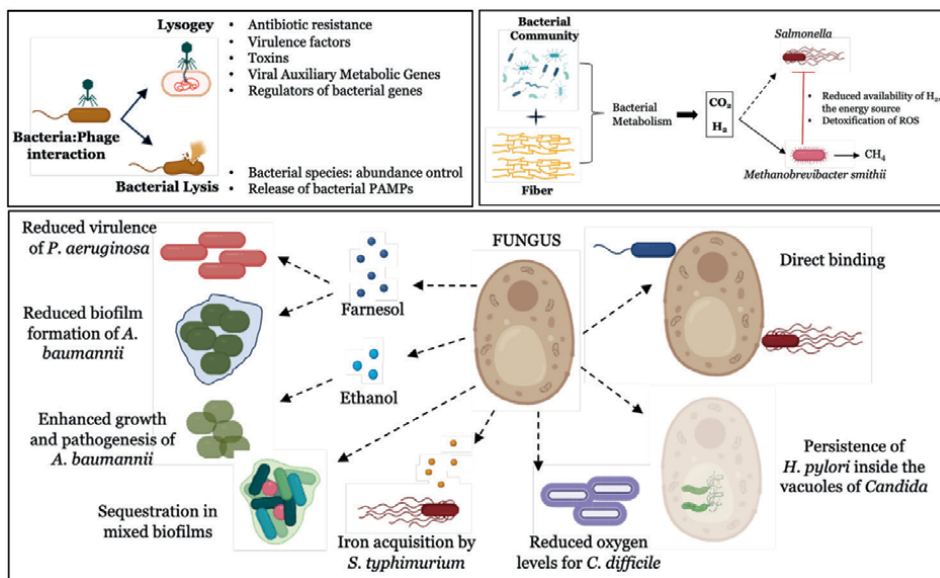


**Figure 2.**  
 Trans-kingdom interactions between bacteria-viruses; bacteria-archaea; bacteria-fungi [Figure is created with BioRender.com].

inhibit bacterial metabolic rates by changing the redox potential. *Methanobrevibacter smithii*, an archaeal methanogen, has the ability to convert hydrogen to methane by combining it with CO<sub>2</sub> [58]. This activity of *M. smithii* (*Methanobrevibacter smithii*) is beneficial to the commensal bacteria and inhibitory to the pathogenic *Salmonella*. Similar to the methanogenic archaea *M. smithii* (*Methanobrevibacter smithii*), *Salmonella* also uses hydrogen as energy source. Therefore, by competing for H<sub>2</sub>, *M. smithii* (*Methanobrevibacter smithii*) inhibits the growth of *Salmonella*. Another way it suppresses *Salmonella* pathogenesis is by inhibiting the inflammation and ROS production caused by this pathogen. *M. smithii* (*Methanobrevibacter smithii*) expresses anti-oxidant genes in response to the presence of *Salmonella* [59]. Similarly, intestinal fungi and bacteria influence the existence of each other either through direct interaction or by competing for nutrition, metabolism and growth [60]. For instance, fungi reduce the binding of pathogenic *E. coli* and *S. typhimurium* to gut epithelium through a direct interaction [61]. Bacteria and fungi also form mixed biofilms, protecting them from the actions of antimicrobial drugs and immune system [62]. Ethanol secreted by *S. cerevisiae* can enhance growth of *Acinetobacter* species [63]. Farnesol, a quorum-sensing molecule is secreted by *C. albicans* (*Candida albicans*) which, increases vancomycin resistance in *S. aureus* (*Staphylococcus aureus*) [64]. On the other hand, farnesol also prevents biofilm formation by *S. aureus* (*Staphylococcus aureus*) through a synergistic action with antimicrobials [65]. *H. pylori* can use vacuoles of *Candida* species as hiding places. *C. albicans* (*Candida albicans*) supports the growth of the anaerobe, *C. difficile*, by reducing oxygen tension in its vicinity [66]. *S. boulardii* is shown to protect against *C. difficile* infection by the action of its secreted protease, which degrades bacterial toxins or by internalizing and neutralizing a bacterial subunit [67]. Further, *S. typhimurium* can use siderophores secreted by fungi to sequester iron from its environment ([67, 68] and references therein).

## 2.3 Mechanisms of interaction between gut microbiota and human host

Experimental outcomes, mainly from the studies with animal models, either germ-free (GF) or where the microbiota was perturbed through environmental incursions, support that the establishment of gut microbiota is important for healthy growth of an individual. Role of mature gut microbiota in healthy growth is also emphasized by the studies, which showed that transplantation of microbiota from malnourished infants to GF animals hampers weight gain in the recipients [69]. Early-life microbiota programs the host for future state of physiology and therefore its impact is long-lasting. Although, as compared to the intestinal bacteria, much less is known about the specific activities performed by the members of fungal-, protista-, archaeal- and viral taxa, it is established that they interact with the human host. Their interactions affect various biological processes such as the development of immune system, maturation of the gut barrier, intestinal functions, metabolism, circadian rhythm and endocrine signaling through mechanisms that are complex and dynamic. Generally, the mode of interaction includes sensing of the microbial structures and secreted metabolites by the host. The effects are manifested through biochemical reactions and/or epigenetic regulation.

Some of the mechanisms underlying microbial colonization and how the gut microbes affect the host physiology are known. For instance, *Bifidobacteria* which belongs to the phylum *Actinobacteria*, colonize gut of breastfed infants. Colonization and persistence of *Bifidobacteria* in human gut are facilitated by the ability of these bacteria to degrade host-derived glycans, mucins and human milk oligosaccharides (HMOs), as well as diet-derived glycans. They encode enzymes required for degradation (fucosidase,  $\beta$ -galactosidase, sialidase,  $\beta$ -hexosaminidase) and internalization (permeases of ABC transporter system and extracellular solute binding proteins) of HMOs ([70]; and references therein). Similarly, other core members of the gut microbiome encode genes to metabolize and use host-derived mucins as carbon and energy source. In addition, cross-feeding activities within bifidobacterial communities also support colonization by some of the species. Although the molecular mechanisms by which the gut-resident *Bifidobacteria* interact with the host are not fully explored, evidences so far has shown that their capsular structures such as surface-associated exopolysaccharides (EPS) and fimbriae/pili support developmental programming of the host immune system and modulation of the host response under pathological and homeostasis states by providing stimulation ([70]; and references therein). Other gut microbes including the members of the class *Clostridia*, genera *Bacteroides*, *Veillonella*, *Streptococcus*, *Collinsella*, *Lactobacillus* and *Akkermansia* also interact with the host similarly. In the absence of commensal microbes, the levels of IgA antibodies, number of Th17 cells and intraepithelial cells ( $\alpha\beta$  and  $\gamma\delta$  IELs) are reduced but can be restored upon microbial colonization. Polysaccharides from *Bacteroides fragilis* correct T-cell deficiencies and Th1/Th2 imbalance in lymphoid tissues. Microbial diversity is important for establishing an immunomodulatory network to protect from induction of mucosal IgE. Further, microbiome-derived ligands of TLR and NOD act directly on enterocytes, intestinal immune cells and remote tissues to modulate immunity (Figure 3) ([70]; and references therein).

Bacteriophages and eukaryotic viruses also interact with the human host and affect the immune response. Bacteriophages line the intestinal mucosa and block invasion by pathogenic bacteria [71]. Phage-mediated lysis of bacterial pathogens in the gut releases bacterial PAMPs that are recognized by APCs to mount innate immune response and release of proinflammatory cytokines [57]. RNA and DNA of

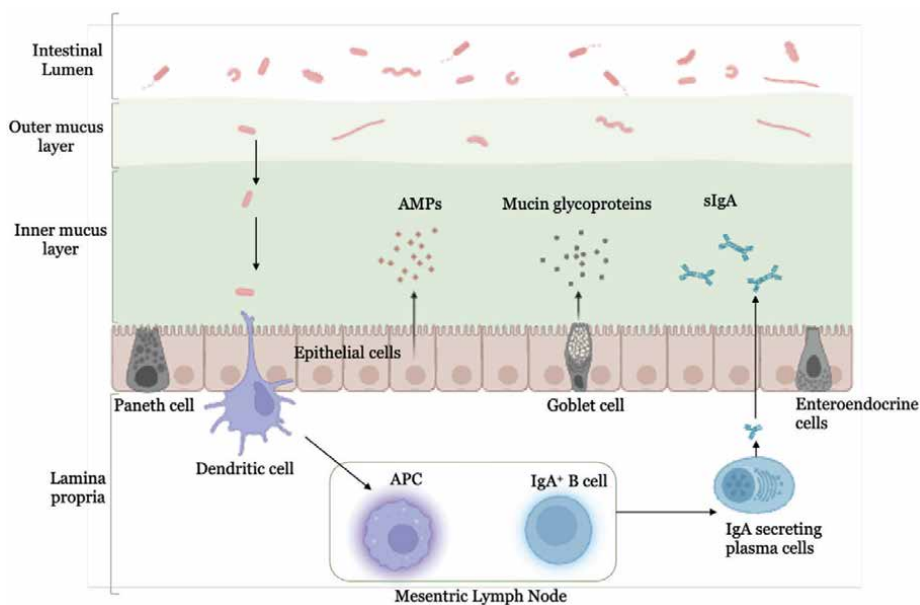


**Figure 3.**  
 Microbial interaction with human host [Figure is created with BioRender.com].

eukaryotic viruses are sensed by APCs *via* RIG-I and TLRs, respectively, leading to the secretion of anti-inflammatory interferon, IFN- $\beta$ . Release of IL-15 as a result of RIG-I activation helps in maintaining the number of IELs (intestinal intraepithelial lymphocytes) and thereby boosting the intestinal defense against bacterial infection. Some viruses induce the secretion of IL-22 by ILCs, leading to enhanced production of particular antimicrobial peptides by the epithelial cells (**Figure 4**) [72, 73].

There are evidences to suggest that the gut-resident fungi contribute to homeostasis and modulation of immune response. Pathogen-associated molecular patterns such as chitin,  $\beta$ -glucan and mannans are recognized by the innate immune receptors, TLR2 and 4, DC-SIGN, Mincle and Dectin-2 of the APCs, macrophages and dendritic cells which, primes the immune cells to act against enteric pathogens. Gut fungi also increases the number of granulocytes and Th17 CD4<sup>+</sup> T cells, which provide protection against enteric infections. Experiments with animal models have also indicated that colonization with certain commensal fungi, such as *C. tropicalis* (*Candida tropicalis*) is important for maturation of lymph nodes. *C. albicans* (*Candida albicans*) is shown to promote T regulatory cell expansion to limit inflammation and maintain immune homeostasis (**Figure 5**) [74].

Although, further research is required, a study has shown that dendritic cells are able to take up commensal archaeal species through phagocytosis and can stimulate immune cells [75]. Archaeal sp. can also contribute to colonization resistance to enteric pathogens either through metabolic competition or *via* the host immune system ([68]; and references therein).

Apart from the microbial structures, gut microbiota also interacts with the host through secreted metabolites to modulate host metabolism, influence the functions of intestine, immune system and nervous system. Microbial metabolites, SCFA, bile acids, vitamins, BCAA, trimethylamine-N-oxide (TMAO), Lipopolysaccharide (LPS), Tryptophan and indole derivatives are associated with energy and nutrient

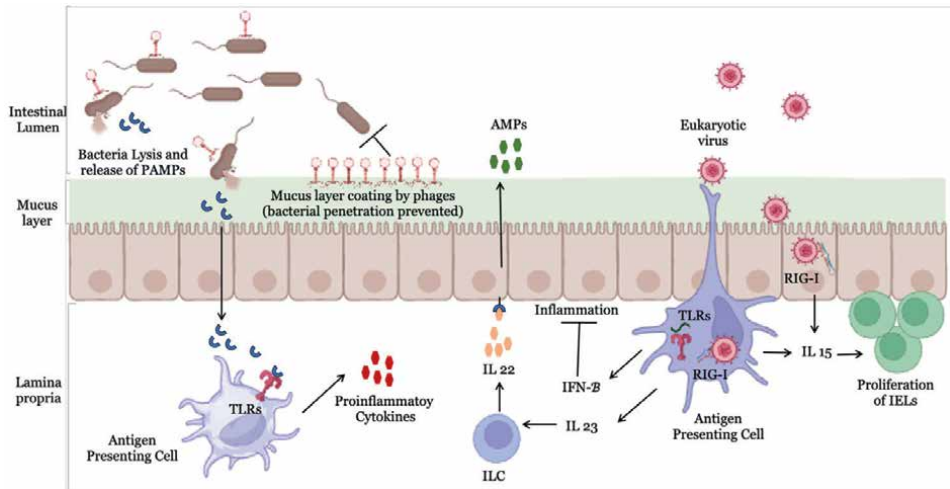


**Figure 4.** Interactions of bacteriophages and eukaryotic viruses in the human intestine [Figure is created with BioRender.com].

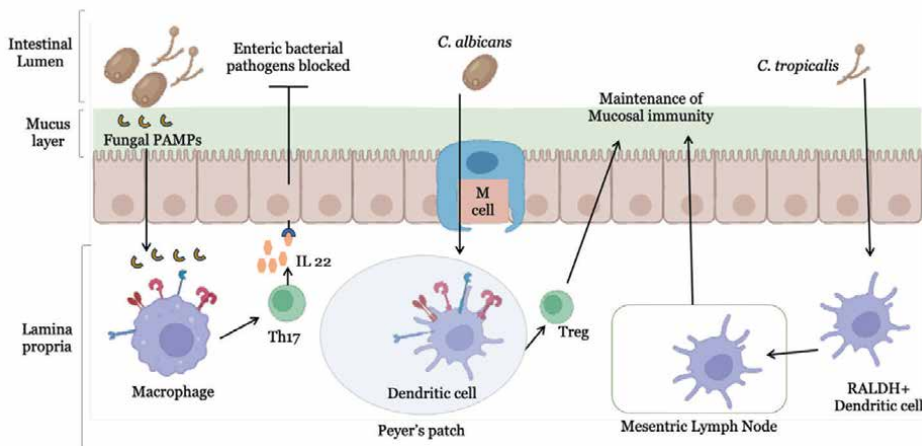


**Figure 5.** Interactions between fungi and the human host [Figure is created with BioRender.com].

metabolism. SCFAs, *via* the receptor, GPR43 affect insulin secretion by  $\beta$  cells [32]. In skeletal muscle cells, they affect the metabolism of proteins, lipids and carbohydrates *via* GPR41, GPR43 and HDACs [28]. In liver and heart cells, they are used as energy resource [29]. In liver, they are also used for synthesis of glucose, cholesterol and long-chain fatty acids [29]. In intestine, SCFAs affect the barrier function of the intestine to prevent pathogen invasion by regulating the genes encoding tight-junction proteins and by stimulating the secretion of mucins and sIgA. SCFAs also regulate immune cell function and the production of cytokines to exert anti-inflammatory effects. In enteroendocrine cells, SCFAs regulate appetite and gut motility by inducing the release of hormones, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) [28]. Bile acids also induce the secretion of GLP-1 and PYY as well as they act as emulsifiers

to facilitate the absorption of fat-soluble vitamins and dietary lipids [33–36]. BCAAs regulate the metabolism of glucose and lipids, protein synthesis, insulin secretion, thermogenesis of BAT and hepatocyte proliferation. Action of BCAA involves phosphorylation of mTOR, c-Jun NH2-terminal kinase and insulin receptor substrate 1. Indole and indole derivatives affect barrier functions of the intestine *via* the epithelial nuclear receptors, PXR, AhR and retinoid-related orphan receptor gamma-t ([52–54] and references therein).

In addition to the local effects in the intestine, metabolites of the gut microbiota also exert effects on extraintestinal organs **Figure 6** ([70] and references therein). SCFA, Bile acids, LPS and TMAO act *via* the immune cells, including T cells, B cells and macrophages and influence the innate and adaptive immune responses systemically. For instance, SCFAs increase acetyl-CoA and regulate metabolic sensors in B cells and support antibody production in spleen. In lungs, *via* the T cells and by decreasing IgE levels, SCFAs protect against asthma and allergy [30]. They relieve arthritis *via* activation of AhR and B cell function. In liver, SCFAs ameliorate inflammation by suppressing the proinflammatory activation of macrophages. SCFAs also modulate bone metabolism [28, 30]. Microbial SCFA, Gamma-aminobutyric acid (GABA), BCAA and indole derivatives can cross blood-brain barrier and exert various effects on the neuronal system such as regulation of appetite, activation of AhR to modulate astrocyte activity, reduce axonal damage and reduce autoimmune encephalomyelitis [31]. High levels of bile acids cause injury to biliary epithelial cells, cause autoimmune liver injury *via* inflammation and modulate bone metabolism. LPS inhibits osteoblastic differentiation and reduces bone mineral density through the activation of TLR4 and TLR2 in mesenchymal stromal cells. TMAO impairs the self-repair capacity of endothelial cells as well as enhances monocyte adhesion *via* activating NLRP3 inflammasome, PKC and NK- $\kappa$ B ([70] and references therein).

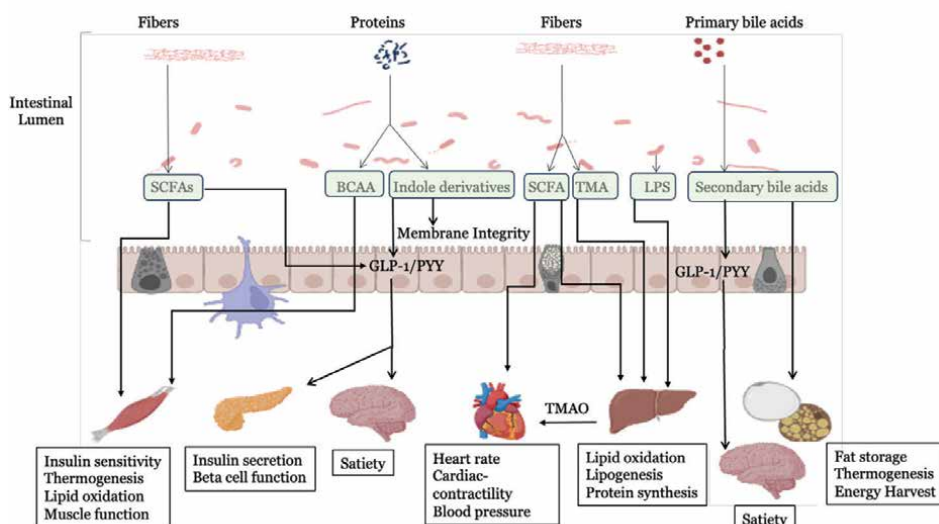


**Figure 6.**  
 Local and systemic effects of microbial metabolites [Figure is created with BioRender.com].

Vitamins and SCFAs are epigenetically relevant microbial metabolites [76]. They regulate patterns of DNA methylation, histone methylation and histone acetylation, which in turn affects gene expression during inflammation and homeostasis. Host uses the folate, vitamin B2 and vitamin B12 produced by the gut *Bifidobacteria* and *Lactobacillus* species to generate the primary substrate, S-adenosylmethionine (SAM), for methylation of DNA and histones by the DNA methyltransferase (DNMT) and histone methyltransferase (HMT), respectively. SCFAs affect the cellular levels of acetyl coenzyme A, which leads to the modulation of ten-eleven translocation (TET) methylcytosine dioxygenases, central enzymes in DNA methylation. SCFAs also affect the acetylation of histones by inhibiting the activity of histone deacetylases (HDACs), through the inhibition of HDACs in various immune cells, including ILCs, CD4<sup>+</sup> T cells, colonic macrophages and dendritic cells, SCFAs program their transcriptional activity toward an anti-inflammatory state during pathogenic infections. It also supports polarization of naïve T cells toward Th1 and suppresses Th17 differentiation. In Tregs, an enhanced expression of Foxp3 protein and thereby, suppression of aberrant inflammatory responses is promoted by SCFAs. Generally, the epigenetic effects of SCFA on T and B cells promote a phenotype that dampens the T-cell mediated autoimmune response, protects against intestinal inflammation and enhances the anti-tumor activity by modulating the expression of effector cytokines and genes associated with B cell functions, through HDAC inhibition. Initial studies have indicated that the microbiota may also confer non-covalent epigenetic regulation of gene expression *via* non-coding RNAs, lncRNAs and miRNAs ([76] and references therein).

## 2.4 Association of gut microbiota with diseases and syndromes

A healthy gut microbiota is characterized by high taxonomic diversity, high gene richness and a stable core microbiome. Although, variations in microbiota composition due to factors such as age, ethnicity, and environments are noted, dysbiosis is associated with diseases and syndromes ([77]; and references therein). The diseases and syndromes that are associated with gut microbiota dysbiosis include gastrointestinal diseases, hepatic diseases, metabolic diseases, immune system-related diseases, oncologic diseases and diseases of nervous system, as summarized in **Table 3** and **Figure 7**.

IBS is characterized by high abundance of fungi, particularly *C. albicans* (*Candida albicans*). Concentration of SCFA and serotonin is high in IBS [78]. In IBD patients, penetration through the intraepithelial cells by luminal microflora causes inflammatory changes. In addition, high abundance of fungi, particularly *C. albicans* (*Candida albicans*), is noted [79, 80].

In non-alcoholic fatty liver disease (NAFLD), there is fat accumulation, mainly of triglycerides, in the hepatic cells. NAFLD can lead to a condition of liver cirrhosis and hepatocellular carcinoma. Gut microbiota composition in NAFLD creates a condition for high energy harvest and low butyrate levels, leading to gluconeogenesis and lipogenesis pathways. Low butyrate levels lead to the accumulation of triglycerides through the activation of lipoprotein lipase activity [81].

Obesity, characterized by accumulation of excess fat and body mass index (BMI) that is more than 30 kg/m<sup>2</sup>. High ratio of firmicutes to bacteroides/prevotella found in obese individuals increases the capacity of gut microbes to harvest energy and accumulate high levels of SCFA. Obesity can lead to metabolic syndrome characterized by diabetes/insulin resistance, hyperlipidemia, liver steatosis, osteoarthritis and cancer [82].

| Affected organ/<br>system                | Disease and<br>references                 | Features of the associated microbiota                                                                                                                                                                                                                                                                                                                                                                            |
|------------------------------------------|-------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gastrointestinal and<br>hepatic diseases | IBS                                       | Quantitative and qualitative changes in the microbiota; elevated levels of Firmicutes, lower levels of <i>Ruminococcus albus</i> , <i>Bacteroides fragilis</i> , <i>R. callidus</i> and <i>B. vulgates</i> [78].                                                                                                                                                                                                 |
|                                          | IBD                                       | Lower abundance of <i>Faecalibacterium prausnitzii</i> , <i>Roseburia</i> and some species of Bacteroidetes and Firmicutes; elevated levels of proteobacteria, <i>E. coli</i> , Pasteurellaceae, <i>N. corrodens</i> , <i>Veillonella parvula</i> , yeast and fungal taxa [79, 80].                                                                                                                              |
|                                          | Non-alcoholic fatty liver disease (NAFLD) | High levels of <i>E. coli</i> and a decreased ratio of Bacteroidetes to Firmicutes [81].                                                                                                                                                                                                                                                                                                                         |
| Metabolic diseases                       | Obesity                                   | High ratio of Firmicutes to Bacteroides/Prevotella; low abundance of <i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Faecalibacterium</i> , <i>Coprococcus</i> , <i>Akkermansia</i> and <i>Methanobrevibacter</i> ; high abundance of some <i>Bacteroides</i> , <i>Staphylococcus</i> , <i>Ruminococcus</i> , <i>Porphyromonas</i> , <i>Campylobacter</i> , <i>Dialister</i> and <i>Parabacteroides</i> [82]. |
|                                          | Diabetes (T1D/T2D)                        | High abundance of Bacteroidetes, low bacterial diversity and a lack of butyrate and lactate producers in T1D. As compared to healthy individuals, higher proliferation of Proteobacteria, Firmicutes and Bacteroidetes in T2D [83, 84].                                                                                                                                                                          |
|                                          | Cardiovascular diseases                   | Reduced abundance of butyrate-producing <i>Roseburia</i> and <i>Eubacterium</i> in atherosclerosis; enrichment of genes involved in peptidoglycan synthesis [85].                                                                                                                                                                                                                                                |
| Immune system-related diseases           | (Allergy/atopic eczema/asthma)            | Low bacterial diversity in childhood but a higher abundance of <i>Bifidobacterium adolescentis</i> at the beginning of allergic symptoms; Some of the bacterial taxa, including <i>Faecalibacterium</i> , <i>Akkermansia</i> and <i>Lachnospira</i> are found in infants at risk for asthma [86].                                                                                                                |
| Oncologic diseases                       | CRC                                       | Presence of <i>Fusobacterium</i> members including, <i>F. mortiferum</i> , <i>F. nucleatum</i> , and <i>F. necrophorum</i> ; Higher abundance of <i>Klebsiella</i> , <i>Escherichia coli</i> , <i>Streptococcus</i> and <i>Enterococcus</i> ; lower abundance of <i>Rothia</i> [87].                                                                                                                             |
| Neurologic diseases                      | Anxiety                                   | Enhanced anxiety response in presence of <i>Campylobacter jejuni</i> ; some species of <i>Lactobacillus</i> and <i>Bifidobacterium</i> can relieve anxiety [88].                                                                                                                                                                                                                                                 |
|                                          | Depression                                | Translocation of Gram-negative bacteria such as <i>Enterobacteriaceae</i> across disrupted intestinal barrier [89].                                                                                                                                                                                                                                                                                              |
|                                          | Alzheimer's                               | Higher levels of Bacteroidetes and Firmicutes; lower levels of <i>Akkermansia</i> and <i>Allobaculum</i> compared with healthy condition [90].                                                                                                                                                                                                                                                                   |
|                                          | Autistic spectrum disorder                | Reduced relative abundance of Bacteroidetes and an increased ratio of Firmicutes to Bacteroidetes [91].                                                                                                                                                                                                                                                                                                          |

**Table 3.**  
Gut microbiota features associated with diseases/syndromes.

In type 1 diabetes, there is a lack of insulin-producing pancreatic  $\beta$ -cells, due to demolition by immune system. However, interaction between the gut microbiota and immune system can impact the predisposition toward the development of T1D [83]. Type 2 diabetes, a chronic metabolic disorder, is characterized by either low levels of insulin or inability to metabolize glucose effectively due to low levels of insulin

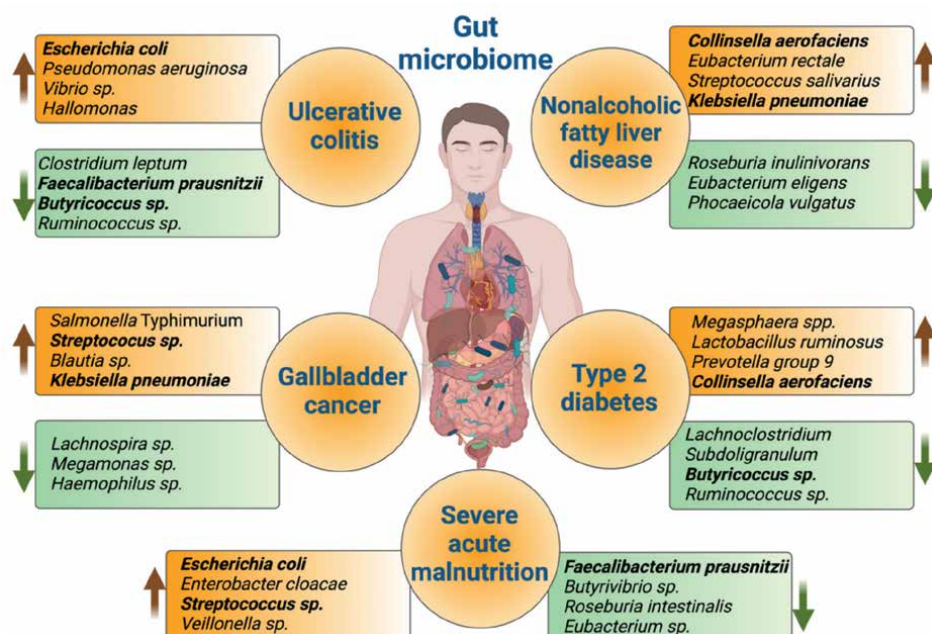


**Figure 7.** Shared and disease-specific microbial signatures in various human diseases [Figure is created with BioRender.com].

receptors/insulin resistance. Microbial metabolite, butyrate, can enhance insulin sensitivity by blocking the translocation of microbial endotoxins responsible for insulin resistance [84].

Digestion of dietary choline and carnitine by gut microbes could increase the possibility of developing cardiovascular diseases. Digestion of dietary choline and carnitine produces trimethylamine (TMA), which, upon oxidation, produces trimethylamine-N-oxide (TMAO). TMAO affects the metabolism of bile acids and cholesterol transport [85].

Oncologic diseases such as CRC could develop *via* various mechanisms involving the gut microbiota. Certain bacteria produce compounds that are harmful to DNA, whereas others could induce a state of persistent inflammation, which is a significant risk factor for developing CRC [87].

Anxiety behavior was found to increase by *Campylobacter jejuni* infection through activation of markers of neuronal activation but without elevating proinflammatory cytokines. Probiotic treatment in animal models normalized anxiety phenotypes *via* the regulation of immunologic factors and GABA receptors [88]. Depression is associated with deficiency of tryptophan metabolism and dysregulation of immune system due to translocation of certain Gram-negative bacteria across disrupted intestinal barrier and causing inflammation [89]. Gut microbiota-derived LPS, inflammatory cytokines and amyloids are found associated with Alzheimer's disease [90]. Autistic spectrum disorder, a neurodevelopmental disorder, is related to a damaged intestinal barrier, a dysfunctional bowel, and a distinct gut microbiota which is characterized by high abundance of fungi, particularly *C. albicans* (*Candida albicans*) [91].

### 3. Harmonizing the gut microbiota

An established role of gut microbiota in host physiology and understanding of the mechanisms by which it affects the functions of various organs, present the possibility of targeting it to develop solutions for several pathophysiological conditions. Toward developing microbiota-directed solutions, multiple approaches have been evaluated in a number of pre-clinical and clinical studies. They include fecal microbial transplantation (FMT) and use of probiotics, prebiotics, postbiotics and/or synbiotics. The goal of these approaches is to modulate and/or assist the establishment of a “healthy” gut microbiota. Demonstration of the beneficial effects and establishing the safety/risk factors are important requirements to achieve the goal [92]. A non-profit collaboration of scientists, International Scientific Association for Probiotics and Prebiotics (ISAPP), is dedicated for the advancement of the science of prebiotics and probiotics. The following sections describe the microbiome-directed therapeutic approaches and the outcomes from some of the relevant studies.

#### 3.1 Fecal microbial transplantation

Fecal microbial transplantation (FMT) is defined as “the transfer of minimally manipulated fecal material from a healthy donor into the gut of a recipient with the goal of treating a disorder associated with intestinal microbiota alterations.” FMT is among the recommended treatment options for recurrent *Clostridioides difficile* infection, due to its consistent success rates (nearly 90%), and it is also a cost-effective option. Procedures, classification and regulations for FMT have evolved over time. For instance, procedural transition from use of fresh fecal material to use of frozen fecal material and incorporation of stool screening with updated concerns toward risks and safety outcomes have come into place over time. In the United States, Canada and France, FMT is regulated as an investigational drug. Whereas, in the United Kingdom, it is regulated as a medicinal product; in Australia and Germany, as a practice of medicine; and in Italy, Belgium and Netherlands, it is regulated as a tissue transplant. However, despite multiple investigations, various issues have prevented a clinical translation of FMT for chronic non-communicable disorders such as IBS, IBD, metabolic syndrome and neuropsychiatric conditions. Various donor-related and recipient-related factors, such as, long- and short-term safety, efficacy of transfer, genetic and immune factors and environmental factors influence the outcomes of FMT. The process of FMT involves steps such as antibiotic priming, bowel cleansing, decision making for number of fecal infusions, amount of infused feces, number of donors, route of delivery and dietary adjuvants. The most challenging step in the process of FMT is the screening and selection of the donors. The two main concerns that prevent the use of FMT for chronic non-communicable disorders are FMT procedures including the lack of microbiome analysis and the consideration of FMT as a single-use therapy ([93] and references therein).

#### 3.2 Probiotics

Probiotics are defined as “live organisms that, when administered in sufficient amounts, confer a health benefit on the host” [94]. Probiotics confer beneficial effects on the recipients either directly or through their metabolites, which are called

“Postbiotics.” The effects are achieved either by (i) interaction with enteric microbes to support growth of the commensals and displacement of potential pathogens, (ii) interaction with the host to impact functions of the intestine and extraintestinal organs, or by (iii) producing essential nutrients for the host. Probiotics can be consumed as part of foods or functional foods such as curd, yogurt, cheese and others. They are also available in pure forms and supplied by various pharmaceutical companies [95]. Apart from the beneficial effects, quality and safety are the important issues to be considered for use of probiotics. They are addressed and summarized as best practices by multiple groups including ISAPP. Issues such as potential short- and long-term risks, the need for long-term studies, the importance of reporting of adverse events, quality of probiotic products and considerations for vulnerable populations were discussed at the annual meeting convened by ISAPP in 2022 [94].

Probiotic formulations have been tested for their effects on various pathophysiological conditions, including IBS, IBD, colorectal carcinoma, infantile colic, *Clostridium difficile*-induced diarrhea, UC and Crohn’s disease, Antibiotic-associated diarrhea, acute gastroenteritis in children under 5 years of age, Colon cancer, etc. Bifidobacteriaceae and Lactobacillaceae families of bacteria and the yeast, *Saccharomyces boulardii* are among the most studied probiotics, both *in vitro* and *in vivo*. Additional probiotics, such as *Streptococcus* species, strains of *Escherichia coli*, *Bacteroides thetaiotaomicron* and *Akkermansia muciniphila* have shown promising results [96–133]. Details of such studies with are summarized in **Table 4**.

### 3.3 Prebiotics

Prebiotics is defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit.” Availability of nutrients such as dietary fiber, dietary protein, secreted host protein, host lactate, 3-hydroxybutyrate, urea, polyphenols, polyunsaturated fatty acids, low molecular weight carbohydrates and resistant

| Probiotic genera           | Microbial types                                                                                                                                                                                                                                                         | Effects                                                                                                                                                                                                                                                          |
|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Lactiplantibacillus</i> | Gram-positive bacteria thrive in a wide range of environments, including fermented foods, several types of meat and plants, as well the mammalian gastrointestinal tract <i>L. plantarum</i> ( <i>Lactiplantibacillus plantarum</i> ) YRL45, CRL681, CRL1506, BSG201683 | Wound healing; anti-inflammatory; upregulation of specific chemokines in subepithelial myofibroblasts; elevation of sIgA, IgA and IgG levels and upregulation of and junctional adhesion molecule 1, epithelial markers mucin 2 and zonula occludens-1 [96–102]. |
| <i>Lactobacillus</i>       | <i>L. acidophilus</i>                                                                                                                                                                                                                                                   | Reduced gut inflammation, increased ILC3, IL-22 and IL-17 [103, 104].                                                                                                                                                                                            |
| <i>Lacticaiseibacillus</i> | <i>Lacticaiseibacillus rhamnosus</i>                                                                                                                                                                                                                                    | Downregulation of proinflammatory IL-18, reduced oxidative stress, induction of tight-junction proteins [105, 106].                                                                                                                                              |
| <i>Ligilactobacillus</i>   | Lactic acid producing, Gram-positive bacteria, commonly found in fermented foods <i>L. salivarius</i>                                                                                                                                                                   | Downregulation of proinflammatory markers, strengthened epithelial barrier and inhibition of adherence of various pathogens to gut epithelial cells [107].                                                                                                       |
| <i>Limosilactobacillus</i> | <i>L. fermentum</i> MN410703 and MN410702, <i>L. mucosae</i> , <i>L. amylovorus</i>                                                                                                                                                                                     | Overexpression of IL-6 and IL-10, downregulation of IL-8. Localization of tight-junction proteins [108, 109].                                                                                                                                                    |

| Probiotic genera         | Microbial types                                                                                                                                                                                                          | Effects                                                                                                                                                                                                                                                                                                                                                                       |
|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Levilactobacillus</i> | Gram-positive bacteria found mainly in fermented foods and in the composition of the intestinal microbiota <i>Levilactobacillus brevis</i>                                                                               | Reduced IL-8 and NF- $\kappa$ B in inflamed HT-29 intestinal epithelial cells [110].                                                                                                                                                                                                                                                                                          |
| <i>Bifidobacterium</i>   | Gram-positive bacteria, is found both in food and in the gastrointestinal tract <i>B. longum</i> , <i>B. breve</i> ( <i>Bifidobacterium breve</i> ), <i>B. lactis</i> , <i>B. pseudocatenulatum</i> , <i>B. animalis</i> | Wound healing, angiogenesis, anti-inflammatory [111–116].                                                                                                                                                                                                                                                                                                                     |
| <i>Pediococcus</i>       | Gram-positive, lactic acid bacterium which plays a significant role in the food fermentation process                                                                                                                     | Body weight restored in mice, downregulation of IL-1 $\beta$ , IL-6 and TNF- $\alpha$ [117, 118].                                                                                                                                                                                                                                                                             |
| <i>Akkermansia</i>       | An aerotolerant anaerobe, Gram-negative bacteria, capable of growing on a viscous substrate such as mucin. <i>A. muciniphila</i>                                                                                         | Degradation of intestinal mucin glycoproteins, strengthening of mucosal barrier, upregulation of anti-inflammatory markers, GPR109A and SLC5A8, and downregulation of proinflammatory TNF $\alpha$ , IFN- $\gamma$ , IL1 $\beta$ , and IL6 [119, 120].                                                                                                                        |
| <i>Rouxsiella</i>        | <i>R. badensis</i> subsp. <i>acadiensis</i>                                                                                                                                                                              | Increase in serum IgA, a decrease in proinflammatory cytokines and increase in the anti-inflammatory subset population of cells in the small intestine [121, 122].                                                                                                                                                                                                            |
| <i>Roseburia</i>         | Gram-positive anaerobic bacteria. <i>R. intestinalis</i> is a major butyrate producer in the gut                                                                                                                         | Immunomodulatory effects and boosts the immune system toward fighting tumorous cells [123, 124].                                                                                                                                                                                                                                                                              |
| <i>Tetragenococcus</i>   | Gram-positive facultative anaerobic lactic acid bacteria. <i>T. halophilus</i> ( <i>Tetragenococcus halophilus</i> )                                                                                                     | Decreased production of IL-1 $\beta$ in animal model [125, 126].                                                                                                                                                                                                                                                                                                              |
| <i>Faecalibacterium</i>  | Extremely oxygen-sensitive commensal butyrate-producer, populating the most anaerobic parts of the GI tract of mammals and is generally recognized as a biomarker of intestinal health. <i>F. prausnitzii</i>            | Amelioration of colitis, decreased disease scores and reduced inflammation in animal model [127, 128].                                                                                                                                                                                                                                                                        |
| <i>Enterococcus</i>      | Lactic acid bacteria, the species of which exert both harmful and beneficial effects. <i>E. faecium</i>                                                                                                                  | Enhanced expression of tight-junction proteins and downregulation of proinflammatory cytokines but potential health risks could be associated with this genus and therefore is not in the category of “Generally Recognized as Safe.” [129, 130].                                                                                                                             |
| <i>Clostridium</i>       | Gram-positive anaerobic bacteria, species of which exert both harmful and beneficial effects. <i>C. butyricum</i>                                                                                                        | Reduction of inflammation in experimental colitis [131].                                                                                                                                                                                                                                                                                                                      |
| <i>Saccharomyces</i>     | Widely used in the food industry but additionally serves as a very potent probiotic. <i>S. cerevisiae</i> , <i>Saccharomyces boulardii</i> , <i>S. boulardii</i>                                                         | Downregulation of neutrophil infiltration and proinflammatory cytokine, TNF- $\alpha$ , upregulated expression of tight-junction proteins and the anti-inflammatory cytokine IL-10 in DSS-induced colitis. Decreased adherence of <i>E. coli</i> , downregulation of the proinflammatory IL-23 and IL-17 and upregulation of the anti-inflammatory cytokine IL-10 [132, 133]. |

**Table 4.**  
Probiotics and their effects.

starch shapes the gut microbiome composition ([134]; and references therein). Most prebiotics are polysaccharides with a polymerization degree of 4 till 30; for example, fructooligosaccharides (FOS), galactooligosaccharides (GOS), chitooligosaccharides (COS), mannanoligosaccharides and xylooligosaccharides. Other examples are phytochemicals, polyunsaturated fatty acids, conjugated linolenic acid, readily fermentable dietary fibers and human milk oligosaccharides [135]. Although, generally administered orally, prebiotics could be administered to other sites, for instance, skin. The possible health benefits could include reduced lipid levels in blood, amelioration of insulin resistance, improved mental health, increased mineral availability, etc. Potential avenues for prebiotics are expanding the range of prebiotics and delivery methods [136–157]. Regulations associated with the use of prebiotics in food industry also needs to align with the advancement in science of prebiotics. Animal model and human subject studies on prebiotics or potential prebiotics and their effects on various conditions are reviewed recently and are summarized in **Table 5** [158].

A combination of both, probiotics and prebiotics is termed as synbiotics. Synbiotics is defined by ISAPP as “a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host.” Two subsets of synbiotics, “complementary” and “synergistic” were also defined by ISAPP. A synergistic synbiotic is “a synbiotic in which the substrate is designed to be selectively utilized by the co-administered (allochthonous) microorganism(s).” A complementary synbiotic is “a synbiotic composed of

| Condition                      | Prebiotics and effects                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Colorectal cancer              | Inulin alone or in combination with GOS: Increased production of SCFA, reduction in the number of colon polyps. Chitosan depolymerized oligomers: higher levels of butyrate-producing <i>Akkermansia</i> and <i>Cladosporium</i> spp.; reduction in levels of <i>Shigella</i> , <i>Escherichia</i> , <i>Enterococcus</i> or <i>Turicibacter</i> . Dextran coated spores of <i>Clostridium butyricum</i> : SCFA production through fermentation of dextran [136].                                                                                                                                                                                                                                                       |
| Cognitive functions            | A beverage containing inulin: improvement in three domains of cognitive functions possibly through increase in bifidobacterial and modulation of some inflammatory markers. Polydextrose: effect on cognitive functions, possibly due to modulation of <i>Ruminococcus</i> 5 [137–139].                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Stress, anxiety and depression | $\alpha$ -Lactalbumin along with sodium butyrate (histone deacetylase inhibitor): relieved symptoms of anxiety, reduced intestinal inflammation, increased motility [140, 141].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Autism                         | B-GOS: effects on levels of sociality, lethargy and improved qualitative composition of gut microbiota [142].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Schizophrenia and Parkinson    | Inulin, resistant starch, resistant maltodextrin, rice bran: reduced the markers of inflammation, such as plasma zonulin and stool calprotectin in Schizophrenia cases; increase in SCFA, positively affected gut microbiota composition, reduced severity of motor and non-motor symptoms, better gastrointestinal function in Parkinson's disease [143, 144].                                                                                                                                                                                                                                                                                                                                                        |
| Inflammatory bowel disease     | Inulin with or without FOS: increase in lactobacilli in cecum, reduction in the colon pH, lower degree of mucosal inflammation and damage. Resveratrol: levels of <i>Bifidobacterium</i> and <i>Lactobacillus</i> were increased, along with decreased levels of <i>E. coli</i> and <i>Enterobacteriaceae</i> . Inulin, FOS in combination with <i>Bifidobacterium</i> : reduced inflammation, TNF and IL-1 $\alpha$ . Fructans: increase in butyrate production and improvement of colitis. Inulin and FOS: improved clinical symptoms in Crohn's disease, increase in <i>Bifidobacterium</i> population. FOS: increased IL-10 positive dendritic cells and fecal concentration of <i>Bifidobacterium</i> [145, 146]. |
| Irritable bowel syndrome       | FOS: reduced the scores of IBS-associated anxiety and increased fecal Bifidobacteria. GOS: increased fecal Bifidobacteria, improvement in symptoms such as abdominal pain and flatulence [147].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

| Condition          | Prebiotics and effects                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Enteric syndrome   | GOS, exopolysaccharides, inulin and catechins: increased growth of <i>Lactobacilli</i> and <i>Bifidobacteria</i> in gut [148].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Obesity            | COS, FOS, GOS: improved gut microbiota dysbiosis and anti-inflammatory activity, possibly due to SCFA production. Flavalons, certain polyphenols, inulin, cinnamon, lignans, vanillin: reduction in the ratio of Firmicutes/Bacteroidetes. Flavanols, cranberry extract, resveratrol, catechins, pterostilbene and apple procyanidins: also promote the growth of <i>A. muciniphila</i> . Inulin-type fructans: can stimulate the growth of <i>F. prausnitzii</i> and modulate gut microbiota. ITF, Resveratrol, catechins, Flavanols: promote the growth of <i>Bifidobacteria</i> , possibly by secretion of the hormone ghrelin that may regulate the sense of appetite; modulation of calprotectin; increase in linolenic acid and rumenic. FOS in combination with <i>B. animalis</i> subsp. <i>Lactis</i> and inulin with a mixture of probiotics: affects inflammation. Soy isoflavones, pomegranate extract, arctic berries, pollen extract, and genistein: positively affected gut microbiota composition and led to weight loss [149–151].                                   |
| Diabetes           | COS: affects gut microbiota composition to favor the abundance of Firmicutes, Bacteroidetes, Actinobacteria, Lachnospiraceae population and Proteobacteria; reduces hyperglycemia, hyperlipidemia and blood glucose levels. ITF: In addition to the effects as shown by COS, IFT also reduced fasting blood glucose; lowers the ratio of Firmicutes and Bacteroidetes; increases the levels of <i>Phascolarctobacterium</i> , <i>Lachnospiraceae</i> , <i>F. prausnitzii</i> and bifidobacterial; produce acetic and propionic acid. Extracts of certain plants (bitter melon and mulberry leaf) and probiotics ( <i>L. casei</i> K11 and <i>Lactobacillus paracasei</i> ): improved insulin sensitivity and reduced blood glucose levels, modulated lipid metabolism, levels of proinflammatory cytokines and oxidative stress; improvement in secretion of glucagon-like peptide-1, SCFA levels, upregulation of free fatty acid receptor 2. Dextran or resistant dextran and maltodextrin: improvement in quality of life. Inulin and glucans: modulation of gut microbiota [152]. |
| Metabolic syndrome | N acetyl-chitooligosaccharide: improved glucose tolerance, lower fasting glucose, promoted insulin secretion, inhibited lipid accumulation in liver. Pro-anthocyanidins: significant increase in glucagon-like-peptide-1, improvement in glucose tolerance, increase in ghrelin and induction of satiety [153].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Osteoporosis       | FOS and GOS: higher levels of serum alkaline phosphatase, increased bone density. FOS and dried prune treatment: significant increase in bone density [154, 155].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Immunosenescence   | Syringaresinol, found in plant foods such as oilseeds, cereal brans and berry seeds: delayed immunosenescence, increased the number of T lymphocytes; reduce the ratio of Firmicutes and Bacteroidetes; increased the populations of <i>Bifidobacterium</i> and <i>Lactobacillus</i> ; adverse effect on opportunistic members <i>Bacteroides vulgatus</i> and <i>Staphylococcus lentus</i> . GOS, B-GOS, FOS, chicory inulin: support the growth of <i>Bifidobacterium</i> and <i>Lactobacillus</i> ; stimulation of SCFA production countering inflammation by GOS, B-GOS, FOS [156, 157].                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

**Table 5.**  
*Prebiotics, their applications and effects. COS: chitooligosaccharides; GOS: galactooligosaccharides; FOS: fructooligosaccharides.*

a probiotic combined with a prebiotic, which is designed to target autochthonous (resident) microorganisms.” Both components of a complementary synbiotic must meet the minimum criteria for the existing probiotic and prebiotic [159].

#### 4. Future directions and challenges

Malnutrition is a significant global challenge. It can be resolved partly through dietary interventions. Scientific evidences has shown that the establishment of

early-life gut microbiome has a lasting impact on the growth of the host, which suggests that the management of gut microbiota is also a promising approach to resolving the issues associated with malnutrition. However, while targeting the gut microbiota, the complexity of the gut ecosystem must be acknowledged. Clearly, most of the current mechanistic understanding is based on our knowledge of gut bacteria. Other microbes, although present at lower levels, are relatively poorly understood. Further understanding of the gut microbiota could reveal additional opportunities.

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# Gut Microbiota-Derived Conjugated Linoleic Acid (CLA) in Health and Disease

*Trisha Dey, Alok Kumar Mishra and Sharon Nagpal*

## Abstract

Dietary fatty acids have a major role to play in human health and disease conditions as they are now recognized as the major biologic regulators. Conjugated linoleic acid (CLA) is a generic term denoting a group of isomers of linoleic acid (C18:2, n-6) with a conjugated double bond. CLA is produced as a result of the biohydrogenation of other fatty acids and has attracted increased research interest because of its health-promoting benefits and biological functions. CLA has been shown to impact immune function and has protective effects against cancer, obesity, diabetes, and atherosclerosis that are evident from both preclinical and clinical studies. Studies investigating the mechanisms involved in the biological functions of CLA are emerging with results from both *in vivo* and *in vitro* studies. The most commonly used isomers of CLA which have a prominent effect on metabolic and homeostatic regulation are mostly concerned with two of its isomers i.e. c9,t11-CLA and t10,c12-CLA. In this study, the role of CLA on various forms of cancers has been given priority along with its mechanism to enable the future research more translational. However, we believe that more intense research is required to further evaluate its efficacious nature and future implication in therapeutics.

**Keywords:** CLA, probiotics, cancer, obesity, diabetes, inflammation

## 1. Introduction

Conjugated linoleic acid (CLA) is a group of naturally occurring fatty acids synthesized from linoleic acid by bacteria present in the alimentary tract of ruminant animals [1]. CLA may also be synthetically produced by partial hydrogenation of linoleic acid or oils rich in linoleic acid (e.g., sunflower or safflower oils) or by alkali isomerisation of the same [2, 3]. CLA is a mixture of positional and geometric isomers of linoleic acid (LA) with double bonds between carbon atoms 7 and 9, 8 and 10, 9 and 11, 10 and 12, or 11 and 13. They occur in *cis* or *trans* configuration. The most prevalent form is the *cis*-9, *trans*-11 CLA isomer found in food derived from ruminants such as milk, cheese, and meat [4]. Since the discovery of CLA, it has shown many health benefits which include antiadipogenic [5–7], anticarcinogen [8, 9],

antiatherogenic [10], antidiabetogenic, [11], and anti-inflammatory [11] effects. Linoleic acid, known as an element of the geometric isomers of CLA, is a class of fatty acids with 18 carbon atoms. This is the common term for a class of position isomers that has two double bonds with a methylene group between both of them. The double bond in this conjugation is often in areas 9 and 11 or 10 and 12, and it could be in either a *cis* or *trans* form (**Figure 1**). To a lesser extent, pigs, chickens, and turkeys also produce CLA naturally in their digestive tracts. This synthesis is caused by the fermentative bacteria *Butyrivibrio fibrisolvens*, which isomerizes the linoleic acid in CLA, or by synthesis via 9-desaturase of 11-*trans* octadecanoic acid. The CLA content of beef fat ranges from 1.7 to 10.8 mg/g, with 9-*cis* and 11-*trans* isomers. Dairy products (milk and derivatives) also contain it. The enzyme 9-desaturase, which encourages the desaturation of the 11-*trans* octadecanoic acid, can be used to obtain CLA. The most well-known CLA isomers are 11-*trans* and 9-*cis* as they can be found in food (**Figure 2**). Additionally, it is feasible to obtain CLA in an industrial form through the partial hydrogenation of linoleic acid or through thermal processing, with the goal of creating a substance with the highest level of biological activity and a specific chemical makeup. Regarding the oxidative degradation intracellular framework, which defines many biological features of fatty acids, CLA plays a significant role in lipid metabolism [12]. With relation to the antioxidant intracellular system, which describes many biological aspects of fatty acids, CLA plays a significant role in the metabolism of lipids. Inhibition of the Peroxisome Proliferator-Activated Receptors (PPARs), particularly PPAR $\gamma$ , mediates the effects of CLA on lipid and glucose metabolism on body composition. CLA isomers inhibit PPAR, leading to inhibiting cell differentiation and modifying the function of proteins that regulate lipogenesis and lipolysis of fat which are two ways that gene expression modification might reduce fat in the body. Evidence suggests that PPAR activation can slow the progression of cholesterol as well as enhance insulin sensitivity, which makes it an appealing treatment option for the management of a variety of illnesses, including diabetes mellitus [13]. The expression of genes involved in lipid metabolism, such as acyl CoA synthetases and lipoprotein lipase, is regulated by PPAR in adipocytes. PPAR additionally controls the production of the fatty acid transport protein involved in adipocytes intake of lipids [14]. The decrease in body fat is caused by a decrease in

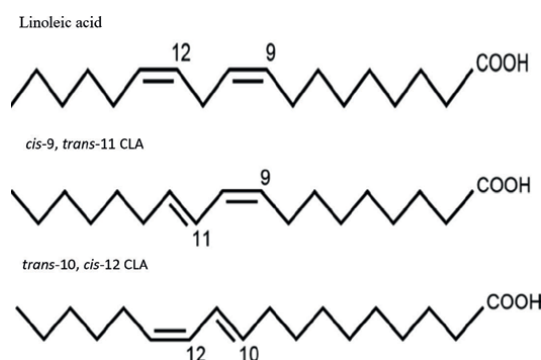


**Figure 1.**

Composition of both elementary conjugated metabolites of linoleic acid. In food supplements, *cis*-9,*trans*-11-CLA and *trans*-10, *cis*-12-CLA are the primary isomers. In the gastrointestinal tract of ruminant species, symbiotic bacteria typically produce CLA. In dietary sources, *cis*,*trans*-11-CLA (*c*-9, *t*-11-CLA) (second panel) predominates as an isomer and makes for up to 90% of the total CLA. Another prominent isomer, *trans*-10, *cis*-12-CLA (*t*-10, *c*-12-CLA) (bottom panel), makes up 1–10% of the total CLA found in dietary sources.

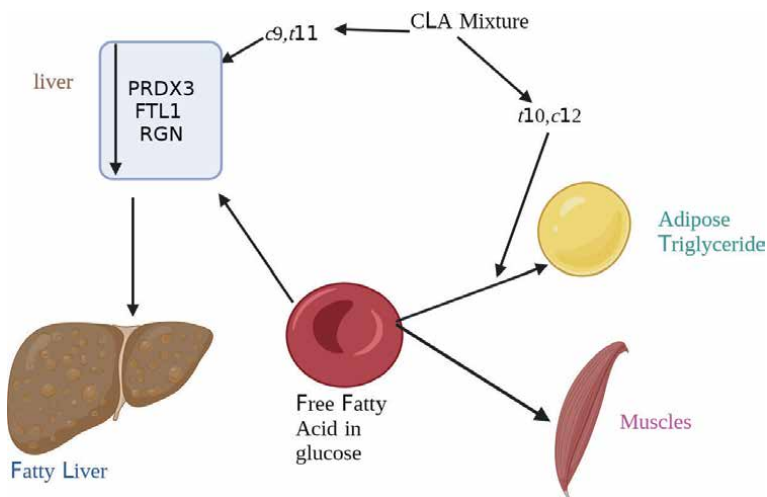


**Figure 2.**  
 Hypothetical mechanism of action of CLA isomers. An assumption has been made for possible role of CLA isomers in liver cancer and obesity. The peroxiredoxin 3 (PRDX3) is a member of conserved proteins having role in proliferation, apoptosis inhibition, pro-inflammatory activity, and the response to oxidative stress. Similarly iron protein ferritin light chain (FTL1) may have crucial role in dysregulation of iron metabolism and initiation of breast cancer. Regucalcin (RGN) is involved in regulation of genes involved in fatty acid synthesis.

adipocyte quantity than by a decrease in the adipocyte size. The triglyceride level of adipose cells directly correlates with their size, thus lowering the concentration decreases cell size. Smaller adipocyte, caused by reduced triacylglycerol, is caused by enhanced oxidation of mitochondrial lipids induced by CLA [15].

## 2. CLA as a probiotic metabolite and its implications

According to the World Health Organization (WHO) and Food and Agricultural Organization (FAO), Probiotics are “live microorganisms which when administered in adequate amounts confers a health benefit to the host” [16]. The probiotics mainly belong to two types of lactic acid-producing microorganisms, *Bifidobacteria*, and the low GC (Guanine- Cytosine) content lactic acid bacteria (LAB) that include *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, and *Streptococcus* genera. The most widely used probiotics belong to the genera *Lactobacillus* and *Bifidobacterium* [17, 18]. The probiotic properties of the genus *Lactobacillus* are important because of their use in various food manufacturers such as fermented milk, fruit juice, cheese, fermented cereal, or vegetable-based foods [19]. In humans, *Lactobacillus* is reported to have various roles such as stimulation of the immune system, prevention and reduction of the intensity and duration of diarrhea, and reduction of lactose intolerance [20]. The other properties which are associated with the *Lactobacillus* are synthesis of vitamin B, improvement of mineral and nutrient absorption, degradation of anti-nutritional factors and modulation of intestinal physiology, and reduced pain perception [20]. In this way, *Lactobacillus* is considered to be effective against certain diseases and confers health benefits. The properties of lactic acid bacteria considered to be potent probiotics are the ability to survive and grow in the *in vivo* conditions that resemble the site of administration [21]. The probiotic

strain should be tolerant toward bile and acidic environment present inside the gut. The strain should have antimicrobial properties along with antibiotic resistance toward selected antibiotics [21]. Cell adherence along with the viability must be taken into account for the strain along with the beta galactosidase activity [21]. The LAB is supposed to produce various metabolites, but the primary metabolites of LAB are acetoacetate, acetate, and lactate [22]. The major short chain fatty acid (SCFA) found in human feces is acetate. The bacteria isolated from the human intestine are capable of producing butyrate from acetate in colon [23]. The other members of the flora use substrates such as acetoacetate and lactate for producing other SCFAs [24]. Within the gut, butyrate is considered to have a potent anti-carcinogenic effect. Butyrate has very clearly demonstrated the anti-carcinogenic effect of SCFA [25]. Butyrate is supposed to control cellular machineries like apoptosis and cell differentiation. It has got some effects at the molecular level such as regulating gene expression via phosphorylation and acetylation of histone protein [26–28]. The production of CLA along with other metabolites similarly has proved to be very useful in the modulation of certain disease conditions. The role of CLA in obesity, asthma, colon-rectal cancer, inflammatory bowel disease (IBD), and certain other diseases is well established [29]. LAB such as *Lactobacillus* or *Lactococcus* along with *Bifidobacterium* are most commonly used in the production of fermented food and can be a potential probiotic [29]. Similarly, some of these microorganisms also have the ability to produce CLA from the linoleic acid (LA) [29–31]. In a study conducted by Luis M. Rodríguez-Alcalá et al., 22 probiotic bacteria were tested for the production of CLA using the UV (ultra violet) screening method and high preference liquid chromatography (HPLC) technique. Out of 22 tested strains, five microorganisms, two strains of genera *Bifidobacterium*, two *Lactobacillus*, and one *Lactococcus* were selected for their ability to produce CLA after incubation in skim milk with free LA (lactic acid) as a substrate [32]. The cells of the immune system upon any stimulus such as cell injury or infection secrete an array of immune responses toward the stimulus. Dendritic cells (DC) are at the front of the innate immune response. They present antigens to the naive T cells along with the secretion of two cytokines IL (interleukins)-10 and IL-12 [33]. The immunomodulatory potential of c9, t11 CLA was examined by Loscher et al. on bone marrow-derived dendritic cells (BMDC). CLA reduced both protein and mRNA concentrations of IL-12p70 and IL-12p40 which is a pro-inflammatory cytokine; meanwhile, the concentration of both protein and mRNA concentration was increased for IL-10 increasing surface expression of the IL-10 receptor. This study also suggested that c9, t11-CLA reduced nuclear factor kappa B (NF-kB) and increased cytosolic nuclear factor kappa B suggesting prevention of degradation IκBa, a protein responsible for sequestering of NF-kB in the cytosol, whereas this effect was reversed in presence of IL-10 neutralizing antibody [34]. Therefore, this study suggests the role of CLA in the treatment of Th1-mediated disease. The potential anti-inflammatory effect of CLA has also been shown in many animal models such as IBD, 2.5% dextran sodium sulphate-induced colitis in mice, and other models [35]. The CLA-fed mice were shown to be protected from colitis-induced weight loss, rectal bleeding, and colonic tissue damage. The mechanism responsible for CLA-mediated protection against colitis was the upregulation of PPARγ and PPARδ and the reduction in NF-kB-mediated inflammation [35]. Rectal bleeding and colitis-induced weight loss were reduced in CLA-fed animals. The anti-inflammatory effect of CLA was also studied in response to lipopolysaccharide (LPS) by Zhao et al. The result demonstrated that both TNF-α and mRNA expression in peripheral blood mononuclear cells (PBMC) was significantly reduced in CLA-fed animals, and this reduction was attributed to the attenuation of NF-kB signaling [36]. In the past

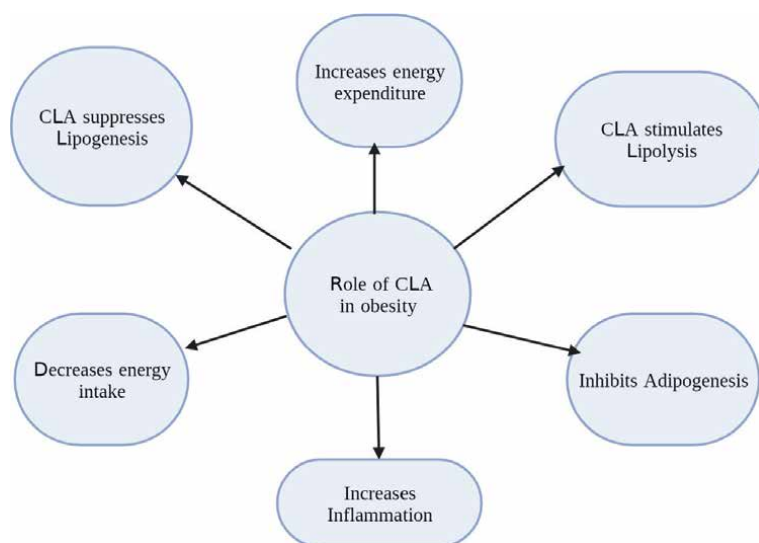


**Figure 3.**  
*Depicts the role of CLA in obesity. CLA plays an important part in reducing body fat. Although it is unclear if this results in less food consumption, CLA may also lessen feelings of hunger. The majority of an individual's weight or BMI does not appear to be reduced by CLA. However, it also can inhibit adipogenesis (This is a potential treatment strategy for overweight).*

30 years, obesity has become one of the major challenges in the society. There has been a substantial increase in the number of patients. The anti-obesity mechanism of CLA was reviewed by Wigham et al. in the year 2007 [37]. The two major isomers of CLA, that is, 10, 12 and 9, 11 when provided in a mixture or the isomer 10, 12 alone have proved to have some anti-obesity effect in many animal and human studies [37, 38]. The major mechanism through which CLA regulates includes a reduction in energy intake, increase in energy expenditure, inhibition of adipogenesis by CLA, increase in inflammation, suppression of lipogenesis, and stimulation of lipolysis (**Figure 3**) [37, 38]. Resistance to insulin and diabetes mellitus type 2 is a result of obesity's challenging impact on the liver, pancreas, and additional metabolic organs as well as on adipose metabolism [39]. The blood flow to the adipocytes declines with obesity and the adipocytes boosting expansion, resulting in hypoxia [40]. Adipocytokines, also called active metabolites, and inflammatory mediators like tumor necrosis factor- $\alpha$  and interleukin-6 (TNF- and IL-6), as well as free fatty acids, have been overproduced as a result of the expansion of adipocytes and hypoxia [41].

### 3. Biological activities of CLA

#### 3.1 Anticancer effects by CLA and prevention of cardiovascular disease

CLA has been shown effective against a number of cancer models such as skin, forestomach, mammary, colon, and liver [11, 42, 43]. It is stated not only to reduce initiation, promotion, progression, and development of cancer but also to reduce metastasis of cancer [11, 42, 43]. The suggested methods for CLA action are reducing eicosanoid production, interfering with cell signaling pathways, inhibiting DNA synthesis, enhancing apoptosis, and inhibiting angiogenesis as shown in reduced

matrix metalloproteinase and vascular endothelial growth factors [11, 42–44]. Modern potential anticancer treatments rely on lifestyle modifications, including dietary changes. CLA, for example, can reduce the risk of developing cancer [45]. Necroptosis was induced by several PPAR ligands, which prevented the development of cells with lung cancer in humans. However, no studies have yet looked into how CLA affects PPAR mRNA and nutrient levels in non-small cell lung cancer cell lines [46].

It has been reported that CLA reduces atherosclerotic lesions in rabbits and hamsters [47–49]. It reduces total cholesterol triacylglycerides low density lipoprotein (LDL), cholesterol and increased High Density Lipoprotein (HDL) cholesterol in various animal models [49–51]. Various factors are involved such as reduction in blood pressure, involvement of PPAR key role in lipogenesis, sterol regulatory element binding protein (SREBPs, a key role in fatty acid synthesis and elongation), and/or stearoyl Co-A desaturase (SCD, a key role for triglyceride and cholesterol formation) [48, 51–53]. Between the bloodstream and the supporting connective tissue, the endothelium is an overlay of flat epithelial cells. It describes important processes that, when they are dysfunctional, cause endothelial dysfunction. The development of vascular disorders is attributed to this syndrome. A mouse ovariectomized model has been utilized to explore the consequences brought on by the lack of sex hormones because it is well-known that they have a cardio-protective impact. The impact of dietary fats like CLA on cardiovascular illnesses has been researched as a means of maintaining vascular health. Antioxidant, anti-atherogenic, and anti-inflammatory properties are some of the benefits of CLA that have been scientifically demonstrated. It increased the antioxidant activity and stopped the orchietomy's effects on prostacyclin, nitric oxide (NO), and cholesterol oxidation products (COPs). These results might help us comprehend how things work. The impact of an eight-week CLA diet supplement on parameters linked to aortic and mesenteric artery vascular dysfunction as well as the quantity of lipid oxidative released after orchidectomy. The inclusion of CLA in the diet prevented the production of proteinoids from rising and preserved the physiological parameters of NO with enhanced antioxidative activity. Also, it stopped the vascular wall's cholesterol and COPs from rising. The antioxidant activity was increased, and the orchidectomy-induced changes to prostacyclin, NO, and COPs were avoided by a CLA-supplemented diet. These results may aid in our understanding of the processes by which CLA prevents cardiovascular illnesses [54].

### **3.2 Body fat reduction by CLA**

In 1995, for the first time, it was reported that CLA has some effect on the reduction of body fat. The multiple effects through which CLA acts on the reduction of body fat include increasing energy expenditure and reducing lipid accumulation in adipose tissue [55, 56]. It may also work by increasing adipocyte differentiation or by adipocyte apoptosis. CLA has also some modulatory effects on adipokines and cytokines such as leptin, TNF- $\alpha$ , adiponectin, or interleukins and increasing fatty acid  $\beta$  oxidation in skeletal muscle [56]. Ruminant fats contain a lot of conjugated linoleic acid which appears to enhance body composition and risk factors for cardiovascular disease. Experimental models have already demonstrated CLA's ability to lower body fat levels and to have positive effects on the glycemic index, atherosclerosis, and cancer. Moreover, CLA supplements may enhance bone mineralization, assist in the resynthesizing of glycogen, and alter immunological function. Additionally, CLA supplementation may boost lipolysis and decrease the build up of fatty acids in adipose tissue. The putative factors responsible may be its ability to decrease lipase lipoprotein activity and increase

carnitine-palmitoyl-transferase-1 (CAT-1) activity, interact with PPAR, and increase mitochondrial uncoupling protein-1 (UCP-1) expression. While human studies have indicated some advantages of CLA supplementation, such as weight loss, the findings are still inconclusive. Additionally, some have demonstrated detrimental effects, such as harm to lipid profiles and glucose metabolism. This article's goal is to summarize the information that is now available regarding the advantages of CLA for energy consumption and muscle mass, with a focus on the mechanisms of action [57]. Although most research on CLA's impact on obesity has concentrated on how much body fat it reduces, other studies have shown that it also boosts muscle mass and improves athletic performance. These effects have been attributed to altered signal transduction pathways in metabolism or energy metabolism, changes in the type transformation of muscle fibers, and other physiological modifications in the skeletal muscle. The exact mechanism by which CLA affects cardiometabolic risk is not fully understood. This review's objective is to provide an overview of what is presently known about how CLA affects skeletal muscle metabolism. There is a lot of potential for using CLA because it not only enhances lean mass but also reduces body fat [58]. When supplemented over a duration of 6 to 16 weeks, CLA may have a somewhat anti-obesity effect on women's body weight (BW), body mass index (BMI), and TBF, especially in post-menopausal and overweight women. This meta-finding analysis needs to be supported by more well-designed studies [59].

### **3.3 CLA in immune and inflammatory responses**

The major anti-inflammatory response of CLA reported is reducing colonic inflammation, decreasing antigen-induced cytokine production in immune-competent cells, and modulating the production of cytokines prostaglandins and interleukin B4 [60–64]. The immune-related response of CLA has been shown by modulating tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), cytokines (i.e., interleukin 1, 4, 6, or 8), prostaglandins, or nitric oxide while reducing allergic-type immune response [65–67]. In Holstein cow, the enhancement of n-3 fatty acid in EM with the use of essential fatty acid (EFA) prevented n-3 FA dehydration. The combined EFA + CLA treatment led to small further alterations in the anti-inflammatory response, and the enhanced n-3 FA (Fatty Acid) status and decreased n-6:n-3 ratio by the EFA treatment showed a more distinct influence on the inflammatory process during the period of transition than the single CLA treatment [68]. Many studies have confirmed the rumen microbiota's vital role in maintaining the rumen epithelium's innate immunity equilibrium. Information on the immunoprotective impacts of various CLA isomers on rumen respiratory epithelium is scarce as they are the natural microbial metabolites of the rumen. The 100 M trans-10,cis-12-CLA in this study reduced dramatically the gene expression associated with inflammation, cell proliferation, and migration in RECs after activation with lipopolysaccharide, exerting greater anti-inflammatory actions than the cis-9,trans-11-CLA. Trans10,cis-12-CLA significantly decreased, when compared to cis-9,trans-11-CLA with enhanced supervision of signaling pathways and cytokine production involving KEGG pathways NF-B, chemokine, NOD-like binding site, Hippo, PI3K-Akt, TGF- and Rap1 signaling. Also, compared to the LPS group, pre-treatment with trans-10,cis-12-CLA dramatically decreased the activation of lipogenic genes and the production of the unsaturated fat pathway in RECs; however, cis-9,trans-11-CLA showed the opposite results. These findings point to different CLA isomer variations in RECs' control of systemic inflammation and work in different signaling, and they will serve as crucial references for figuring out their target application in the future (**Figure 4**) [69].

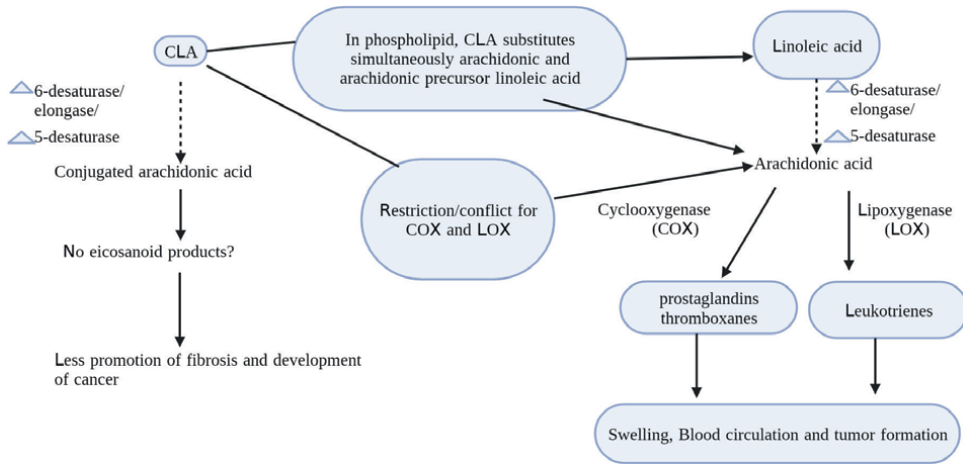


**Figure 4.** A possible explanation for how CLA alters carcinogenesis. The chemical breakdown of arachidonic acid is impacted by CLA, which also reduces Lipoxygenases (LOX) and Cyclooxygenase (COX), Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), and TB<sub>2</sub> levels, and inhibits the activation of NF- $\kappa$ B, significantly the Bcl-2 and Ki-67 proteins, phosphorylating ErbB<sub>3</sub> and Akt, and upregulating caspases and CLA affect apoptosis.

### 3.4 Bone health and CLA

According to the reports, CLA improves bone mass as reported by ash weight, bone density, bone mineral contents, bone dry weight, bone length, or calcium, magnesium, or phosphate content [70]. However, the effect of CLA on body ash and bone mass has not been consistent. This inconsistency has been reported due in part to the interaction between CLA and calcium in diet [70]. However, there have been mixed reports of bone resorption by CLA. Some suggest that CLA decreases the osteoclast activity, thus enhancing bone resorption, while others suggest CLA has no effect on bone resorption [71, 72]. Rats receiving a dietary supply of CLA showed increased levels of CLA isomers in many tissues, including bone compartments. Improvements in the cis-9, trans-11 CLA monomeric concentration of rat bone tissue lipids were linked to changes in bone indicators and bone production rates in developing rats. In contrast to polar lipids, CLA had a stronger impact just on the fatty acid structure of neutral lipids in rat tissues. Rats' bones with dietary CLA supplementation have higher concentrations of CLA isomers. Dietary CLA caused variances in CLA concentration, with the brain showing the lowest quantities of isomers while having the highest concentrations in certain bone tissues. The examination of CLA benefits will yield fresh data on the interactions between fatty acids and osteoblasts [73].

## 4. CLA and cancer

The anticancer effect of CLA has been studied in both in vitro cell and in vivo animal models. They can be found in the food that ruminant animals produce as well as in the seeds of some plants [74]. As specified both the isomer of CLA, that is, c9 t11 and t10 and c12 have shown to inhibit both the promotion and initiation stages of carcinogenesis, suggesting the chemopreventive effect of CLA (Table 1) [86]. The anticarcinogenic effect of CLA was discovered accidentally by researchers at

the University of Wisconsin in 1979. Although the anticancer effect of CLA is well known, it has other known properties such as reduction of cardiovascular disease and inflammation along with the regulation of body weight, reducing body fat, and increasing lean body mass [87]. The mechanism behind the anti-cancer properties of CLA is the activation of PI3/Akt signaling and stimulation of the tumor suppressor gene, p53 [86, 87]. These pathways increase cancer cell apoptosis, decrease cancer cell proliferation, and inhibit tumor growth. Cyclin C and cyclin D modulation is carried by CLA which participates in cell cycle progression. The expression level of PPAR

| Cancer type        | Model         | CLA Source    | Mechanism                                                | Outcome                                                                                                                                                                                                                                                                                                               | Ref. |
|--------------------|---------------|---------------|----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Colon              | SD Rats       | CLA Mixture   | Inducing TGF- $\beta$ - producing macrophages and T cell | This decreased the epithelial integrity and proinflammatory influx in the conjugated sodium sulfate-induced colon model.                                                                                                                                                                                              | [75] |
| Colon              | SD Rats       | C9, t11- CLA  | Reduced tumor frequency, aggregate apoptosis             | CLA could have anticancer activity by causing apoptosis in the rectal epithelial cell by alteration of the transmitter.                                                                                                                                                                                               | [76] |
| Colon              | BALB/c mice   | CLA Mixture   | Diminished metastatic foci, stimulation PPRn             | The proportion including both cells' abdominal canal malignant regions was reduced significantly, As MKN28 and Colo320 were pretreated with LA, and glycogen replenishment indicated a decline in the antibody for epithelial stimulating elements and a rise in Bax.                                                 | [77] |
| Mammary            | SD Rats       | C9, t11 - CLA | Reduce the weight and volume of the tumor                | In empirical mammary antitumor, the leading CLA isomer found in the human diet shows antineoplastic properties.                                                                                                                                                                                                       | [78] |
| Mammary and Breast | SD Rats       | CLA Mixture   | Impede PUFA oxidation                                    | CLA's efficacy for reducing simultaneous non-infectious disorders                                                                                                                                                                                                                                                     | [79] |
| Breast             | SCID Mice     | CLA Mixture   | Inhibit the growth of cancer cell                        | Dietary polyunsaturated fats may alter a prostate cancer patient's survival, presenting novel therapy possibilities.                                                                                                                                                                                                  | [80] |
| Prostate           | Big Blue Rats | CLA Mixture   | Inhibit the formation of PhIPin DNA                      | The first study to demonstrate CLA's protection from PhIP-induced mutagenesis in the prostatic in terms of mutation frequency and gene mutation diversity. It may be used in human cancer prevention studies because of human chemoprevention because of CLA's antagonistic effect against PhIP-induced genotoxicity. | [81] |

| Cancer type | Model           | CLA Source    | Mechanism                                                                | Outcome                                                                                                                                                                                                                                                                                                     | Ref. |
|-------------|-----------------|---------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Prostate    | Copenhagen Rats | CLA Mixture   | Cytotoxicity                                                             | Without performing a fatty acid compositional study, the optical properties can be utilized as a quick and easy way to manage the hydroformylation activity of vegetable oils to achieve the desired conjugated linoleic concentration.                                                                     | [82] |
| Liver       | Male F344 Rats  | C9, t11 - CLA | Inhibit Tumor formation                                                  | Pomegranate seed oil high in c9,t11, and c13-CLN can reduce AOM-induced colonic carcinogenesis and that this inhibition is partly mediated by increased liver and colon CLA content and/or colon stromal PPARgamma protein expression.                                                                      | [83] |
| Liver       | Donryu Rats     | CLA Mixture   | Helps to generate hepatic lipid peroxidation and aggregation             | Conjugated palmitoyl hydroxylase II, a percentage protein in the energy metabolism beta-oxidation pathway, wasn't really activated by dietary CLA. Although injected rats tended to have increased levels of natural killer cell function in the spleen, no detectable effect of nutritional CLA was found. | [84] |
| Breast      | BALB/c mice     | C9, t11 - CLA | Inhibited the growth of the tumor when combined with chemotherapy drugs. | A nucleotide analog drug against a wide range of cancers is gemcitabine. Fast decomposition in vivo, which causes a short circulatory period and reduced anticancer activity, is a limitation that has to be resolved.                                                                                      | [85] |

**Table 1.**  
*Preclinical evidence of CLA against various forms of cancer.*

gamma is also increased by CLA suggesting its role in metastasis and decrease of the tumor size [86, 87].

#### 4.1 CLA and breast cancer

Breast cancer is the second most common cause of cancer death among women worldwide. Cell proliferation is supposed to be downregulated by CLA in breast cancer [88]. CLA induces apoptosis via Liver X Receptor (LXR) in the MCF-7(Michigan Cancer Foundation-7) cancer breast line [89]. Cell growth and invasions inhibited through PI3/Akt signaling in the breast cancer cell line. It has also been shown that apoptosis is stimulated by a mixture of CLA through p-53-mediated cell

signaling in the MCF-7 breast cancer cell line [90]. Intravenous injection of CLA in nude mice-xenografted human breast MCF-7 tumors showed an enhanced tumor growth inhibition effect. Many clinical studies have also suggested the anticancer effect of CLA in stages I–III of breast cancer. According to one study, the breast cancer tissue expression of S14 was decreased after a short course of treatment of 7.5gm CLA/day [90, 91]. Another study showed a reduction in proliferative marker Ki-67 in primary invasive breast cancer tissue on the supplementation of 7.5 gm CLA for 10 days. The limitations of these two studies were a small population and less duration of dose. But the safety and tolerability of CLA were measured to be 7.5 gm/day for a period of 20 days [91, 92]. In breast cancer, CLA was proven to reduce cell proliferation [74]. Certain *in vivo* experimentation on rats, fed on proportions of CLA with linoleic acid and simultaneous tumor induction showed The protective benefits of CLA. However animals fed with HFD and varying concentration of CLA showed no impact on tumor growth. Along with that neither linoleic acid nor arachidonic acid replaced CLA in the mammary tissue [93].

#### **4.2 CLA and colon cancer**

Colon cancer also called colorectal cancer is cancer due to uncontrolled growth in the colon or rectal region [94]. Rectal bleeding and anemia are the typical symptoms associated with colon cancer. The major cause of colorectal cancer can be associated with lifestyle and increasing age with only a very few due to genetic disorders. It is more common in developed countries, and it is the third most commonly diagnosed cancer in the world [94]. CLA has been shown to have an anti-proliferative effect on colon cancer. Trans 10, cis 12 isomer of CLA induces cell death through reactive oxygen species (ROS)-mediated endoplasmic reticulum stress in the human colon cell line [95]. This isomer of CLA exhibited an anti-proliferative effect in the Caco-2 cancer cell line. It induces cell cytotoxicity via both PPAR gamma and APC/beta-catenin signaling pathways [95, 96]. Cell proliferation by CLA has been carried out through p53-dependent mechanism, and it is also supposed to regulate the expression of G1 restriction point in colon cancer cells [97]. In animal *in vivo* studies, it has been demonstrated that CLA mixture ameliorates inflammation-induced colorectal cancer through activation of the PPAR gamma signally pathway. Similarly, the mixture of CLA isomers was effective in inhibiting colon cancer metastasis in BALB/c (Bagg and Albino) mice [96, 97]. The inhibitory effect of CLA on growth and metabolism in the human HT29 colon cancer cell line has been demonstrated with special regard to the conversion of trans 11 trans 13 CLA isomer. CLA has also been shown to reduce colon carcinogenesis in azoxymethane-pretreated rats [97, 98]. Shiraishi et al. mentioned research on malignant rats fed beef tallow over an extended period. Carcinogenic rats fed beef tallow over an extended period of time. The combination of CLA isomers was employed to prevent the development of colon cancer. After administering azoxymethane to the animal model first, the impacts of the CLA combination were examined. According to the findings, a combination of 1% CLA in the triglyceride form and 1% CLA in the free lipid form reduced the risk of colon cancer. Although CLA-FFA was better at colony carcinogenesis, its exact apoptotic mechanism remained unknown [74]. Kim et al. also considered the implicit parcels of the two main CLA isomers against colon cancer cells Caco-2, *in vitro*. The results indicated that t10, c12-CLA cure dropped the situations of the insulin growth factor stashing and IGF-binding proteins, thus reserving the Caco-2 cell growth. The study concluded that only one isomer t10, c12-CLA

convinced cell apoptosis and dropped DNA conflation, whereas c9, t11-CLA had no effect [99].

### **4.3 CLA and endometrial cancer**

In developed countries, particularly among post-menopausal women, endometrial cancer is the most common malignancy found. Endometroid adenocarcinoma which is the most common subtype occurs within a few decades of menopause, and it is mainly associated with obesity and excessive estrogen exposure which often develops in endometrial hyperplasia [100]. In the endometrial cancer cell line, RL 95–2 when treated with cis 9 trans 11 isomers of CLA, the expression of caspase 3 and Bax/bcl ratio was significantly increased, but no change was observed in Akt and p-Akt [100, 101]. According to one of the studies, the expression of total ER $\alpha$  level was unchanged in the treatment of cis 9 trans 11 isomers in the RL 95–2 cell line. Also, the protein expression level of p-ER $\alpha$  was downregulated upon treatment with 80 Mm of cis 9 trans 11 isomers [101]. From this study, it can be concluded that cis 9 trans 11 isomer of CLA induces apoptosis by estrogen receptor alpha (ER $\alpha$ )-mediated signaling pathway in the RL 95–2 endometrial cancer cell line. As there is not much information available on the possible role of CLA in endometrial cancer, it opens an aspect for further research in this direction [102]. Additionally, it was revealed that ER $\alpha$  was mediated by a number of signaling pathways, including phosphoinositide 3-kinase/Akt and extracellular signal-regulated protein kinase  $\frac{1}{2}$  [103]. The impact of isomer-specific CLA on RL 95–2 cells is evident from the fact that as the cells were exposed to c9, t11-CLA at doses of 10–160 M for 24, 48, and 72 h, respectively, the viability of the RL 95-2 cells was decreased. When the cells were exposed to c9, t11-CLA at doses of 10–160 M for 24, 48, and 72 h, respectively, the viability of the RL 95-2 cells was decreased [103]. It is demonstrated that the formation of total ER $\alpha$  was unaltered in RL 95-2 cells treated with the cis 9-trans 11 CLA isomer, but that at an 80 mM concentration, the isomer downregulated the level of p-ER $\alpha$  protein expression. According to this finding, cis 9-trans 11 CLA isomer causes apoptosis in RL 95-2 endometrial cancer cell lines via an ER $\alpha$ -mediated signaling mechanism [104]. In a research paper, it is observed that ATP-CVA was previously used to assess the effectiveness of chemotherapy in uterine cancer cell lines. In order to identify the inherent therapeutic efficacy of cytotoxic medications, researchers conducted the ATP-CVA on patients with endometrial cancer as part of this study to examine the viability of doing so. The SF50 (survivability percentage at 50%) of the PPC of 32 of the 33 ovarian cancer samples could be evaluated using ATP-CVA. In comparison, cisplatin (0.71), topotecan (0.93), paclitaxel (0.68), doxorubicin (1.0), etoposide (0.70), 4-epidoxorubicin (0.88), and carboplatin's median SF50 (0.33) was significantly lower [105].

### **4.4 CLA and renal cell carcinoma**

Cancer which starts in the cells of the kidney is said to be associated with kidney cancer. Renal cell carcinoma (RCC) and urothelial cell carcinoma (UCC) are the two most common types of kidney cancer of the renal pelvis [106]. These different types of cancer develop in a different way, and their outcome is also different and need to be staged and treated in different ways [107]. A lipid class distribution study was performed by Hoffman et al. showing that CLA was incorporated in the neutral lipid compared with the phospholipid class [108]. The content of the trans 10 cis 12 isomers

of CLA was found to be different in normal and cancerous cells of the kidney suggesting a key role for the above-said isomer of CLA in kidney cancer. Much work needs to be done due to the lack of mechanistic study in this regarding the effect of CLA on kidney cancer [109]. Significant reductions in tumor weight, fluid volume, and live Ehrlich cells were seen in TTM that had been treated with CLA. Histopathology showed negligible effects, whereas hematological and biochemical profiles returned to more or less normal levels. Due to the synergistic and additive effects of its isomers, the current study demonstrated the safety and cytotoxic efficacy of milk protein CLA and provided a scientific foundation for its therapeutic usage as an anticancer agent [110].

#### **4.5 CLA and pancreatic cancer**

Pancreas is a glandular organ located behind the stomach. Pancreatic cancer is a cancer within the pancreas. The signs and symptoms of pancreatic cancer include abdominal or back pain, yellow skin, unexplained weight loss, light-colored stool, dark-colored urine, and loss of appetite [111]. Ductal pancreatic adenocarcinoma is the most prevalent form of pancreatic carcinoma, and it is said to be the fifth leading cause of death in Western countries. The relationship between dietary fat intake mainly n-6 polyunsaturated fatty acids (PUFA) and cancer was studied by many researchers [111]. CLA is an essential fatty acid with known anticancer potential. However, LA is reported to promote tumor growth caused by high sensitivity to nonenzymatic lipid peroxidation [111, 112]. The impact of dietary LA and CLA on liver metastasis and lipid peroxidation (LPO) was examined by Kilian et al. in N-nitroso bis-2-oxo-propylamine (BOP)-injected Syrian hamster for 12 weeks [112]. No difference was observed between tumor groups in liver metastasis and lipid peroxidation. Therefore, these results suggested that there was no difference between the LA and CLA-fed groups according to the impact on liver metastasis in ductal pancreatic cancer. Again Kilain et al. in 2003 investigated the impact of the high content of CLA along with dietary intake on ductal pancreatic carcinoma. The result suggested that CLA increased the weight of the pancreas in the tumor and non-tumor group compared to LA [112]. It was also found that LA and CLA do not influence lipid peroxidation or activity of anti-oxidative enzymes GSH-px (glutathione) and SOD in tumor-free control groups. Therefore, according to the study, it can be confirmed that the content of CLA in the diet does not influence pancreatic tumor growth. However, further research is required by increasing the content of the CLA dietary model [112, 113]. The frequency of pancreatic cancer in relation to CLA and LA dietary consumption of CLA and LA was examined in a subsequent investigation. Four groups of 60 male hamsters each were chosen. While the third and fourth groups received injections for 12 weeks, the initial group had 0.9% NaCl once per week. The findings demonstrated that neither CLA nor LA had an impact on the prevalence of pancreatic cancer. However in both the groups, pancreatic intratumoral tissue had higher lipid peroxidation and enhanced glutathione peroxidase activity [114].

#### **4.6 CLA and liver cancer**

Cancer that originates in the liver can be termed liver cancer. It is the third largest cause of cancer death in the world and fifth in the case of women due to cancer [115]. The symptoms associated with liver cancer are abdominal mass, abdominal pain,

yellow skin, nausea, or liver dysfunction, and it can be detected by medical imaging equipment [115]. The liver is the most common site for metastasis, and it is mostly derived from the colorectal and neuroendocrine primary tumor [116]. The effect of CLA has been studied in hepatocellular carcinoma cell lines, and it exhibits an inhibitory effect on hypoxia-induced factor 1 (HIF 1)  $\alpha$  stabilization under hypoxic conditions [117]. Along with this, the proapoptotic effect of CLA is mediated by PPAR  $\alpha$  and PP2A signaling pathway in the SK-HEP-1 (human hepatoma cell line) [117, 118]. Moreover, mitochondria-related apoptosis and lysosomal destabilization are demonstrated in rat primary hepatoma cells by trans 10 cis 12 isomers of CLA [97]. The same isomer is known to exhibit a strong cytotoxic effect on dRLH-28 rat hepatoma cells via the activation of caspase 9 followed by cytochrome release from mitochondria [97, 119]. In animal studies, CLA inhibits azoxymethane-induced rat colon cancer by alteration of lipid composition and elevation of colonic PPAR gamma expression [119]. The cytotoxic effect of trans 10 cis 12 isomer on rat hepatoma was observed, and it was modulated by other fatty acids such as tocopherol and tocotrienol [119, 120]. The difference between in vivo and in vitro results could be attributed to the duration and isomer of CLA. In the cell line study, we mostly use the trans 10 cis 12 isomer of CLA, whereas in the animal model, CLA mixtures were used. Also, the duration of the dose is also a considerable factor; hence, further studies with purified isomers can give a better result [119, 120]. A study on anti-carcinogenic properties of pomegranate seed oil also demonstrated 70% of the c9, t11 CLA isomer. Acute otitis media (AOM) was injected directly into male F344 rats once every week for 2 weeks. The higher dietary content of c9, t11-CLA in the lipid component of the colonic mucosa and liver was related to the prevention of colonic adenocarcinomas [121]. On rat hepatoma dRLh-84 cells, CLA has been shown to possess anti-mutagenic and anti-carcinogenic activities in vitro. It has been previously established that the geometric disparity between the two main CLA isomers was the cause of their divergent anti-tumor responses. On the dRLh-84 rat hepatoma cell line, different studies revealed variations in the anticancer activity of CLA isoforms [110]. Under 1% O<sub>2</sub> circumstances, the anti-cancer actions on the human liver cancer cell line HepG2 have been documented. Under low oxygen conditions, both the isomers c9, t11-CLA and t10, c12-CLA reduced cell proliferation and brought on apoptotic cell death (HIF-1 stabilization). This unique investigation was demonstrated to be of considerable potential for future trials because the precise process remained unknown (Table 1) [122].

## **5. Mechanism of action of CLA**

The potential mechanisms by which CLA alters body composition include metabolic adjustments that favor a decrease in lipogenesis and potentiation of lipolysis, along with the oxidation of fatty acids in skeletal muscle, as a result of elevated carnitine in palmitoyl-transferase-1 movement and activity or perhaps due to the inhibition of adipocyte differentiation [123]. Consequently, experts have assessed how the addition of CLA to a diet affects hormonal and lipid levels as well as the functioning of oxidation-related enzymes [124]. Studies have shown that CLA isomers that are 10-trans and 12-cis, as opposed to the 9-cis 11-trans, dramatically promote lipolysis in human adipocytes and also serve to reduce triglyceride production [125]. This would substantially explain any potential techniques through which CLA affects body composition. The control of the expression of genes that

regulate the transformation of this was before the adipocytes into mature adipocytes, or more specifically, the communication of these genes would lead to reducing lipogenesis, which was the foundation of metabolism hypotheses to explain the body fat-reducing action of CLA, despite the fact that various studies were in vitro [126]. The nuclear transcription factors known as peroxisome proliferator-activated receptors play an essential role in the preservation and metabolism of fatty acids (FA). They are a subset of nuclear channels that are related to the nuclear receptor family which includes the steroid, retinoid, and thyroid receptors. The nuclear receptor has already been divided into three isoforms: PPAR, PPAR, and PPAR. Lipid metabolism (particularly, the proteins associated with FA oxidase) and glucose are both regulated by the PPARs, and PPAR is also involved in the development of adipocytes. The co-activator unit (acetyltransferase capacity) and the dissolution of the co-repressor compound (histone deacetylase function) by the binding agent are required for the activation mechanism. Through transcription-competent competent structure develops when the activating PPAR:R $\times$ R complex attaches to the PPRE-responsive zones, altering the chromatin's structural makeup. Therefore, it appears that the CLA interacts with PPAR, enhancing the transcription of genes involved in adipocyte differentiation, lipolysis (oxidation), mitochondrial biogenesis, and insulin sensitivity, all of which are associated with the weight loss impact [127]. The activity or inactivity of the PPARs, particularly PPAR, mediates the effects of CLA on the metabolism of lipids and glucose on body composition. By modulating gene expression in a way that prevents cell differentiation and changes the functioning of proteins associated with lipogenesis and lipolysis, CLA (isomer 10-trans, 12-cis)'s suppression of PPAR causes a reduction in body fat [128]. According to the available data, PPAR activation may slow the development of atherosclerosis and improve insulin sensitivity, making it an attractive target in the management of a number of conditions, particularly type 2 diabetes mellitus (DM2) and dyslipidemia. The activity of genes that regulate lipid metabolism, such as acyl CoA-synthetase and lipase, also called lipoprotein lipase (LPL), is regulated by PPAR in adipocytes. PPAR also regulates the production of the fatty acid transport protein that regulates adipocytes' intake of lipids [129]. The decrease in fat in the body is caused less by a decrease in adipocyte quantity than by a decrease in the adipocyte size. Given that the triglyceride level of adipose cells directly correlates with their size, lowering it leads to smaller cells. Triacylglycerol synthesis may have been reduced as a result of the increased process of cellular fatty acids brought on by CLA, which did not result in their deposition in adipocytes but did result in a reduction in their size [130]. The carnitine-palmitoyl-transferase (CPT) unit carries the fatty acid into the mitochondria. CPT-1, CPT-2, and carnitine acyl carnitine translocase (CATC) are the three enzymatic players. By producing an activated complex (fatty acyl-CoA) that includes the carnitine-palmitoyl-transferase (CPT-1) enzyme, the acyl-CoA synthetase enzyme activates the fatty acids. The intermembrane gap is reached after this complex passes through the mitochondrial membrane. Following the breakdown of carnitine in the CPT-2 reaction, acyl-CoA is replenished. The large chain fatty acid (LCFA) is oxidized once it enters the mitochondrial matrix to produce adenosine triphosphate, also known as ATP through the oxidation of the fatty acids [131]. The amount of and function of CPT-1 would rise with the addition of CLA. Thus, the build up of fatty acids in the adipose and muscular tissues is decreased as a result of increased lipolysis, decreased lipase lipoprotein activity, and enhanced carnitine-palmitoyl-transferase-1 (CAT-1) activity. The researchers frequently address these action mechanisms [130]. By

oxidation processes and the Krebs Cycle (CK), which take place inside the mitochondria, fatty acids are converted to  $H^+$  and  $e^-$  which are then transported (via  $NADH+2$  and  $FADH_2$ ) to the pulmonary chain (1). The ATP synthesis protein (2) either produces ATP (in conjunction response) or heat (uncoupling protein (3)) in response to the gradients of  $H^+$  and  $e^-$  across the intermembrane gap and the matrix. The argument that CLA promotes lipolysis only by raising CPT-1 is, however, only true in circumstances where fatty acyl-CoA complex transport to the matrix of mitochondria is less effective than oxidation, which is the ability to produce ATP through the sequential breakdown of fatty acid carbon. This makes it plausible and logical to conclude that CLA supplemental intake (such as increasing CPT-1 concentration in order and stimulation) would likely only have a marginally positive impact on people who are physically active, especially for those whose oxidation is more effective compared to the movement of fatty acids to the mitochondrial matrix. The interaction of CLA with the separating enzyme of the chain of respiration, which increases its ability for oxidation, may, on the reverse, account for the weight loss that occurs when CLA administration is used [132]. A collection of transport substances found in the membrane on the inside of the mitochondria makes up the respiratory chain, also known as the electron transport chain. Cytochrome oxidase, the last among these substances, is the only one in existence that meets all requirements to transport electrons to  $O_2$  directly. But not all of the power that comes from the electrons will end up in ATP as some of it turns into heat to preserve the unpredictability of the subsequent transfers. The respiratory chain causes the electrons to lose their inherent energy as they go through it. In order to create ATP using ADP and inorganic phosphate, some of this energy can be captured and stored. The remaining energy that is free, which is not used to re-synthesize ATP, is emitted as heat is produced, boosting the uncoupling proteins (UCP's) function [133]. The membrane that surrounds the mitochondria contains proteins

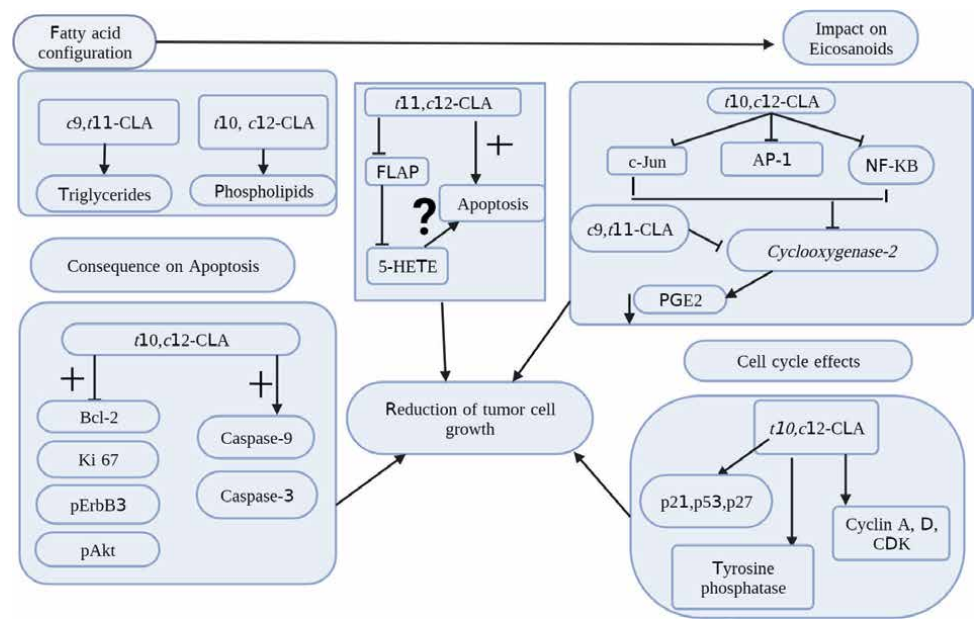


**Figure 5.**  
The overall mechanism through which CLA inhibits the formation of eicosanoids from arachidonic acid.

called UCPs that enable proton transport from the intermembrane gap to the mitochondria matrix. However, the protons' return to the matrix of mitochondria does not result in ATP energy storage, which would release heat. When UCP-1, additionally referred to as thermogenin, is stimulated, it can cause weight loss because it frequently accelerates up the hydrogen return to the mitochondria matrix, causing energy from the Krebs cycle, which comes from the decomposition of energy substrates (including lipids), to be lost in the form of heat [134]. UCPs can be subdivided into UCP-1, UCP-2, and UCP-3. They differ in their distribution among tissues and possible function. UCP1 is exclusively expressed in brown adipose tissue; UCP3 is expressed in muscle and a number of other tissues; and UCP2 is expressed in a variety of tissues including white adipose tissue (WAT) and is the most highly expressed UCP. In rodents, it has been established that supplementation of a particular CLA conjugation or 10,12 CLA causes UCP2 translation in WAT [135]. Nevertheless, it is uncertain if it affects energy loss. It appears that CLA combines with PPAR, boosts CPT-1 (carnitine palmitoyltransferase-1) and UCP-1 expression, and promotes the ability of lipolysis, which stored fat for weight loss (Figure 5) [132].

## 6. Future prospects

The role of CLA is well established in many inflammatory diseases and has drawn significant attention in the last two decades for its various biological effects. CLA reduces body fat, modulates immune and inflammatory responses, reduces cancer and cardiovascular disease, and improves bone mass. As far as obesity-related cancer is concerned, it has shown potent anti-cancer effects and management of tumor growth and metastasis. The overall result of CLA is due to its two main isomers, that is, cis 9, trans 11 and trans 10 cis 12. However, there is some controversy between in vitro and in vivo studies as most in vivo animal model uses CLA mixtures, and the effect of CLA on cancer cell proliferation and its molecular mechanism is yet to be fully understood. In the case of clinical application, the role of CLA is limited for cancer patients due to low bioavailability, that is, poor water solubility and short half-life. Moreover, when using a mixture of CLA, the result is varying, and it is lacking in scientific basis. The different isomers are shown to have different molecular mechanisms, and some are shown to have opposite effects on each other. Hence, direct extrapolation of the result needs to be more studied, and further investigation is needed to show the short- and long-term effect of CLA in a human trial in order to determine the safety for application to human. Therefore, these drawbacks are yet to be looked into for the CLA to be implemented as for potent remedy for many inflammation-related disorders.

## 7. Conclusion

CLA research has drawn much attention in the last two decades ranging its effect from cancer to obesity. Even being a relatively simple structure, the more diverse effect of CLA in different diseases such as cancer, obesity, inflammation, asthma, cardiovascular disease, and bone health is intensively studied. The different effect of CLA is attributed to its different isomers, namely, cis 9 trans 11 and trans 10, cis 12. Both the isomers have different molecular mechanisms which are yet to be examined more intensively. CLA is known to have a potent anti-cancer effect which is studied

extensively, especially in the case of obesity-related cancer such as colon cancer, endometrial cancer, liver cancer, kidney cancer, and breast cancer. The mechanism by which the CLA acts are activation of the PI3K/Akt pathway, stimulation of the tumor suppressor gene, p53, followed by activation of caspase 9. As far as human consumption is concerned, the safety parameters are also been evaluated in a number of human clinical trials. About 90% of the studies were conducted with either of the two isomers of CLA. The daily consumption of 3–6 g/day in human beings is tested, but no such adverse effect has been reported to date. However, there are still concerns regarding the potential safety of CLA as a therapeutic agent. Therefore, better experimental designs are required to clarify the CLA activity's mechanism.

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## **Authors' contribution**

TD wrote the paper and analyzed data. AK designed the study and carried out proof reading. AKM designed the study, conducted proof reading and editing, and had primary responsibility for the final content.

## **Conflict of interest**

The authors declare no conflict of interest.

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# The Promotion of Biotication in Controlled Fermentation Using Kefir Grains and Kombucha Membranes as Starter Cultures

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## Abstract

Through the centuries, fermented foods have demonstrated their bioactivity, usefulness, and sustainability in promoting the increase of life quality through multiple actions *in vivo* and *in vitro*. Fermented foods, through the complex biotransformation of substrate components, enrich the final products with useful biotics, i.e., prebiotics, probiotic microorganisms, and their metabolites named postbiotics. The artisanal cultures, milk, water kefir grains, and kombucha (SCOBY) membranes are functional and sustainable microbial symbiotic cultures that offer approachable opportunities for obtaining valuable functional foods and ingredients. These consortia are formed by the natural association of the bacteria (lactic acid and acetic acid bacteria), yeasts, and bacteriophages. Various conventional and unconventional substrates can be transformed into functional fermented products based on the complex metabolism of these microorganisms in controlled fermentations targeting the improvement of the biotication, i.e., increase the contents of pre-, pro-, and postbiotics. Thus, the complex biochemical activities of the multiple microorganisms from the natural consortia could be exploited in controlled fermentation conditions to obtain the beneficial effects for *in vitro* and *in vivo* features of the obtained fermented products.

**Keywords:** artisanal starter cultures, milk and water kefir grains, SCOBY membranes, metabiotics (pre-pro-post-and paraprobiotics), functional fermented products

## 1. Introduction

Fermented foods have been a part of the human diet for almost 10,000 years. In the twenty-first century, a wide variety of fermented foods are available. Initially, food fermentation was used as a preservation method to reduce the contamination risk of harmful microorganisms [1]. Fermented foods are based on natural processes, targeting the diversification of goods (food, feeds, nutraceuticals, ingredients, etc.), agri-food waste valorization, and environmental protection. They extend the shelf life of foods, provide safety assurance, and increase the need for preservatives by

applying different procedures; those are naturally preferred because they are friendly to the consumers and environment and resource utilization. This feature makes them an essential element of sustainable food and feed systems development [2].

Moreover, fermentation processes change texture, sensory properties, and bioactive characteristics, which are considered significant advantages. Scientific research has indicated that consuming fermented foods offers various health benefits due to the biochemical changes in food components, increased nutritional and biological values, and the introduction of beneficial microorganisms with probiotic potential. The controlled growth of microorganisms in fermented foods allows for manipulating their metabolism in desired directions, with the implication on the beneficial properties [3–5].

Further, the new concept of metabiotics will be briefly discussed in the context of the actual transition of the probiotics to the sustainable complex of the prebiotics, probiotics, and derived paraprobiotics obtained by controlling the fermentation process or by different treatments of the probiotic cells to destroy them [6, 7].

Artisanal cultures such as kombucha, milk kefir grains, and water kefir grains are microbial consortia rich in wild probiotics, beneficial microorganisms that support gut health, and other functional actions *in vivo* [8]. Artisanal cultures can produce bioactive compounds like exopolysaccharides (EPSs), organic acids including SCFAs, bioactive peptides, vitamins, amino acids, phenols, and polyphenols, minerals [9, 10]. The fermented products obtained with artisanal cultures have positively impacted the immune system, physiology, and cognition [11]. The complex microbiome of these fermented products plays a crucial role in their bioactive functions, which include probiotic effects, antimicrobial activity, and antioxidant properties.

Kombucha is a fermented tea beverage made with a symbiotic culture of bacteria and yeasts (SCOBY), naturally immobilized in a thicker gelatinous membrane named zooglea. The key microorganisms typically found in SCOBY membranes microbiome include predominates yeasts from the genus *Saccharomyces* spp. and *Zygosaccharomyces* spp., as well as bacteria from the genus *Acetobacter* spp., *Gluconobacter* spp., and *Lactobacillus* spp. [12–14].

These microorganisms work symbiotically to biotransform the tea's sugar medium, producing various organic acids (such as acetic and gluconic acids), vitamins (especially B vitamins), and trace amounts of alcohol [15].

Milk kefir grains are a complex microbial community comprising bacteria and yeasts embedded in a matrix of proteins, lipids, and polysaccharides, named kefiran. The grains microbiome includes the most predominant species of bacteria belonging to the genera *Lactobacillus* spp., *Lactococcus* spp., *Leuconostoc* spp., and *Acetobacter* spp., as well as yeasts from the genera *Saccharomyces* spp., *Kluyveromyces* spp., and *Candida* spp. [16, 17]. During the fermentation of medium based on the milk or derivatives (whey, colostrum), lactic acid, ethanol, and various other bioactive compounds, such as bioactive peptides, SCFAs, exopolysaccharides, etc., are produced [9].

Like its dairy counterpart, water kefir is a fermented beverage well-known for its probiotic properties and health benefits [18]. It uses water kefir grains, a symbiotic artisanal culture of bacteria and yeasts. Typically, the water kefir grains microbiome consists mainly of special lactic acid bacteria such as *Lactobacillus* spp. and *Leuconostoc* spp., as well as yeasts like *Saccharomyces* spp. and *Zygorhizopus* spp. strains, and some acetic acid bacteria like *Acetobacter* spp. strains [19, 20].

The wild symbiotic consortium ferments a sugary water solution but can be adapted to other food matrices, such as the flour mixtures used to produce sourdoughs [21, 22].

The microorganism part of the kombucha and kefir grains microbiome plays a crucial role in producing beneficial substances (postbiotics). Through fermentation, these probiotic beverages not only become more nutritious but also produce metabolites and bioactive compounds [9, 23, 24] that support gut health, for example, by improving digestion [25], boosting the immune system [26], protecting against oxidative stress [27], and enhancing overall well-being [28].

Merging the concepts of kombucha, kefir grains, probiotics, and metabiotics provides insight into how these wild communities and their metabolites contribute to health by producing bioactive compounds. Both kombucha SCOBY membranes and kefir grains are actively implied to produce many biotics, which are responsible for many of the health benefits associated with the gut microbiome and further with the gut-brain, gut-lung, and gut-skin axis [29].

Various drugs (including metabiotics), food supplements, and functional food and feed products have demonstrated their efficiency in functional actions targeted at maintaining and restoring the human and animal microbiome ecology [30].

Although the concept of metabiotics has gained momentum recently, Shenderov has mentioned this term and the concept of metabiotics since 2007 [31]. Thus, as derived from the novel probiotics, metabiotics include prebiotics, probiotics, postbiotics, and paraprobiotics [32, 33].

Developing novel probiotics used as bioingredients for the formulation of foods and feeds, nutraceuticals, pharmaceuticals, or cosmeceuticals was proposed as the new fermentation biotechnologies based on symbiotic microbial cells or the structural components, metabolites, autoregulators, or biosynthetic analogs [31, 34]. Metabolic, epigenetic, informational, regulatory, transport, immune, neurohormonal, and behavioral reactions of the symbiotic wild starter cultures microbiome on the host organisms can be modulated by metabiotics, which are defined as the structural components, metabolites, and signaling molecules of probiotic microorganisms with known chemical structure [35].

The well-known probiotics consist of live microorganisms that provide health benefits to the host when ingested in sufficient quantities. Postbiotics are the metabolites of probiotic microorganisms, which exhibit *in vitro* and *in vivo* bioactive properties [36]. Prebiotics are non-digestible compounds that promote probiotic bacteria' growth and/or activity [37]. Paraprobiotics are newly discovered, and they can be obtained by processing cells to lose their viability. Thus, the paraprobiotics consist of inactivated microbial cells or cell fractions that have lost their viability due to exposure to factors that damage microbial cell structures, such as DNA breakage, disruption of the cell membrane, or mechanical damage to the cell envelope. Despite being nonviable, paraprobiotics still offer valuable health benefits [38].

Overall, the metabiotics derived from the fermentations with the artisanal cultures are bioactive components (chemical compounds, viable and nonviable cells) produced during the bioprocessing of different substrates in controlled biotechnological conditions that have health benefits on humans and animals [39–41].

## **2. Novel insights in the transition from probiotics to metabiotics using artisanal cultures**

Due to the growing popularity of artisanal cultures in Europe, ASIA, and the United States, numerous studies have been conducted to improve the fermentation process using artisanal cultures [15, 42, 43]. This involves understanding the impact

of biotechnological factors (intrinsic and extrinsic) and microbial composition (biological) on the nutritive and sensorial characteristics and bioactive properties of the obtained fermented products, considering the consumers' safety assurance.

One of the most important biotechnological factors is the chemical composition of the fermentation substrate. Further, will be described the types of substrates that are more used in artisanal fermentations with kefir grains and SCOBY membranes.

Kombucha beverages are usually made by adding tea leaves and sugar to boiling water and settling the mixture. Then, the SCOBY membrane is added to initiate fermentation. The time of the fermentation process varies based on factors like substrate composition, inoculum concentration, temperature, and the size and shape of the fermentation vessel [44]. The fermentation process consists of a liquid tea phase and a floating layer of microbial cellulose membrane, carried out between 7 and 14 days [45]. Additionally, producing a beverage from sugar-containing raw materials could take between 15 and 60 days [46]. The optimal fermentation temperature ranges from 20 to 26°C [47].

Kombucha production involves fermenting the substrate to obtain a base with a high organic acid content, mainly acetic acid. The acetic acid concentration must be at least 1.2% to ensure microbiological safety. Kombucha manufacture is associated with various risks, so controlling the entire technological process at every stage is crucial to maintaining quality and safety. Monitoring the fermentation process based on temperature, pH, acidity, acetic acid content, ethyl alcohol content, and residual sugar content is also part of the safety of the final product [44].

The chemical composition of fermented drinks depends on several factors, including the type of substrate and carbohydrates used, the composition of SCOBY, and the presence of biologically active substances such as polyphenols, vitamins, and organic acids [44]. Using the yeast starter culture in a sweet medium, or adding *Lactobacillus* spp. biomass, and introducing aeration to the fermentation process can enhance the production of biologically active compounds (organic acids such as acetic, succinic, citric, tartaric, oxalic acids, vitamins, polyphenols, and bioactive peptides) in the final product and promote biomass growth [46].

Black tea and sugar are the more frequent conventional substrates for kombucha beverage preparation. Sugar is the main carbon source for microbial metabolic pathways and is preferred for its low cost and easy availability. However, kombucha is increasingly being prepared with unconventional substrates such as green, white, oolong, and red (rooibos) teas, herbal infusions, and sweeteners such as coconut sugar, cane sugar, maple syrup, or honey [48]. Unconventional substrates are preferred to enhance the functional composition and properties of kombucha.

The components of black tea include caffeine, theobromine, theophylline, amino acids, and flavonoids. It contains catechins, flavonol glycosides, and polyphenols such as catechins, theaflavins, and thearubigins [49]. Kombucha is abundant in tea polyphenols, particularly catechins, which have prebiotic effects. Tea phenolics are considered unconventional prebiotics, similar to classic prebiotics such as oligosaccharides (galacto-oligosaccharides and fructo-oligosaccharides), being able to modulate the gut microbiota and promote the growth of beneficial bacteria, such as *Bifidobacterium* spp. and *Lactobacillus* spp. species, which produce health-promoting metabolites [50].

Green, oolong, white, yellow, and black teas derive from the *Camellia sinensis* plant. The specific type of tea produced is determined by the degree of oxidation used during processing [49]. Green tea has the highest antioxidant properties, while black tea has the lowest values. Additionally, green tea kombucha exhibits less degradation

of epicatechin isomers when compared to black tea kombucha [51]. Owing to its antioxidant properties, specifically produced from red and green tea, kombucha is safe for people who experienced physical or mental stress. However, because the fermented product has a low pH, those who have gastrointestinal reflux disease or ulcers should avoid it [52]. Due to its high polyphenol content, particularly the phenolic compound aspalathin, unfermented and green rooibos from the *Aspalathus linearis* plant has the potential to improve chronic inflammatory gastrointestinal disorders by acting as a prebiotic for the gut microbiota [53].

Kombucha beverage stimulates beneficial microorganisms by acting as prebiotics through certain polysaccharides, such as cellulose, a carbohydrate polymer made of repeating glucose units. The presence of insoluble fibers helps maintain beneficial microbes [33]. Kombucha polysaccharides can alleviate gut microbiota imbalance and prevent colitis by helping maintain intestinal barrier integrity, modulating the gut microbiota, and remodeling metabolism pathways [54].

Fermented products obtained by fermentation with the milk kefir grains are another source of prebiotics. During the milk fermentation process, compounds with prebiotic effects are produced. These compounds include exopolysaccharides (EPS), galacto-oligosaccharides (GOS), and kefiran (grains matrix), and they can enhance the growth of beneficial gut bacteria and improve digestion [55, 56]. The conventional substrate is typically cow milk with 3.5% fat, and the fermentation process consists of 2–3 days of fermentation at 18–25°C. However, more unconventional substrates are being explored due to lactose intolerance and other digestive issues. Due to the microbial diversity of grains, multiple unconventional substrates can be used, such as goat, sheep, buffalo, and camel [57, 58], bovine colostrum [59], or plant-based milk like coconut, almond, soy, rice, and oat [59].

Each type of milk has specific chemical and organoleptic properties. For example, cow milk is rich in proteins, fats, and lactose, which support robust fermentation. It also contains vitamins and minerals like calcium and vitamin D [60, 61]. Goat milk is easier to digest due to its protein structure, contains less lactose, and is often tolerated by those with mild lactose intolerance [43]. Sheep milk is high in protein and essential fatty acids [62]. Buffalo milk contains more protein and fat than cow's milk and is known for its thick and creamy texture [58]. Camel milk contains unique proteins, vitamins A, B2, E, and C, and minerals Ca, Na, K, Zn, Mg, and Fe [63]. Colostrum is an excellent substrate. It contains essential nutrients such as proteins, fat, lactose, vitamins, and minerals. In addition, it plays a fundamental protective role with its antimicrobial substances, including immunoglobulins, lactoferrin, lactoperoxidase, lysozyme, and cytokines. Fermentation also improves the preservation of colostrum, making it a good source of bioactive peptides [64–66].

As for plant-based milk, coconut milk contains healthy fats like medium-chain triglycerides (MCTs) [67]. Almond milk is low in calories, has high fiber content, and is a good source of vitamin E and polyphenols [68]. Soy milk is high in protein and a good source of isoflavones aglycones [69]. Rice milk is hypoallergenic and suitable for those with nut or dairy allergies [70]. Oat milk contains beta-glucans, which are beneficial for health [71].

Plant-based milk can also be used for water kefir grain fermentation. However, the conventional substrate for water grains (6–20%) is water with sugar (6–30%), and figs are used for 24–48 h at 20–25°C [72]. Other unconventional substrates for water grains are fruit juices (apple, orange, grape/wort, red pitaya) [21, 73, 74], legume juices (red beetroot, black beans) [41, 75], or herbal infusions and dried fruits such as raisins, plums, or dates [76]. It is essential to consider the kind of fruit,

how it was processed (such as dried), whether preservatives were used, and whether the fruit contains any microorganisms that could contaminate the water kefir, such as enterobacteria or *Pseudomonas* spp. strains. Additionally, it is suggested that the high sucrose concentration and the impact of osmotic pressure on microorganisms are the reasons for the sluggish fermentation process—furthermore, various sucrose sources, such as honey [20].

The main component of the water kefir grain matrix is dextran, a bacterial-derived exopolysaccharide (EPS) synthesized by indigenous lactic acid bacteria, especially by strains of *Liquorilactobacillus* spp. and *Levilactobacillus* spp. [77, 78]. Dextran is a bioactive molecule with great potential to be considered a prebiotic [18]. Dextran has various industrial applications: it is used as a blood plasma substitute, viscosifier, stabilizer, gelling agent, sweetener, and emulsifier in food production, as well as in the preparation of bakery products [79].

Understanding the health benefits of fermented foods and beverages is closely tied to the concepts of probiotics, postbiotics, and paraprobiotics. These terms signify different stages of beneficial microorganisms and their byproducts.

Artisanal cultures are shaped by various factors such as their source, origin, temperature, fermentation substrate (e.g., tea, milk, whey, colostrum, or water), and initial ingredients (e.g., sugar, honey, figs, or raisins). These factors can lead to changes in the microbial community, producing a varied range of metabolites or compounds with distinct activities [39, 80–83].

Regarding probiotics, it can be noted that kombucha commonly contains strains of *Lactobacillus* spp. and *Bifidobacterium* spp. [84]. In a recent study, Majid et al., fermented kombucha using butterfly pea flower as a substrate for probiotic growth. The probiotic strain *Lactiplantibacillus plantarum* at a concentration of  $10^6$  CFU/mL was added to enhance the probiotic effect and the organoleptic qualities. The organoleptic properties were improved, acetic bacteria and yeast metabolism were stimulated, and lactic acid production was increased [85]. In contrast, milk kefir grains contain diverse probiotic microbiota, including *Lactobacillus* spp., *Lactococcus* spp., and *Leuconostoc* spp. strains. Water kefir grains also contain similar probiotic bacteria adapted to thrive in a non-dairy environment [86]. All consortia contain the probiotic yeast *Saccharomyces* spp. strains. **Table 1** presents the metabiotic potential of certain strains from artisanal starter cultures microbiota.

In terms of postbiotics, kombucha fermented on teas contains organic acids (acetic and gluconic), vitamins (B complex and vitamin C), and polyphenols [83], while milk kefir contains bioactive peptides, exopolysaccharides (kefiran), and a variety of vitamins [9]. Fermented products (fermented on water or juices) obtained with water kefir grains contain organic acids (such as lactic acid), vitamins (B complex and vitamin C), and exopolysaccharides [101, 102].

Bioactive peptides, organic acids, bacteriocins/beta-glucans, and vitamins have been the most researched postbiotics in recent years.

Elkhtab et al. [103], observed that milk fermented with 1% kombucha beverage produced ACE (Angiotensin-I Converting Enzyme) inhibitory peptides and other peptides with low IC<sub>50</sub> values. The milk proteins may be the source of these peptides, but it is also worth considering the potential *de novo* synthesis of these peptides by the SCOBY microbiota [103].

Other studies have shown that products obtained using kefir grains for fermentation of cow's milk have antihypertensive properties and can reduce ACE activity *in vivo* as the effect of the 35 ACE inhibitor bioactive peptides detected by proteomics [104].

| Class of Microorganisms | Strains names according to the new taxonomy                                                                         | Source                      | Metabiotic                                                    | Properties                                                                                                                                          | Ref.     |
|-------------------------|---------------------------------------------------------------------------------------------------------------------|-----------------------------|---------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Lactic acid bacteria    | <i>Lactiplantibacillus plantarum</i>                                                                                | Kombucha tea                | Bacteriocin, SLG10                                            | Antibacterial activity against Gram-positive and Gram-negative bacteria, including multidrug-resistant strains.                                     | [87]     |
|                         | <i>Pediococcus pentosaceus</i>                                                                                      | SCOBY                       | Strain with the probiotic potential,                          | Bacteriocin production and high tolerance to bile salts.                                                                                            | [88]     |
|                         | <i>Lactobacillus harbinensis</i> ,<br><i>Lactocaseibacillus paracasei</i> ,<br><i>Lactiplantibacillus plantarum</i> | Kefir from milk kefir grain | Strains with the probiotic potential.                         | Survive at low pH, tolerance to bile salt, and ability to adhere to HT-29                                                                           | [89]     |
|                         | <i>Lactocaseibacillus rhamnosus</i> ,<br><i>Lactocaseibacillus paracasei</i>                                        | Milk kefir grain            | Strain with the probiotic potential.                          | antagonistic activity against multidrug-resistant Gram-negative bacteria ( <i>E. coli</i> , <i>P. aeruginosa</i> and <i>Klebsiella pneumoniae</i> ) | [90]     |
|                         | Multiple strains                                                                                                    | Water kefir grain           | Symbiotics and synbiotics.                                    | The removal of aflatoxin AFB1 by absorption and biotransformations.                                                                                 | [91]     |
| Acetic acid bacteria    | <i>Lactocaseibacillus paracasei</i>                                                                                 | Water kefir grain           | Superoxide dismutase and glutathione peroxidase biosynthesis. | Regulation of the expression of several genes related to glucose homeostasis and metabolism of lipids.                                              | [92, 93] |
|                         | <i>Lentilactobacillus hilgardii</i>                                                                                 | Water kefir grain           | Exopolysaccharides such as dextran.                           | Prebiotic effect.                                                                                                                                   | [94]     |
|                         | <i>Komagataeibacter</i> sp.                                                                                         | Kombucha tea                | Bacterial cellulose-producing.                                | Prebiotic effect.                                                                                                                                   | [95]     |
|                         | <i>Gluconacetobacter xylinus</i>                                                                                    | Kombucha tea                | Bacterial cellulose-producing                                 | Prebiotic effect.                                                                                                                                   | [96]     |
|                         | <i>Gluconobacter oxydans</i>                                                                                        | Water kefir                 | Antimicrobials                                                | Inhibitory effect against human enteric pathogens.                                                                                                  | [86]     |
|                         | <i>Acetobacter</i> spp.                                                                                             | Milk kefir grains           | Strain with the probiotic potential.                          | Probiotic effect and cytotoxicity against cancer cell lines.                                                                                        | [97, 98] |

| Class of Microorganisms | Strains names according to the new taxonomy | Source             | Metabiotic                                   | Properties                                                                                                                                                                                                                                                                                        | Ref.  |
|-------------------------|---------------------------------------------|--------------------|----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Yeasts                  | <i>Zygotritilaspova florentina</i>          | Water kefir grains | Source of nutrients in fermentation process. | Provide essential amino acids for lactobacilli.                                                                                                                                                                                                                                                   | [99]  |
|                         | <i>Kluyveromyces marxianus</i>              | Milk kefir grains  | Strain with the probiotic potential.         | The cholesterol-lowering effects (30% cholesterol reduction rate) and ability to adhere to Caco-2 cells (5.3 log CFU/well)                                                                                                                                                                        | [100] |
|                         | <i>Saccharomyces cerevisiae</i>             | Kombucha tea       | Strain with the probiotic potential.         | High levels of antioxidant activities, cell surface hydrophobicity (64.60–83.87%), co-aggregation, resistance to eight tested antibiotics (ampicillin, chloramphenicol, colistin sulfate, kanamycin, nalidixic acid, nitrofurantoin, streptomycin, and tetracycline) and auto-aggregation (>98%). | [14]  |

**Table 1.**  
*Metabiotics produced by the microorganisms from the artisanal cultures microbiota.*

Bioactive peptides were also obtained by fermenting a medium based on tomato seed and protein isolate with the water kefir grains for 24 h at 37°C. This might be explained by the grain's microbiota's capacity to break down and transform the proteins from tomato seeds into bioactive peptides [105].

Other peptides, such as bacteriocins, are produced by strains of *Lactobacillus* spp. present in artisanal cultures. For example, *Lactiplantibacillus plantarum*, found in kombucha products, can produce bacteriocin SLG10. This compound exhibits antibacterial activity against both Gram-positive and Gram-negative bacteria and can also inhibit *Staphylococcus aureus* biofilms [87]. Bacteriocins weighing between 1 and 10 kDa with bacteriostatic effects are produced by bacterial strains found in milk kefir. FK-1000, an antimicrobial compound from milk kefir fermented with grains consisting of phenylalanine, alanine sequences, monosaccharides, and tyrosine, listed a bactericidal effect against weak-acid-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains at pH 5.0, and bacteriostatic against both pathogens at pH 7.0 [106].

Polyphenols, organic acids, and vitamins are the main postbiotics in fermented kombucha beverages. The most abundant organic acid in kombucha is acetic acid (1.55 g/L), followed by tartaric and citric acids. In terms of mineral compounds, kombucha contains the highest amounts of manganese (1.57 mg/L) and zinc (0.53 mg/L) [107]. Additionally, copper, iron, nickel, and cobalt were also detected. As for vitamin content, kombucha contains vitamin C (1.51 mg/mL), B<sub>12</sub> (0.84 mg/mL), B<sub>6</sub> (0.52 mg/mL), and B<sub>1</sub> (0.74 mg/mL) [108].

The inhibitory properties of milk kefir obtained by fermentation with the milk kefir grains are due to organic acids and bioactive peptides. The lactic, acetic, and citric acids were detected. Glutamic acid, histidine, serine, tyrosine, alanine, methionine, and lysine were also identified [58]. During the fermentation process, levels of vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>5</sub>, B<sub>6</sub>, B<sub>12</sub>, K, folic acid, calcium, and amino acids are increased [109].

Water kefir fermented products contain many postbiotics. For example, citric, gluconic, succinic, lactic, and acetic acids were detected in fruit water kefir [106]. Also, the complex of B vitamins is predominant in water kefir [20].

Ultimately, it's important to note that when a fermented product is subjected to pasteurization or another process to destroy the living microbes, the remaining nonviable cells and nonviable intact or cell fragments are paraprobiotics [110, 111]. These components can interact with the host's immune system and provide health benefits. Pasteurized kombucha, milk kefir, and water kefir contain beneficial microbial cell components, cell wall components, metabolites, and microbial fragments [110, 112–114].

The kombucha microbiota inactivation process includes heat treatment, which means pasteurizing at 65–75°C for 15–30 minutes. According to Jayabalan et al., the heat treatment at 60–90°C for 1 min produces total inactivation of bacteria (from  $15 \times 10^3$  CFU mL<sup>-1</sup>) and yeast (from  $7 \times 10^6$  CFU mL<sup>-1</sup>). Analyzing heat-treated kombucha beverages for 90 days, it was observed that the tea's constituents degraded nonlinearly, and its ability to scavenge free radicals decreased linearly [115]. Alternatively, other methods, such as UV irradiation or high-pressure processing, can be used [116]. The research of Podolich et al., provides information on how UV radiation affects the ability of bacteria and yeasts within the kombucha microbial community (KMC) to survive in simulated spaceflight and Martian conditions. It was observed that under stressful conditions of higher-dose UV irradiation (1000–10,000 J/m<sup>2</sup>), the KMC diversity became unstable. The crucial point for the survival of the KMC was exposure to UV doses higher than 10,000 J/m<sup>2</sup>. Some microbial organisms entered a dormant and uncultivable state at these high doses. Only *Bacillus* spp. from kombucha could tolerate up to a 10,000 J/m<sup>2</sup> dose [117]. In the case of

High-Pressure-Pasteurization (HPP), it exposes the fermented product to extremely high pressures, ranging from 400 to 600 MPa, which inactivates microorganisms present in the beverages. This process maintains the kombucha beverage flavor, vitamins, antioxidants, and fresh-like qualities, often lost in thermal pasteurization [111].

The inactivation process for milk kefir grains and their products includes heat treatment. Pasteurizing the milk before kefir production was made by McGovern et al. at 62°C for 30 min [118]. Other studies say that equol (the main bioactive metabolite in milk) content is not affected by pasteurization at 65°C for 30 min [119]. Also, the pasteurization of milk at 95°C for 10 min decreased the  $\alpha_s$ -,  $\beta$ -, and  $\kappa$ -casein content, while the percentages of  $\gamma$ -casein and peptides were higher. While the concentrations of immunoglobulin,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin remained unchanged [120]. Also, kefir grains can be freeze-dried or dehydrated to inactivate the microbes while preserving cell structure. Conde-Islas et al., freeze-dried kefir grains for preservation at -20°C for 3 h, at 0.6 bar pressure for 15 h. The dried milk kefir grains had a survival rate of 8.5 logs for lactic acid bacteria and 8.6 logs for yeasts [121].

The inactivation process for water kefir grains or their products involves heat treatment. Pasteurizing the water kefir, heating to 65–75°C for about 15–30 min, destroys the microorganisms. Drying can also be used by freeze-drying or dehydrating the water kefir to achieve inactivation while maintaining the structural integrity of the microorganisms [122, 123]. A study by Calatayud et al., found that pasteurizing water kefir at 72°C for 3 min has protective effects on inflammation-induced intestinal barrier disruption. This is because pasteurized products have the highest modulatory activity on gut microbiota and an increased release of bioactive molecules derived from water kefir yeast and bacteria during inactivation. Pasteurized water kefir has also been shown to enhance intestinal barrier function and promote the production of the anti-inflammatory cytokine IL-10. Microbial metabolites and yeast-derived molecules may be involved in upregulating IL-10, crucial for maintaining mucosal tolerance and reducing gut inflammation [110].

Heat-treated kombucha retains its beneficial acids and enzymes, while inactivated milk and water kefir maintain their bioactive peptides and polysaccharides [115, 124].

### **3. Conclusions**

Through their microbial complexity, artisanal cultures represent valuable starter cultures able to produce a large diversity of fermented products through controlled fermentations. These products have functional properties that have great physiological effects on hosts, expressed by the boost of the immune system, reduction of inflammation, and promotion of a healthy balance of gut bacteria, which has beneficial effects on the gut-brain axis.

These effects are induced by all metabiotics (pre-, pro-, post-, and paraprobiotics) derived from the substrate' composition, diversity, and the metabolic activity of the complex microbiota (symbiotics) and the control of the biotechnological parameters (intrinsic, extrinsic, and biological).

Known and utilized for a long time ago, artisanal starter cultures (kefir grains, SCOBY membranes) are being researched based on their complex bioactive potential, large capacity for adaptation, and protection against microbial contamination. In this context, using the milk and water kefir grains and SCOBY membranes to produce foods, feeds, ingredients, nutraceuticals, or cosmeceuticals rich in active biotics offers

new challenges to the production of the goods with an impact in increasing the life quality and natural resources valorization, especially by intelligent and fast biotransformation of the agri-food byproducts.

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## **Conflict of interest**

The authors declare no conflict of interest.

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# The Role of Inulin in Human Health and Sustainable Food Applications

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## Abstract

Inulin, a naturally occurring prebiotic soluble fiber, has been a staple in our diets since prehistoric times. It has been identified in over 36,000 plant species- including chicory, Jerusalem artichoke, onion, garlic, bananas, and agave. The polysaccharide has gained recognition for its positive effect on gut microbiota via fermentation and cultivation of gut healthy bacteria. However, inulin has also exemplified numerous significant health benefits beyond the gut including weight loss, improved glucose and lipid metabolism, increased mineral absorption, immunomodulator effects. Additionally, the robust, versatile, and prevalent nature of inulin offers opportunities to ameliorate growing challenges in sustainability. This chapter sheds light on the role of inulin as a prebiotic, including its impact on gastrointestinal and overall health; as well as its applications in fostering sustainability, particularly in the food, socio-economic, and environmental sectors.

**Keywords:** inulin, gut microbiota, prebiotic, sustainability, fermentation

## 1. Introduction

Within the corridors of the gastrointestinal tract resides a lively ecosystem, known as the gut microbiota. This biome plays a crucial role in maintaining health and preventing disease [1, 2]. Advancements in research have shown that diet significantly affects these microbial communities [3].

Prebiotics are non-digestible dietary fibers that selectively promote beneficial bacteria in the gut, such as *Bifidobacteria* and *Lactobacilli* [4]. Unlike probiotics, which are foreign live microorganisms that are then ingested; prebiotics act as substrates to nourish already residing beneficial microbes present in the gastrointestinal tract. Although prebiotics have silently played a crucial role in human nutrition since ancient times, the definitive term of prebiotic was first introduced in 1995 by Marcel Roberfroid and Glenn Gibson; defining substances that induced a growth or activity of beneficial microorganisms in the gut [5]. In the compass of prebiotics, one of the most highly researched topics is inulin.

This review aims to elucidate the growing significance of prebiotics such as inulin in the context of health management practices. Moreover, this chapter will explore

the benefits of inulin beyond simply the context of healthcare; to encompass escalating concerns of public health, sustainability, and economic adversities.

## 2. Role as prebiotic and applications in health

One of the many ways inulin exerts a positive influence on the gut microbiome is by cultivating beneficial bacteria. It accomplishes this through numerous mechanisms including selective fermentation, production of short chain fatty acids, supplying nutrients, establishing a competitive advantage regarding beneficial bacteria; in effect wholly improving the gut microbial population, and gut barrier [6, 7].

Prebiotics, such as inulin, may even be considered superior to probiotics in certain facets. These benefits lie chiefly in the character of the prebiotic as a nondigestible fiber; serving as a food source for further stimulation and growth of local beneficial gut bacteria. In doing so, it creates a more stable and diverse microbiota, that is sustainable over a longer period. Probiotics, on the other hand, introduce new bacteria which could immediately boost certain bacterial strains; however, the effects may be short-lived and require continuous intake to maintain beneficial effects [8]. Additionally, prebiotics can survive the digestive process reaching the colon intact. Probiotics may be more sensitive to the acidic environment, leading to a lower number reaching the intestines [9]. Moreover, probiotics carry the risk of bacteremia, sepsis or endocarditis, particularly in the immunodeficient population. Of note these risks have not been reported in studies with prebiotics [10]. This is likely due to their slower mechanism of action on resident local bacteria rather than introduction of foreign microbes.

Beyond its influence on the gastrointestinal biome, inulin contributes to vitamin synthesis, absorption, weight management; and glucose and lipid metabolism. It has also been studied for its potential role in secondary cancer risk reduction, via its immunomodulatory and anti-inflammatory properties [1, 2]. Other studies, regarding the gut-brain pathway, suggest that gut dysbiosis may translate to mood, behavioral, and cognitive disorders [1, 11].

### 2.1 Fostering beneficial gut bacteria

Inulin resists digestion in the upper GI tract. Its molecular structure-  $\beta$ -(2- > 1) glycosidic bonds- is largely unaffected by salivary amylase and pancreatic enzymes like  $\alpha$ -amylase and maltase [9, 12]. This is an anticariogenic benefit. When inulin reaches the colon, it is selectively fermented by beneficial bacteria like *Bifidobacteria* (bifidogenic effect) and *Lactobacilli*. These bacteria possess the enzymes required to break down inulin into simpler compounds that can be utilized for growth and energy [13]. Specifically, *Lactobacillus* and *Bifidobacteria spp* contain an enzyme called inulinase, a  $\beta$ - fructofuranosidase, that breaks down inulin into fructose by cleaving the  $\beta$ -2,1-glycosidic bonds [14–16]. Through selectively promoting the proliferation of these beneficial bacteria, inulin helps beneficial bacteria outnumber and establishes a competitive advantage against pathogenic bacteria [17].

### 2.2 Gut barrier

In addition to preventing diseases by diminishing the pathogenic bacteria population, the beneficial bacteria stimulated by inulin also enhance the gut barrier integrity,

specifically the mucosal lining. This fortified gut barrier prevents the passing of harmful substances and pathogens which can contribute to cancer development and illnesses. *Lactobacillus* spp. strengthen the mucosal barrier by promoting tight junction (claudin-1, zonula occludens-1 and occludin) integrity, and in doing so, reduce permeability, and prevent pathogen or toxin entry [18, 19]. On the other hand, the *Bifidobacterium* species enhance gut barrier integrity by producing short chain fatty acids- acetate, butyrate. Of note, butyrate is produced indirectly through the bifid shunt. The byproduct of this shunt, lactate, is utilized by other bacteria like *Eubacterium Halii* and *Anaerostipes caccae* to produce butyrate [20, 21]. These SCFAs aid in health maintenance of intestinal epithelial cells and support immune responses in the gut.

### 2.3 Short chain fatty acids

Short chain fatty acids (SCFAs) have many benefits. They serve as a major source of energy for colonocytes, help maintain the integrity of the gut barrier; and hold anti-inflammatory properties [7, 22].

Inulin produces SCFAs through fermentation in the colon by *Bifidobacteria* and *Lactobacilli* [23]. After the enzymatic breakdown of inulin into simpler sugars, the bacteria metabolize these compounds through various anaerobic fermentation pathways; of which the primary fermentation products are SCFAs—acetate, propionate, butyrate. The specific SCFA produced depends on the bacterial species. Acetate is the most abundantly produced SCFA that is involved in influencing gut pH and fostering beneficial gut bacteria. Propionate can be absorbed through the bloodstream and has effects in glucose, lipid metabolism and improved insulin sensitivity [24–27]. Butyrate is typically produced by “cross-feeder” bacteria, *Anaerostipes spp* and *Eubacterium Halii*, through metabolization of *Bifidobacteria*’s major end products acetate and lactate [19].

SCFAs provide significant energy for colonocytes. This energy is utilized in enhancing performance of colonocytes, maintaining the integrity and function of the gut barrier, promoting healthy cell growth, and reducing the risk of conditions like leaky gut syndrome. SCFAs additionally support the barrier function through their capability of enhancing the expression of tight junction proteins, which helps restore the epithelial barrier function that is often compromised in diseases such as inflammatory bowel disease (IBD) [18, 19]. SCFAs also decrease pH; creating an acidic colon environment that is hostile to pathogenic bacteria and favors beneficial bacteria. Moreover, SCFAs play a feature role in immunomodulation. In response to stimulus markers like concanavalin-A and immobilized anti-CD3 monoclonal antibodies, SCFAs stimulate T cells, cytokines, and regulatory T cells. Through this, SCFAs prevent the migration and over-activation of lymphocytes, thereby reducing excessive immune activation and colonic inflammation [28].

SCFAs, particularly butyrate, have anti-inflammatory properties. Through inhibition of the activation of nuclear factor kappa B (a key character of the inflammation pathway), SCFAs reduce the production of pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ), and interleukin-6 (IL-6) [22].

Clinically, the mechanisms of SCFAs have been employed to manage various GI conditions such as IBD (Crohn’s disease, ulcerative colitis); and diversion colitis. Notably, clinical research has revealed low total SCFA levels ( $p = 0.014$ ); and low butyrate producing bacteria ( $p < 0.0001$ ) in ulcerative colitis; highlighting SCFAs pivotal role in chronic inflammatory processes. More pertinently, this conveys the potential of inulin supplementation as adjunctive therapy in IBD conditions [29].

Studies have been conducted regarding SCFAs' role in reducing carcinogenic compounds. There are several mechanisms which have been postulated to do this, including many we have already discussed: reducing the pH (inhibiting harmful bacteria which produce carcinogenic byproducts); anti-inflammatory effects (chronic inflammation has been linked to cancer development); providing energy to colonocytes (maintain energy for gut barrier integrity and prevent exposure from translocation of malignant compounds); cellular signaling (ex: HDAC inhibition [which can stimulate and employ the inhibitory role of regulatory T cells]); regulating gene expression and inducing apoptosis for normal cell turnover [28].

## **2.4 Weight loss and modulation of lipid, glucose metabolism**

Inulin has been linked with weight loss through several potential mechanisms including decreased caloric intake, increasing satiety, modulating hunger hormones, enhancement of gut health, improving insulin sensitivity, reducing fat absorption, promoting thermogenesis, and long-term effects on body composition. Inulin is postulated to be about 2.5 kcal (potentially overestimated as inulin and other NDFs' interactions with digestion of proteins and fats, has been posited to decrease the caloric value of the meal as a whole) [30–32]. Inulin forms a gel-like substance in the digestive tract which: through distension, stimulates vagal receptors in the gut and aids in promoting feelings of fullness and satiety; and through the morphology of the gel-like substance itself, aids in slowing down carbohydrate absorption (in turn preventing blood glucose spikes) [32]. This reduces overall food intake and controls appetite which can contribute to weight loss [33]. Clinical trials have found that oligofructose (a subgroup of inulin) may influence hormones involved in appetite regulation; specifically decreasing ghrelin and increasing peptide YY (PYY) [24]. Ghrelin is a peptide hormone which is simply known as the “hunger hormone” because it stimulates appetite. Hence blood levels are highest before meals. PYY, on the other hand, suppresses appetite. Inulin, by reducing ghrelin levels and increasing PYY levels, reduces appetite and in turn promotes weight loss. Additionally, the study showed, in comparison to placebo, reduction plasma glucose and insulin levels in individuals with obesity. A randomized controlled clinical trial showed significant reduction in fasting blood sugar, HbA1c, total cholesterol, triglyceride, LDL-c, and increased HDL-c, further positing improvement of the metabolic profile with inulin supplementation [34].

Some clinical trials have shown a significant increase ( $p = 0.003$ ) in GLP-1 secretion with inulin supplementation which supported additional metabolic and comorbidity benefits including improved diastolic blood pressure, reduced waist circumference, and reduced fasting blood sugar [35, 36]. Additionally, GLP-1 agonists, such as Ozempic, have been increasingly used to promote weight loss and glucose metabolism. GLP-1 is secreted from the L-cells in the intestine which increases insulin secretion and reduces glucagon secretion, thereby reducing insulin resistance and stabilizing blood sugar levels in diabetic patients. GLP-1 is also involved in appetite regulation by acting on the central nervous system. Inulin induced increases in GLP-1 can promote feelings of satiety and reduce food intake by activating anorexigenic neurons in the hypothalamus [37]. This action helps in managing body weight and reducing obesity-related complications. GLP-1 promotes the sustenance of pancreatic beta cells (via stimulation of cAMP, insulin receptor substrate-2 activity, and finally Akt, which is responsible for insulin movement from the intracellular compartment to the cell membrane by GLUT transporters, where it proceeds with its function of

glucose uptake) [38]. This dual action helps in maintaining effective glucose control and improving metabolic health.

Through the production of SCFAs, especially butyrate, inulin has been linked with improved insulin signaling and sensitivity. This is done through activation of G-protein coupled receptors (GPCRs). SCFAs bind to receptors like GPR41, GPR43 and GPR109A which increase insulin sensitivity through their effects on inflammation and energy metabolism pathways [39]. SCFAs are associated with lower fasting insulin levels and improved Homeostatic Model assessment for Insulin Resistance (HOMA-IR) which is indicative of better insulin sensitivity. Inulin additionally may aid in weight loss through binding with bile acids; thereby promoting their excretion and reducing fat absorption [40]. This process would also lower cholesterol levels. SCFAs have also been linked to promote thermogenesis by stimulating brown adipose tissue, increasing energy expenditure and thermogenesis [41]. Inulin has been linked with changes in body composition. Through the apparent effect of increasing satiety, reducing overall calorie intake, inulin contributes to better weight management and a decrease in obesity related complications [42].

## 2.5 Mineral absorption

The bioavailability of calcium in our current food products is insufficient. For example, the bioavailability of calcium from dairy products is estimated to be around 30–35%. Lamentably, for individuals with dietary restrictions, the option of calcium from vegetables- like spinach, kale, and broccoli- contain calcium bound to oxalates, which can form insoluble salts and diminish calcium absorption. The bioavailability of calcium from these sources is much lower, generally around 5–15% [43].

Many studies support inulin's influence on mineral absorption. One study showed daily consumption of inulin, over 8 weeks and 1 year time periods, significantly increased calcium absorption and enhanced bone mineralization during pubertal growth [44]. Another study found increased calcium absorption over a 3-week period with inulin supplementation in normocalcemic young females at or near menarche [45]. Inulin supports mineral absorption through increasing passive diffusion, solubility and bioavailability, enhancement of gut microbiota, improvement of intestinal barrier function, ion exchange, modulation of intestinal transporters, and effects of the byproducts of fermentation [28, 46–51].

Inulin is composed of multiple hydroxyl groups (-OH)- which allows for chelation and formation of complexes with calcium ions ( $\text{Ca}^{2+}$ ) [22]. This complex formation helps keep calcium ions in a soluble form throughout the digestive process; and increase bioavailability and absorption. Advancements in biotechnology have already developed modified inulin, in the form of calcium phosphorylated inulin complex. These groups contain atoms with negative charges which are then able to form additional, fortifying bonds with  $\text{Ca}^{2+}$  ions. The extra phosphate groups themselves allow for enhanced chelation and stability through additional interactions. Moreover, the phosphate groups can contribute to the overall hydrophilicity of the molecule, improving solubility and stability [52]. Additionally, inulin (through SCFAs) increases calbindin levels and influences transcellular active calcium transport [46]. Several animal studies report an increase in calcium and magnesium with inulin and/or SCFA supplementation; some also note an increase in transporters TRPV6, Calbindin-D9k, PMCA1b [47–51]. Additionally, in 2007, a study by Abrams et al., posulated that with inulin supplementation, increased calcium absorption occurred in the colon, rather than the small intestine [53]. TRPV6 is highly expressed in the colon

of humans, rats, and mice [54, 55]; with other studies also noting increased mRNA expression levels of TRPV6 in the colon [48]. This raises further support regarding the colonic influence of inulin (through its byproduct SCFA) in increasing calcium absorption through increased transporter activity in the colon; as well as increased overall solubility of calcium due to reduced environmental pH.

## 2.6 Vitamins

These beneficial bacteria also assist in the synthesis of essential vitamins- primarily Vitamin B, and K. These vitamins are crucial in cognition, energy production, fat and amino acid metabolism, DNA repair, hemoglobin synthesis, coagulation, and much more. Many studies have linked reduced levels of vitamin B6, B12 with development of GI cancers [56–58].

Other studies demonstrate *Lactobacilli*'s ability to produce SCFA and vitamins such as B2, B9, B12. Additionally, the research demonstrates that various *Lactobacillus* isolates produced more or less of certain vitamins. For example, KGL3A isolate produced the most B2, while WTS4 produced the highest amounts of folate [59]. This supports the advantages of fostering a diverse microbiome with inulin or other prebiotics; as opposed to pre-formed, limited strains available in probiotics.

In addition to inulin aiding in the synthesis of vitamins through stimulation of various beneficial bacterial strains; inulin also supports absorption through its other functions. These previously discussed mechanisms include fortifying the tight junction integrity to allow for efficient nutrient transport while preventing pathogen entry; and preventing inflammation. Inflammation damages areas of absorption and decreases surface area for absorption. Vitamin deficiencies due to inflammation are apparent in IBD patients. Inulin prebiotic supplementation may be pivotal for patients suffering from vitamin deficiencies due to IBD, leaky gut, short bowel syndrome, post resection, post radiation, restricted diets, autoimmune disorders, infections (with *Helicobacter Pylori* or *Clostridium Difficile* which cause increased intestinal permeability), or aging (also linked to increased tight junction permeability); among others [60].

## 2.7 Colorectal cancer

Inulin can lower the risk of colon cancer through its beneficial effect on SCFAs. Butyrate, a SCFA, induces apoptosis in colon cancer cells, stimulating colon motility, reducing inflammation, and inhibiting tumor cell progression [22]. Butyrate potentially induces apoptosis through the JNK MAP kinase pathway; and histone deacetylase inhibition, which leads to hyperacetylation of histone proteins [61]. This change in histone acetylation affects gene expression involved in cell cycle regulation and apoptosis, promoting the expression of pro-apoptotic genes such as P21, WAF-1/CIP1; while down regulating anti-apoptotic genes such as BCL-2 [52]. This alteration facilitates apoptosis of cancer cells by disrupting mitochondrial function and activating the caspase cascade. As aforementioned, butyrate induces apoptosis through the mitochondrial pathway by reducing the mitochondrial membrane potential. This reduction triggers a release of cytochrome C from the mitochondria into the cytosol where it forms complexes with apoptotic protease activating factor-1 (apaf-1) and caspase-9, which activates caspase-3 leading to execution of apoptosis [62, 63]. Butyrate also elevates the levels of reactive oxygen species which further damages the mitochondrial membrane, enhances the release of cytochrome C and promotes

apoptosis via the cytochrome C/caspase- 3 pathway [64–66]. Butyrate interacts with specific GPCRs (GPR41, GPR43), modulating signaling pathways that influence cell survival and apoptosis. GPCRs are also known as free fatty acid receptors (FFAR). Of note, FFAR 2/3 has been found significantly in colon regulatory T cells (T reg) compared to other tissues; suggesting activation of these receptors can inhibit pathways such as NF- $\kappa$ B (which is involved in inflammation and cell survival) thereby promoting apoptosis [67]. Activation of GPCRs by short-chain fatty acids can reduce inflammation in the colon which is a major risk factor for colon cancer. GPR43 enhances the production of mucus and tight junction proteins, which protect the gut lining from wandering harmful pathogens and toxins that could induce carcinogenesis [22].

Through epigenetic modulation, butyrate can alter acetylation of histone and non-histone proteins. In this way, butyrate can modulate gene expression patterns involved in apoptotic signaling and the immune response [61].

| Application                             | Characteristic/Role                                                                                                                                                                                                                                                                                                                                                                 |
|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gut bacteria                            | <ul style="list-style-type: none"><li>• Production of beneficial bacteria like <i>Bifidobacteria</i> and <i>Lactobacilli</i> [13]</li><li>• Competitive advantage [17]</li><li>• Positive effects as below, notably thru SCFA byproducts [23]</li></ul>                                                                                                                             |
| Gut barrier                             | <ul style="list-style-type: none"><li>• <i>Lactobacillus</i> involved in tight junction integrity [18, 19]</li><li>• Bacteria produce SCFAs which maintain intestinal epithelial cells and support immune responses in the gut [23]</li></ul>                                                                                                                                       |
| Short chain fatty acids                 | <ul style="list-style-type: none"><li>• Serve as sources of energy for colonocytes, help maintain the integrity of the gut barrier, create an acidic environment; hold immunomodulatory, and anti-inflammatory properties [7, 22]</li></ul>                                                                                                                                         |
| Weight loss                             | <ul style="list-style-type: none"><li>• Decrease caloric intake, increase satiety, modulate appetite hormones, enhance gut health, improve insulin sensitivity, reduce fat absorption; promote thermogenesis, and long-term effects on body composition [30, 32]</li></ul>                                                                                                          |
| Modulation of lipid, glucose metabolism | <ul style="list-style-type: none"><li>• Lipid: through weight loss, as above; reduced LDL-c, and increased HDL-c. [30, 32, 34].</li><li>• Glucose: through weight loss, as above; as well as increased GLP-1 [35, 36]; activation of G-protein coupled receptors (GPCRs) [39]</li></ul>                                                                                             |
| Mineral absorption                      | <ul style="list-style-type: none"><li>• Multiple hydroxyl groups (-OH) which allows formation of complexes with calcium ions (Ca<sup>2+</sup>) [22]</li><li>• Calcium complexes stay in a soluble form; with increased bioavailability and absorption [22, 52]</li><li>• Increases calbindin levels [46] and influences transcellular active calcium transporters [47–51]</li></ul> |
| Vitamins                                | <ul style="list-style-type: none"><li>• <i>Lactobacilli</i>: produces vitamins such as B2, B9, B12. [59]</li><li>• Tight junction integrity: efficient nutrient transport [18, 19]</li><li>• Anti-inflammatory [22]; Inflammation damages areas of absorption and decreases surface area for absorption.</li></ul>                                                                  |
| Colorectal cancer                       | <ul style="list-style-type: none"><li>• Induces apoptosis at a cellular level. Various pathways, including acetylation in malignant cells, JNK-MAP pathway, mitochondrial pathway [61–63]</li><li>• Through promotion of healthy gut microbiome; reduced inflammation [68–70]</li></ul>                                                                                             |

**Table 1.**  
*Summary of the role of inulin in health applications.*

Inulin and its byproducts prevent dysbiosis by maintaining a healthy gut microbiota. Dysbiosis is a condition linked to colon cancer due to chronic inflammation. Pathogenic bacteria- which can produce proinflammatory compounds such as lipopolysaccharides- can trigger cyclical inflammatory responses, causing DNA damage and carcinogenesis. It is noteworthy that approximately 20% of cancer diagnoses have been linked to chronic inflammation [68–70]. Similarly, some pathogenic bacteria produce secondary bile acids such as deoxycholic acid and lithocholic acid (through fermentation of primary bile acids, such as: cholic acid, chenodeoxycholic acid) which have been shown to damage DNA and promote tumor formation [71]. Other harmful bacteria have been known to produce toxins such as colibactin which directly induces DNA damage [72]. Dysbiosis and the resulting decreased SCFAs production, can weaken mucosal variant and lead to increased permeability allowing harmful bacteria and thereby products to enter the bloodstream and surrounding tissues again promoting inflammation and carcinogenesis. Dysbiosis is also associated with epithelial to mesenchymal transition (EMT), a process where epithelial cells acquire migratory and invasive properties facilitating metastasis. Pathogenic bacteria can induce EMT by producing proteases that disrupt cell adhesion and increase cell motility [73]. A balanced gut microbiota can produce more SFAs, reinforcing the protective effects of butyrate against colorectal cancer and other malignancies (**Table 1**).

### **3. Role in sustainability**

Inulin can be found in various renewable plant sources and can contribute to growing sustainability efforts globally.

#### **3.1 Inulin plants**

The chicory root is the most common and economically significant source of inulin. It can be cultivated in most environments, and requires fewer fertilizers and pesticides compared to other crops [74]. The Jerusalem artichoke is another source of inulin; which can grow in diverse climates and soil conditions. It can be employed in crop rotation systems to enhance soil fertility, reduce the need for harmful chemical use; and thus, improve soil conditions [75]. The agave plant- more commonly associated with tequila, mezcal production- is also gaining popularity for inulin extraction. Tequila itself is sourced from the “piña,” which is the core or heart of the plant. Further use of other parts of the plant for inulin allows for reduced waste. Moreover, agave plants are highly water efficient and drought tolerant, making them well-suited for regions with limited water- like its native homeland, Mexico [76]. Yet another source is the dandelion root, from which inulin can be harvested from wild or cultivated plants. The dandelion root shines in its contribution to biodiversity and the lack of need for sensitive farming practices [77–79].

Overall, these renewable inulin sources contribute to sustainability efforts in that they are widely available, hardy, do not require intensive agricultural practices; and can be replanted and harvested annually or even more frequently- ensuring a sustainable supply of inulin. Increased focus and implementation of these plants offers additional benefits such as reduction of atmospheric carbon levels and greenhouse gasses. Their deep root systems, particularly of chicory and Jerusalem artichoke, can improve soil aeration and health. Pertinently, the multitude of plant options that

produce inulin encourages biodiversity; providing ecosystems for pollination and habitats for wildlife.

### **3.2 Antibiotic resistance**

Exposure to antibiotic raised meat can disturb the delicate ecosystem of the animal, as well as the human, gut. Resistance caused by exposure can make it difficult to treat more severe infections [80]. Prebiotic in animal feed can provide increased nutrition and the growth of beneficial bacteria; bestowing increased immuno-protective effects in livestock [79]. This, in turn, reduces antibiotic necessity in livestock farming, and antibiotic residue in the environment. Additionally, this promotes healthier, more well-nourished livestock, which can utilize feed more efficiently and have better growth rates; thereby reducing the overall resources needed for farming and minimizing waste production.

### **3.3 Synthetic additives**

Synthetic additives, including artificial sweeteners (such as aspartame, sucralose, sacralin) and fat replacements (such as Olestra, Caprenin) can have various consequences on the environment. Artificial sweeteners can be toxic to land and aquatic ecosystems. Through its toxic by-products [81], it has been linked to inducing behavioral disorders and malignancy in humans, and aquatic life [81–85]. Artificial sweeteners can also induce plant morbidity and death [86]. They have also been noted to impair nutrient cycling and soil fertility; which further diminishes plant health, agricultural product activity and ecosystem stability [87].

Inulin, with its milder taste and beneficial properties, can be employed as a natural alternative to harmful artificial sweeteners in foods and beverages. By replacing these chemicals with the biodegradable inulin, we can improve public health and reduce production and disposal of these toxins in our environment-thereby decreasing their long term (and some not fully understood) effects.

### **3.4 Plastic reduction**

Plastic presents significant detrimental disruptions to the environment through resource depletion. The extraction process from fossil fuels itself contributes significantly to greenhouse gas emissions. Notably, microplastics have been detected in more than 100 aquatic species; including those that are staple to the human diet [88]. Another disturbance is worsening waste management. This is most apparent in developing countries, where garbage management is often nonexistent due to cost [88].

Due to its biodegradability, inulin-based bio-products- such as bioplastic and biodegradable films- offer eco-friendly alternatives to pursue amidst growing environmental concerns regarding excess plastic use. One 2018 study by Cao et al., explored inulin and chitosan blends to create films with a bonus antioxidant and antimicrobial component; another research discussed inulin enhanced PVA-films with antibacterial properties for potential medical wrap use [89, 90]. Iolanda et al., experimented with the transformation of cardoon inulin into polyhydroxyalkanoate with applications in the food packaging sector [91]. These bio-products would naturally break down into carbon dioxide and water through microbial action. Implementation of these products would lead to reduced dependency on fossil fuels; and offer a solution to most of the pressing issues discussed above (regarding plastic, wildlife, habitat destruction,

climate change, waste management). Veritably, as this field is new, the concept of inulin based plastic products faces challenges in developing cost-effective processing techniques and ensuring that these bio plastics meet mechanical and durability standards that traditional plastic offers. Despite these challenges, inulin-based bio-products stand as valuable commodities through their prevalence, potential for being a plastic replacement, biodegradability, and even more so through their antimicrobial and antioxidant properties.

## **4. Role in economic growth**

The Global market for prebiotics was evaluated at USD 5.3 billion USD 2020 and is predicted to be worth around 9.6 billion USD by 2025. The compound annual growth rate is estimated at 12.5% between this period. The market includes Beneo (Germany), ADM (US), DuPont (US), among others [92]. This growth can be attributed to the rising interest in healthier lifestyles, preventative healthcare; awareness of digestive health benefits, environmental concerns, and the growing use of inulin in common products. Well-known brands such as Chobani, FiberOne, Nestle, General Mills, Bayer, and Loreal have already implemented inulin across a variety of their products.

### **4.1 Healthcare cost reduction**

Inulin can reduce the risk of chronic diseases such as diabetes, obesity, cardiovascular diseases, and dental caries [93]. The medical costs and productivity losses due to diagnosed diabetes, in 2022, was estimated to be approximately \$413 billion USD [94]. Similarly, the medical costs of obesity have been projected to reach around \$173 billion USD each year [95]. Cardiovascular diseases result in losses of nearly \$251 billion per year and additionally inflict approximately \$156 billion loss in the context of diminished occupational productivity. Notably, losses from cardiovascular conditions are projected to top \$1 trillion by 2035 [93]. Dental care results in a loss of 34 million school hours each year, and in terms of monetary value dental disease costs our healthcare system approximately \$46 billion [96, 97]. Lower incidence and complications of these diseases reduce healthcare costs for both individuals and governments, freeing up resources that can be allocated to other sectors of the economy.

### **4.2 Food industry growth**

Inulin is increasingly used in the food industry as a functional ingredient. The growing demand for inulin could spur further innovation and investment in food technology and processing sectors, thereby stimulating economic activity and creating jobs. Some yogurt brands, such as certain varieties of Activia yogurt; and Yoplait's Light yogurt, use inulin as a fat replacer to achieve smoother consistency without increasing fat levels. Similarly, inulin can be used in baked goods like cookies, muffins and cakes to replace fat content while maintaining taste, moisture, and texture. It is noteworthy that recently inulin has also been making its rounds into beverages with a new young crowd favorite being the Poppi prebiotic soda. This soda brand posits less sugar, less calories, new intriguing flavors, and advertisement of inulin's health benefits of improving gut health, immunity, and skin health. Although the company was only created around March 2020; it has been postulated that the company had

made over 100 million USD in sales in 2023, just 3 years after its creation [98, 99]. It respectably has joined the functional soda category which is estimated to be valued at approximately \$173 billion USD by 2025 [98].

Moreover, inulin's function in prolonging shelf life of food products can reduce food waste [100]. Globally, about 1.3 billion tons of food is wasted annually, which is a loss of approximately 1 trillion USD per year [101]. By extending the shelf life of food, inulin can help reduce these losses. Even if implementation can reduce food waste by just 1%; that would equal around 10 billion USD saved annually. This also results in cost savings for manufacturers and retailers. Similarly, products that last longer are more liked by consumers, leading to increased sales loyalty. A survey found that 60% of shoppers were concerned that the shelf life of foods had decreased; and more than 70% were mindful of food waste when purchasing food [102]. Longer shelf life also translates to a more efficient logistics and inventory management, reducing cost related to frequent restocking and transportation.

Inulin's attributes as a calorie, sugar, fat, and stabilizer substitute, is attractive to customers seeking healthy options. The growing consumer demand, as well as rapid financial growth of emerging businesses in the prebiotic field, beckons food manufacturers to develop new products and expand their market share. Additionally, this spurs further innovation and investment in food technology and processing sectors, thereby stimulating economic activity, and creating jobs.

### **4.3 Agricultural sector**

Another potential field of influence includes agriculture. The agriculture industry notably contributed 1.530 trillion USD to US gross domestic product (GDP) in 2023, which is a 5.6% share. Agriculture, including forestry, fishing and related activities, employs approximately 2.6 million people directly; indirectly that number stretches out much further [103]. Cultivating crops rich in inulin can provide income opportunities for farmers, especially in regions where these crops can be grown effectively. Chicory root is a hardy plant that grows in dry climates and does not require sensitive farming, which allows for use of arid land deemed "unusable" for cultivation of most crops and provides new opportunities for farmers specializing in these crops, potentially leading to increased agricultural production and income. This diversifies agricultural production and can improve rural economies by providing stable markets for these crops.

### **4.4 Research and development**

Studies exploring the health benefits of inulin, especially topics of public interest increase consumer interest and demand. The current inulin extraction process is complex and costly, first through a high heat diffusion method (70–80°C), then additional purification processes due to impurities from the high temperatures. This process has led to potential innovations such as pulsed electric fields, ultrasound, and microwaves for extraction. The benefits of these processes include saved energy, time, money, resources; and increased production due to milder temperatures which would decrease impurities and eliminate further purification steps [104, 105]. There are also significant ongoing developments being made in the field of inulin-based bio-products [89–91]. Additional new advancements- like inulin use for creation of nano- medicines for cancer therapy [106] or modification of inulin to create enriched mineral supplements [107] can attract more funding, encourage collaboration

between academia and industry, and lead to development of new products and processes which can positively influence public health at large, offer new solutions to improved management of chronic diseases and drive economic growth.

## **5. Conclusion**

The multifaceted exploration of inulin has illuminated a broad spectrum of opportunities spanning various disciplines: from disease prevention and health promotion; to its applications in enhancing agricultural practices and addressing environmental challenges. The myriad of benefits inulin offers paves way for scientific advancement and socioeconomic development. As public interest in personalized health care grows, so does research and funding into the applications of inulin in various sectors.

## **Conflict of interest**

The authors declare no conflict of interest.

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# Review of Microbial Therapy

*Alexander Suvorov*

## Abstract

The human microbiome is an important integral part of the organism. This reflects on multiple molecular connections between the organs and microbiota, which is controlled by the innate immune system and makes the organism function properly as a holobiont. This explains the reasons behind the malfunction of the multicellular organism in cases of dysbiotic conditions. Any infection or pathology, including cardiovascular, gastrointestinal, or cancer diseases, is accompanied by dysbiosis. Microbial therapy with bacteria, fungi, or viruses grown outside the body and taken as a medicine or the drug interferes with the microbiota of the host. It is based on the ability of probiotic microbes to exclude a certain pathogen out of the organism employing the tools of antagonism or improve certain metabolic pathways due to the specific metabolic activity of therapeutic microbes. However, it is difficult to expect the longitudinal effect of such a therapy due to the exclusion of the exogenous microbe by the host immune system. An autoprobiotic approach based on careful selection of indigenous bacterial strains as personal microbial medicine allows to avoid this problem. Here, we present an overview of different approaches for microbial therapy, making the emphasis on autologous microbial therapy by autoprobiotics.

**Keywords:** microbiome, holobiont, microbiota, probiotic, autoprobiotic, dysbiosis

## 1. Introduction

All multicellular organisms on Earth are believed to harbor numerous microorganisms that colonize various niches within their bodies. These microorganisms, collectively known as the microbiota, comprise bacteria, viruses, archaea, fungi, and protozoa, together forming the genetic entity known as the microbiome.

Focusing on the bacterial component of the microbiota, it is now well-established that the human gut ecosystem is home to thousands of microbial species, consisting of trillions of microbial cells that outnumber human cells. The total number of bacteria alone exceeds the number of human cells, and the combined population of all microbial organisms, including viruses, surpasses the number of human cells by several orders of magnitude [1].

The human microbiome, like that of other organisms, is not an exception and likely began to develop long before Cro-Magnon humans migrated to Europe from Africa [2]. It is now widely accepted that this is no coincidence and that the human microbiome co-evolved alongside the evolution of the human species.

Current scientific evidence strongly supports this concept, driving modern biological science to explore the molecular mechanisms underlying the interactions between microorganisms and their hosts, which together form a holobiont—the host and its associated microbiota functioning as a single ecological unit [3].

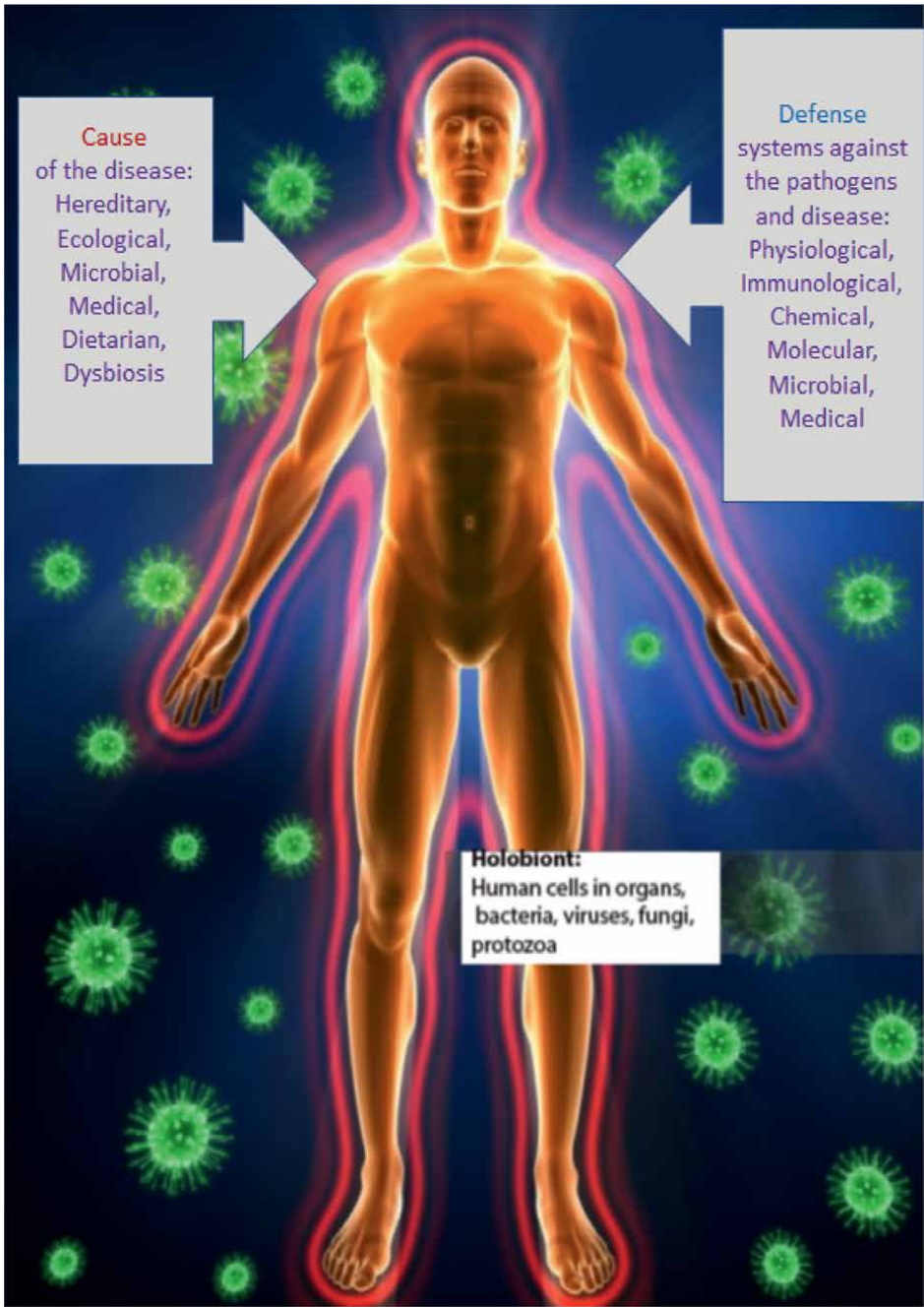


**Figure 1.**  
*Factors affecting human health and providing resistance against diseases.*

There are now numerous examples illustrating that the holobiont theory is a robust framework for explaining the functioning of any organism. Looking back at the history of biological evolution, it is likely that the emergence of multicellular life became advantageous due to atmospheric changes, specifically the rise of oxygen levels produced as a byproduct of photosynthetic bacteria and plants. Anaerobic bacteria, sheltered within multicellular structures, were able to survive and proliferate in this new oxygen-rich environment.

Of course, these complex micro-macro systems required fine-tuning and were influenced by numerous factors such as nutrient availability, temperature, radiation, atmospheric conditions, and interactions with pathogenic microorganisms. As multicellular organisms increased in complexity, so too did their microbiota. The primary regulator of microecological balance within the organism was the innate immune system, which learned to recognize the unique features of the individual microbiota during the first years of life. In humans, this process of immunological education takes approximately 3 years, during which the immune system becomes familiar with the antigens of its resident microorganisms and recognizes them as part of the host [4, 5].

Once this immunological surveillance system is established, significant changes in the microbiome typically occur only in response to sudden shifts in diet, chronic stress, inappropriate medical interventions, the development of chronic diseases, or infections. When the holobiont encounters antigenically foreign microorganisms, the immune system responds with a range of molecular and physiological reactions, including the recruitment of killer cells (such as macrophages or neutrophils), elevated body temperature, vomiting, diarrhea, itching, sneezing, coughing, and other defensive responses. Other defensive mechanisms, including chemical or enzymatic protection against the pathogens, mucosa on the epithelium, IgA antibodies in chime or saliva, altogether support the individual in a healthy condition (**Figure 1**).

## 2. Microbial therapy: An overview

Microbial therapy involves the use of beneficial bacteria, fungi, or viruses as therapeutic agents. The fundamental principle behind this approach is that the introduction of exogenous microbes can restore disrupted microbiota to its normal, individualized state. Historically, nearly every culture worldwide has utilized various microbe-containing foods, which, through millennia of trial and error, have been recognized for their health-promoting properties. Following the pioneering work of the Russian scientist and Nobel laureate Metchnikoff, it became clear that certain bacteria, particularly those in the lactic acid bacteria (LAB) group, could confer health benefits. Since Metchnikoff's studies, some LAB strains have been investigated clinically as adjuncts in the treatment of diseases associated with dysbiosis [6–8].

Later, bacteria and microbe-containing foods were classified as *probiotics*—live microorganisms that provide health benefits when administered in adequate amounts. Today, the most common probiotics on the market belong to the LAB group, including *Lactobacillus*, *Enterococcus*, and *Lactococcus* species. However, several strains of *Bifidobacteria*, *Escherichia*, *Akkermansia*, *Bacillus*, and even some fungi are also available [9].

Newer probiotics are increasingly being designed as targeted therapeutic agents aimed at eliminating specific bacterial pathogens or modulating immune responses, either by promoting or suppressing inflammation. However, even in cases of successful probiotic therapy, the beneficial effects are often temporary. This is due to

the immune system's clearance of the introduced bacteria or competition with the host's native microbiota.

In addition to probiotics, several substances capable of improving the composition of the microbiota upon ingestion have been identified. These substances, which provide nutritional support to the native microbiota, are termed prebiotics [10]. The most commonly used *prebiotics* include fibers and oligosaccharides, such as lactulose and inulin. The biological rationale for using oral pro- or prebiotics is to enhance the microbiota composition, restoring the microenvironment to a state prior to dysbiosis. When consumed in sufficient quantities, probiotics provide a temporary boost to the growth of native bacteria, while prebiotics stimulate the production of metabolites such as short-chain fatty acids (SCFAs).

*Bacteriophages*, or viruses that infect bacteria, are also a form of microbial therapy. Phages, which propagate and lyse bacterial cells, are typically strain-specific and are mainly used as an alternative to antibiotics for the targeted elimination of specific pathogens from microbial ecosystems [11]. This approach for microbial therapy being very promising at first and forgotten lately presently is under very intense study by the scientific community.

### **3. Mechanisms of action in microbial therapy**

As with any stable biological system, an individual's microbiome requires protection from external threats or sudden environmental changes. Upon infection or the appearance of pathogens in the microenvironment, even in the absence of disease symptoms, the initial response of the holobiont's microbiota is to rapidly exclude the pathogen. Indigenous microbiota employs various strategies to achieve this, including competitive exclusion and the production of antimicrobial compounds.

Competitive exclusion is facilitated by the metabolic characteristics of each bacterial species within the microbiome, which are adapted to specific colonization niches—whether in planktonic form, on mucosal surfaces, or within biofilms. In addition, microbiota members synthesize a variety of antimicrobial compounds, including organic acids and bacteriocins. Genomic analyses have revealed that bacterial genomes frequently contain genes encoding bacteriocins, along with the necessary machinery for their expression and immunity to their own antimicrobial peptides. On the host side, mucosal surfaces respond to pathogens by producing a range of antimicrobial peptides, such as  $\alpha$ - and  $\beta$ -defensins (HD5, HD6), RegIII, and cathelicidins [12–15].

In cases of metabolic deficiencies, such as lactose intolerance or the absence of specific metabolic pathways, indigenous bacteria can compensate by providing the host with missing enzymes or essential metabolites. Probiotics function similarly to indigenous microbiota but on a larger scale. For instance, many LAB probiotics produce and secrete bacteriocins with high efficiency or synthesize biologically important molecules such as enzymes or vitamins. The inclusion of certain bacteria on the list of approved probiotics is based on medically validated evidence of their efficacy, particularly their ability to produce antagonistic substances that support host health.

#### **3.1 Introduction to autologous microbial therapy (autoprobiotics)**

The use of indigenous bacteria as a form of microbial therapy offers a promising way to address the limitations associated with conventional probiotics. According

to current immunological concepts, the establishment of innate immunity requires approximately 1000 days from birth. This period is essential for the development of immunological tolerance toward self-antigens, including those of the microbiota—comprising fungi, bacteria, and viruses. During this time, the body learns to distinguish its own microbiota from exogenous microorganisms, allowing it to displace undesirable (potentially harmful) microbes with its native microbiota. In theory, bacteria isolated from an individual's own microbiota and cultivated *ex vivo* should not provoke immunological conflict with the host and may also be capable of prolonged colonization. In contrast, probiotics, which are foreign to the host, typically fail to establish long-term colonization, limiting their effectiveness in restoring the microbiota in cases of dysbiosis.

Another argument in favor of replacing probiotics with microorganisms from the individual's own microbiota is the ability of native microbial communities to form complex symbiotic relationships with other microbiota components, thereby supporting the growth of other indigenous bacteria. The term “autoprobiotic” refers to indigenous bacteria selected from the host's microbiota that confer health benefits. This concept was originally introduced by Russian scientists V. Korshunov, B. Shenderov, and M. Manvelova, who were the first to propose using native *Bifidobacterium* and *Lactobacillus* strains as probiotics [16].

### 3.2 Evaluation of the probiotic effects of indigenous bacteria

The probiotic effects of indigenous bacteria were initially evaluated in laboratory animals with dysbiosis [17]. The key innovation in *autoprobiotic* development lies in the use of genetic algorithms to evaluate the strains, which allows for the exclusion of indigenous strains harboring potential virulence factors in their genomes.

The technology was tested by assessing the ability of these strains to restore the microbiota in laboratory animals with dysbiosis [18]. A series of experimental studies involving indigenous strains of *Enterococcus*, *Lactobacillus*, *Bifidobacterium* species, and various microbial consortia demonstrated that administering autoprobiotics to animals with intestinal dysbiosis effectively restored the microbiota to a state comparable to that of healthy organisms [19]. The advantage of autoprobiotics was their potent antagonistic activity against pathogens, along with their ability to persist in the intestine for extended periods (over 45 days) without suppressing resident microbiota. This long-term colonization presents a major advantage over even the most effective conventional probiotics.

Genomic and bioinformatic analyses confirmed the validity of the initial hypothesis. Following administration of autoprobiotics, either as mono-strain preparations or as mixtures of indigenous *Bifidobacterium* and *Enterococcus* species, an increase in anti-inflammatory cytokine production (particularly IL-10) was observed [19].

Autoprobiotics also significantly influenced the enzymatic activity of the intestinal epithelium across various regions of the gut. Specifically, autoprobiotic *Enterococcus* and *Bifidobacterium* strains were shown to enhance the activity of enzymes involved in carbohydrate metabolism within the chyme while reducing their production in the epithelium through compensatory feedback mechanisms. Moreover, the activity of alkaline phosphatase in the chyme increased following exposure to autoprobiotics, potentially aiding in the detoxification of lipopolysaccharides (LPS). In the blood serum, alkaline phosphatase levels were restored to those seen in healthy animals, suggesting a role in restoring metabolic functions and countering the endotoxins of gram-negative bacteria.

Mass spectrometric analysis of the metabolomes (involving more than 100 metabolites) in the blood serum of laboratory animals treated with autoprobiotics revealed that indigenous bacteria, especially *Bifidobacterium* spp., could significantly neutralize the harmful effects of Proteobacteria. Preclinical studies involving autoprobiotics, including *Lactobacillus*, *Enterococcus*, and combinations of *Bifidobacterium* and *Enterococcus*, were followed by clinical trials investigating their efficacy in treating irritable bowel syndrome (IBS), *Campylobacter* and *Helicobacter* infections, Parkinson's disease, colorectal cancer, metabolic syndrome, bacterial vaginitis, and inflammatory oral diseases [20–23].

None of the clinical trials reported any adverse effects, and patient compliance with autoprobiotic therapy was extremely high. In all studied diseases, patients demonstrated a reduction in Enterobacteriaceae (gamma-proteobacteria) levels, normalization of stool, resolution of pain in IBS cases, and reduction of spondyloarthritis symptoms across the conditions studied [24, 25].

### 3.3 Other current approaches to microbial therapy

An alternative approach to microbial therapy that has gained significant attention recently is fecal microbiota transplantation (FMT). This technique is based on the premise that microbiota from a healthy donor can be transferred to a person with a dysbiotic condition. In heterologous fecal microbiota transplantation, feces from a “healthy” donor is transplanted into a recipient suffering from illness. FMT has been particularly successful in treating infectious diseases such as recurrent *Clostridioides difficile* infection (CDI), with cure rates as high as 90% [26–29]. Due to limited responses to antibiotic treatments, FMT has become a key intervention for CDI in humans. Following its success with CDI, FMT has been explored as a potential therapy for other conditions, such as inflammatory bowel disease (IBD). However, the clinical outcomes for IBD have been inconsistent and less effective [30, 31]. This variability in response may be due to the distinct etiologies of CDI and IBD; while CDI is caused by a clostridial infection, IBD is a complex, microbiome-driven inflammatory disorder that involves immune dysregulation in genetically susceptible individuals. Despite its potential, FMT for intestinal infections has several significant drawbacks, including potential incompatibility with the recipient's microbiota. The primary concern with FMT is the difficulty in ensuring safety, as foreign viruses and DNA from the donor inevitably accompany the transplanted bacteria, making the long-term consequences of microbiota transplantation unpredictable [29, 32].

An alternative to heterologous FMT is autologous fecal microbiota transplantation (a-FMT), which involves banking an individual's own microbiota during a healthy state, preserving it through deep freezing, and using it later to restore gut microbiota after dysbiosis. Unlike FMT, a-FMT avoids the introduction of foreign microbiota and is conceptually similar to autoprobiotic technology. Autologous microbiota transplantation has been explored for various conditions, including obesity, oncohematology, irritable bowel syndrome (IBS), and antibiotic-associated diarrhea [33, 34].

This approach has proven to be safe and well-tolerated, even among immunocompromised patients. Following a-FMT, indices of  $\alpha$ -diversity return to baseline levels, and microbial community composition is restored to its original state. It is particularly beneficial for patients undergoing intensive chemotherapy or antibiotic treatments, as it facilitates the reconstruction of the gut microbiota. In a pilot clinical study, a-FMT demonstrated its effectiveness in restoring the gastrointestinal

microbiome's taxonomic composition, reducing the number of antibiotic resistant strains, and enhancing metabolic capacity [35]. Patients with IBD also showed positive clinical responses, supported by metabolome analysis [36].

The advantages of autologous fecal microbiota transplantation (a-FMT) are evident. While donor selection has become a critical factor for the success of conventional fecal microbiota transplantation (FMT), the use of heterologous donor stool continues to pose a risk of introducing infectious or pathogenic microorganisms to the recipient. Additionally, even in the context of a patient in a healthy state, there remains the possibility of preserving pathobionts in the stool sample that could become harmful, particularly in immunocompromised individuals. A promising alternative to a-FMT is the use of a consortium of indigenous bacteria grown *ex vivo* on artificial culture media [37]. This approach allows for the selection of a bacterial consortium that is free from potentially pathogenic bacteria or viruses, as the indigenous non-pathogenic bacteria are grown under selective conditions, outcompeting harmful organisms. The resulting consortium is then tested and applied as a health-promoting product.

4. Conclusion and future perspectives

Microbial therapy as an approach for the treatment of dysbiosis and reestablishing the indigenous microbiota seems to be an effective tool to fight the majority of the human pathologies. All the approaches described above have the potential for better development in the future (Table 1). Probiotics need to be targeted and selected in good correlation with the physiological condition of the host and the existing microbial pathways expressed in the bacteria which are introduced into the host as functional

| Means of microbial therapy            | Advantages                                                                                                                                                                                                                                                       | Future                                                                                                                                                                        | Setbacks                                                                                                                                                                |
|---------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Probiotic                             | Many probiotics are selected out of health-beneficial food.<br>Many probiotic strains have a good history of being safely consumed.<br>Clinical evidence for beneficial effect in diarrheal conditions or constipations in case of dysbiosis<br>Generally, safe. | Good potential for targeted probiotics.                                                                                                                                       | Alien for the host immune system.<br>Do not stay in the host for long.<br>Very few serious clinical trials for clinical efficacy.                                       |
| Prebiotics and functional foods       | Very safe<br>Good evidence for changing individual microbiota<br>Some evidence of the effect in the case of dysbiosis.                                                                                                                                           | Good potential for personified improvement of microbiota by consuming an individual diet.                                                                                     | Temporary effect<br>Not enough evidence for the efficacy in clinical condition.                                                                                         |
| Fecal microbial transplantation (FMT) | Great results for the treatment of <i>C. difficile</i> infection.<br>Several studies show efficacy in the case of metabolic diseases and IBD.<br>FDA approval for treatment of <i>C. difficile</i> infection.                                                    | There is a potential for use in other diseases associated with dysbiosis.<br>Best donors' microbiota might be used for getting new bacterial or fungus strains as probiotics. | Difficult and expensive selection of fecal donors.<br>Foreign viruses and DNA inevitably enter the system with unpredictable results.<br>Long-term effects are unknown. |

| Means of microbial therapy                   | Advantages                                                                                                                                                                                                          | Future                                                                                                                                                                                                                               | Setbacks                                                                                             |
|----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| Autologous microbial transplantation (a-FMT) | Safer than FMT for being composed of the same patient. Several clinical studies with good outcomes.                                                                                                                 | With the expansion of the biobanks, microbiota can be stored for years and used in case of necessity with little danger.                                                                                                             | Some pathobionts still might survive harming immunocompromised patient.                              |
| Autoprobiotics                               | Very safe, no side effects determined. Good effect in case of IBS, metabolic diseases (type II diabetes, obesity), Positive effect on microbiota in dysbiotic conditions, IBS, type II diabetes, colorectal cancer. | Selection of more different species of autoprobiotic, including anaerobic bacteria such as <i>Bacteroides fragilis</i> or <i>Akkermansia muciniphila</i> . Autoprobiotic consortium can be improved by adding selective supplements. | Difficulty in rapid selection of the indigenous strain with no virulence factors in severe dysbiosis |

**Table 1.**  
*Methods of microbial therapy (pluses and minuses).*

food or probiotic drug. Autoprobiotic technology is a very promising approach for the therapy of different human diseases due to the tolerance of the host immune system to the bacterial strains that originally belonged to the host ecosystem. However, before the introduction of the autoprobiotic strain as a food product, a good microbiological analysis of the host microbiota seems necessary. In any case, making preliminary biobanks of the original microbiota is vitally important. Biobanks for donor microbiota are being actively developed and established across various countries worldwide. Presently, the biobanking of microbiota concept needs to change from the biobanks of donor microbiota to the biobank of microbiota of people in healthy conditions.

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# Probiotics and the Microbiota-Gut-Brain Axis

*Aisan Asalipisheh*

## Abstract

There is strong evidence that bidirectional interactions exist between the gastrointestinal tract, the enteric nervous system, central nervous system, and important relationship among probiotics, psychobiotics, and cognitive and behavioral processes, which include neural, metabolic, hormonal, and immunological signaling pathways. The latest researches approved that gut flora has a significant role in the interaction between the brain and gut. Moreover, gut microbes could play an important role in many psychiatric disorders and could be a potential therapeutic target. Thus, any changes in the complex and structure of gut flora could be correlated with several neurological disorders, such as depression, Parkinson's disease, autism, Alzheimer's disease, and stress. Microbiome-based therapies such as probiotics can be cautiously recommended for nervous psychiatric disorders. This review will focus on new and novel therapeutic treatment approaches on probiotics and the gut-brain axis of various neurological, psychiatric, and nervous psychiatric disorders.

**Keywords:** probiotics, gut-brain axis, psychiatric, neurological disorders, psychobiotics

## 1. Introduction

Psychiatric disorders are characterized by significant heterogeneous problems that disturb emotional regulation, behavior, cognition, feeling, and thoughts of people [1]. It is estimated that disorders such as schizophrenia, depression, bipolar disorder, and anxiety include approximately 12% of the global disease burden. Additionally, depressive disorders are the leading cause of disability worldwide, with a huge cost for healthcare institutions [2]. Alzheimer's disease (AD) and Parkinson's disease also are categorized as a progressive multifactorial neurodegenerative disorder. So far, several researches and studies emphasize the role of gut microbiota in the regulation of behavior (stress) and the pathophysiology of several psychiatric disorders [3]. Human gut is colonized by more than  $10^{13}$ – $10^{14}$  microorganisms known as gastrointestinal microbiota that plays an important role in human health and contributes to different diseases and also in nervous system disorders. Most of them consist of 500 to 1000 different species of bacteria, virus, fungi, protozoa, and archaea, which the number of probiotics and, also stability and diversity of them is varied during development and among different human populations [4]. The microbiota composition is specific in individual

hosts and relatively stable which matures in the first 3 years of life; plays a fundamental physiological role in intestinal motility and the development of the metabolic and immune system (both mucosal and systemic); consequently protects the host against pathogens; and has a significant impact on the host neurophysiology, behavior, and function of the nervous system [5]. Gut microbiota is essential for a range of physiological processes and offers health benefits to the host. Several researches and studies show that there is a bidirectional communication between the gastrointestinal tract (GIT) and brain that is referred to as the gut-brain axis (**Figure 1**). This interaction could affect brain function, cognition, and behavior via different pathways including endocrine, neural, and immune pathways [7]. GIT, along with the immune system, central nervous system (CNS), autonomic and enteric nervous system (ANS), (ENS), and neuroendocrine system, forms the axis of gut and brain [8]. An imbalance in a community of gut microbiota (dysbiosis) can lead to unhealthy signals sent to brain that causes inflammation [9], increased oxidative stress, and rise in cellular degeneration and asymmetric energy homeostasis [10]. Several studies discovered that dysbiosis can contribute to a plenty of neurological diseases such as anxiety, depression, and neurodegeneration; furthermore, scientists suggested that neuroimmune modulation by the microbiota plays a significant role in symptoms of psychiatric disorders such as Alzheimer's disease (AD), Parkinson's disease, autism spectrum disorders (ASD), depression, and anxiety [11]. Thus, maintaining a healthy microbiota community is important in the regulation of the gut-brain axis [6].

According to the WHO organization, probiotics are defined as “living microorganisms, which when administered in adequate amounts confer health benefits on the host.” As beneficial active microorganisms, probiotics can colonize in human gut, change the gut microbiota and regulate it, and improve host immunity [12]. Probiotics have a significant role in the inhibition of growth and colonization of pathogen bacteria in intestine [13]. Thus, the presence of specific and beneficial

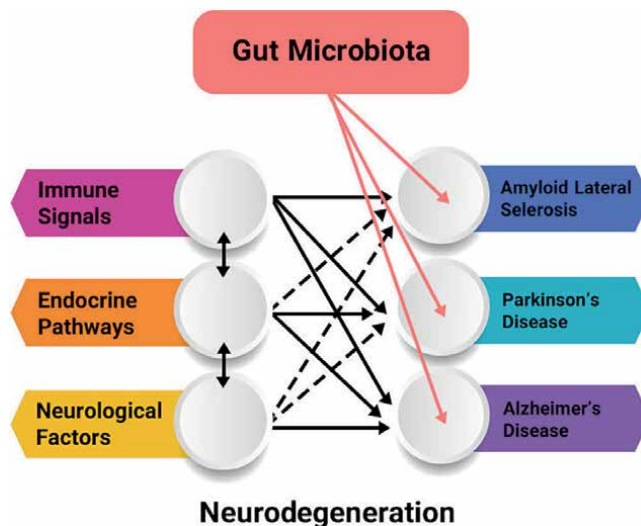


**Figure 1.** Three main modes of communication between the gut and the brain. Various pathways that gut microbiota influences neurodegenerative diseases [6].

microorganisms as well as the certain composition of gut microbiota has critical consequences for host physiological and metabolic processes [14].

## **2. Gut microbiota and human health**

Microbiota is a collection of living microorganisms that live in their host's body [15]. The human gut microbiota is one of the most complex ecologies of various microbial populations. The whole of the microorganisms, their genes, and metabolites is called the microbiome which is specific for each individual [16]. The gut microbiota could be changed with the type of birth even with food diet. The gut microbiota changes over the years, maternal microbiota during birth, breastfeeding microbiota and environments factors are important items in development of microbiota and its changes [17]. The gut flora has great contact with intestinal mucosa which is the largest surface in the human body. The gut microbiota together with mucus forms the mucosal barrier which is the key system to defense against pathogenic microorganisms and potential immunogenic agents of lumen [18].

These microorganisms have fundamental role in the consumption of nutrients in the intestine along with their metabolic activities [19]. Furthermore, they remarkably can affect the immune system function and development as well as other functions of GIT. Each person's microbiota has a specific "bacterial fingerprint," a characteristic of its own species that differs from other people [20].

Indeed, recent research has convincingly demonstrated that the colon and its microbiota form a strong symbiotic relationship and interact with each other. This two-way interaction plays a very important role in the functioning of the intestine as well as the functioning of other important activities of the body, including the immune system, endocrine activity, and brain function [21]. In this symbiosis, the combination of the gut flora and its structure as a key element has important effects as follows:

- Interaction with colonic epithelial cells and GALT to modulate cell differentiation, gene expression, receptor expression, and metabolic activities.
- In the case of dysbiosis or eubiosis can has role respectively cause or reduce the risk of intestinal deceases such as constipation, diarrhea, colon cancer, inflammatory bowel diseases and systematic disorders like allergy, atopy, obesity, diabetes, autism [22].

The human gut is a highly complex ecosystem where a vast number of bacteria, archaea, viruses, and unicellular eukaryotes exist, which inhabit the human gastrointestinal tract (GIT) and interact extensively with the host cells [23]. The microbiome consists of two main bacterial phyla, namely, Bacteroidetes and Firmicutes. Microbiota is an active component of intestinal physiology that has many functions, and the most important of it are metabolic, barrier effect, and nutritional functions [24]. The human intestinal microbiome in each individual is an identifiable core set of genes and pathways. Results from several studies and researches suggested that the genes required for essential biological pathways in the human intestine can be functionally complemented by specified genetic elements from the intestinal

microbiota that may have been missing or incompletely encoded in the human genome. These genes are more than 300 times larger than the whole complement of genes in the human genome [25].

Two major characteristics of the human gut microbiota include the following:

1. The adherence capacity to the host proteins such as fibronectin, collagen, and fibrinogen.
2. Intestine microbiota is able to ferment different carbohydrates [26].

The interplay and connection between the microbiota and the host provide benefits of various kinds to both. The main functions of microbiota that have positive effects on the host organism are as follows [27]:

(1) participation in intestinal wall formation [28], (2) modulation of gene expression and cell differentiation in the intestinal wall (especially endocrine L cells in the colon) [29], (3) resistance to replacement and inhibition of colonization of intestine by potentially pathogenic microorganisms (i.e., improved colonization resistance) by effectively competing with invasion of pathogens for ecological niches and metabolic underlayers [30]; in 1916, Nissel first demonstrated how the human gut microbiota can play role in resistance against *Salmonella* typhoid infection, and later, it has been confirmed that gut flora is the initial line that defense against the invasion of pathogenic microorganisms [30]. (4) Short-chain fatty acids (SCFAs) production, metabolites that are essential for physiological functions such as propionic acid for gluconeogenesis, acetic acid for function of muscles, heart, and brain, and butyric acid for the function of enterocyte [31]. (5) Crucial vitamin generation: particularly K and B vitamins [32]. (6) Interaction with the mucosal immune system [33]. (7) Destruction of xenobiotics, with genes capable of synthesizing enzymes that have catabolic activity toward these compounds [34]. (8) Important sources of energy for intestinal wall cells (e.g., providing more than half of the daily energy requirement for host colonocytes) via the fermentation of carbohydrates to main short-chain fatty acids, such as acetate, butyrate, and propionate [35]. The gut microbiota has a significant effect on the human immune response. In 1989, Strachan suggested that the poor microbial population in the developed countries could lead to an increase in autoimmune diseases [36]. In fact, it is discovered that the gut microbiota has a wide range of effects on inflammatory and autoimmune disease, such as allergy and cancer, and disturbance of the natural intestinal flora causes many diseases [37]. Gut microbiota can affect the function of the gastrointestinal tract by altering gene expression; furthermore, probiotics can have an important effect on gene expression patterns of intestine flora [38]. Scientists suggested that the various diets may be able to influence and change the gut microbiota composition that can have direct effects on biochemical reaction patterns in the intestinal lumen [38]. Different experiments improved that high-fat, high-sugar diet demonstrated rapid changes in intestinal microbial community structure resulting in decreasing abundance and number of phyla *Bacteroidetes* and *Firmicutes*. Thus, healthy gut microbiome could result in prevention of different diseases related to immune system, intestinal lumen, and brain function [39]. Moreover, gut flora is crucial for maturation of the gut-associated lymphoid tissue (GALT). GALT, by producing IgA and the controlled inflammation that induces by pathogenic bacteria colonization, is a key element of forming the immunological complex of GIT [40]. Mucosal immune system function and development can be affected by some parts of gut bacteria such as lipopolysaccharide (LPS),

lipoteichoic acid (LTA), and polysaccharide A [41]. Particular ability to recognize potentially pathogenic microbes from safe antigens through pattern recognition receptors (PRRs) and innate immunity is a substantial mechanism of the mucosal immune system. Toll-like receptors (TLRs) that are present on innate immune system are able to identify specific molecules called pathogen-associated molecular patterns (PAMPs) [42]. When particular TLRs recognize a pathogen, the NF- $\kappa$ B signaling system activates, which leads to an increase in the production of cytokines and T cells activation. Nevertheless, in the absence of gut microbiota, the function of TLRs has low or no expression in the gastrointestinal tract (GIT), and consequently, threatens the appropriate immune response to pathogens [43].

### 3. Probiotics and their effect on gut microbiota

A decade ago, probiotics were considered to be live microorganisms that exert a beneficial effect on the health of the host by improving the microbial balance of the gut. A decade ago, probiotics were considered to be live microorganisms that exert a beneficial effect on the health of the host by improving the microbial balance of the gut. Nevertheless, Frick provided a newer and more comprehensive definition for probiotics: probiotics are known as microorganisms that are basely of human origin and are non-pathogenic, that have beneficial effects on their and, when consumed in sufficient amounts, prevent or ameliorate certain diseases [44]. The most frequently used probiotics belong to *Lactobacilli*, *bifidobacteria* genera, and also some non-pathogenic microorganisms such as some yeasts and *Streptococcus* spp. Some of main probiotics are *Lactobacillus rhamnosus* GG, *Saccharomyces cerevisiae* Boulardii, *Lactobacillus casei* Shirota, and *Bifidobacterium animalis* which are mostly studied. The only yeast that has been used is *S. cerevisiae* Boulardii [45]. The most common used probiotics are listed in (Table 1). Some parts of probiotic microorganisms are known as bioactive elements consisting of nucleic acids, organic acids, SCFAs, cell wall surface proteins and/or lipoic acids antimicrobial proteins, and some other components which are less soluble [47]. Fermented and natural products are certain sources of probiotics such as dairy products like Maasai milk, kefir, Kurut, and fermented milk. Fermented milk is one of the main and important probiotic sources due to its various lactic bacteria species [48]. Due to WHO and FAO guidelines, the microorganisms that are used in food as probiotics should be able to survive through the intestine and its ingredients such as gastric juice and resist against bile. Furthermore, they must have the ability to colonize in gut and be effective and stay effective during the product shelf life. In addition, used probiotics must be safe and do not have side effects on their host [49].

They should have below characterization as probiotics:

- High cell viability, low pH resistance, and gastric and bile acids.
- They should be able to stay in the gut even if the probiotic strain fails to colonize the intestinal.
- Adherence to the gut epithelium to eliminate the inflammatory effects of peristalsis [50].
- Must be able to interact or send signals to gut-associated immune cells.

| <i>Lactobacillus</i> sps.   | <i>Bifidobacterium</i> sps. | <i>Streptococcus</i> sps.               | <i>Saccharomyces</i> sps. | Others                   | Yeast                          |
|-----------------------------|-----------------------------|-----------------------------------------|---------------------------|--------------------------|--------------------------------|
| <i>L. Acidophilus</i>       | <i>B. Bifidum</i>           | <i>S. Thermophilus</i>                  | <i>S. boulardii</i>       | <i>Bacillus cereus</i>   | <i>Saccharomyces boulardii</i> |
| <i>L. casei (rhamnosus)</i> | <i>B. Breve</i>             | <i>S. salivarius subsp.thermophilus</i> |                           | <i>Escherichia coli</i>  | <i>Saccharomyces boulardii</i> |
| <i>L. fermentum</i>         | <i>B. Lactis</i>            |                                         |                           | <i>Enterococcus</i>      |                                |
| <i>L. gasseri</i>           | <i>B. Longum</i>            |                                         |                           | <i>Propionibacterium</i> |                                |
| <i>L. johnsonii</i>         | <i>B. Infantis</i>          |                                         |                           | <i>freudenreichii</i>    |                                |
| <i>L. lactis</i>            | <i>B. adolescentis</i>      |                                         |                           |                          |                                |
| <i>L. Paracasei</i>         |                             |                                         |                           |                          |                                |
| <i>L. plantarum</i>         |                             |                                         |                           |                          |                                |
| <i>L. reuteri</i>           |                             |                                         |                           |                          |                                |
| <i>L. salivarius</i>        |                             |                                         |                           |                          |                                |
| <i>L. bulgaricus</i>        |                             |                                         |                           |                          |                                |

**Table 1.**  
Most frequently used probiotics [46].

- They must be of human origin.
- Must be non-pathogenic [51].
- Resistance and survival in the technological process of construction.
- Must have the capacity to influence local metabolic activity [46].

The mode of action of probiotics is not clear and still remains as a main problem considered by the European Food Safety Authority (EFSA). The main and major mechanisms of action of probiotics are improvement of the epithelial barrier, immune system modulation, producing some substances that have anti-microorganism effects, and blockage of the adherence of pathogens to intestinal mucosa by enhanced adherence ability to intestinal, and they can competitively prevent pathogenic microorganisms [52].

### 3.1 Regulation of intestinal flora by probiotics

Several studies approved that probiotics have an important role in gut microbiota composition and regulation [53]. Treatment with probiotics can reduce dysbiosis, modulate the immune system, and also treat inflammatory diseases and disorders [54]. *Lactic bacteria* as a probiotic can have several effects such as local effects or stimulation of gut immunity (stimulation of secretory IgA production for example) or systemic. Some probiotics produce enzymes like beta-galactosidase and some other enzymes that can have beneficial effect on gut microbiota [55]. The prerequisite for the colonization of probiotics in the intestine is their ability to bind to the intestinal mucosa, which is an important factor in the interaction between the host and probiotics, and this factor is also very useful for fighting against pathogenic microorganisms and modulating the immune system [56]. Thus, adherence ability of probiotics is the main factor of probiotic strain selection. Several surface determinants of lactic acid bacteria (LABs) are important factors involved in their interaction with mucosa and intestinal epithelial cells (IECs). IECs secrete mucin, which is a major complex glycoprotein of mucus, that prevents the adhesion of pathogenic bacteria [57]. In addition, there are other substances in the mucous fluid, such as lipids, immunoglobulins, free proteins, and salts [58].

### 3.2 Maintenance of the epithelial barrier

One important mode of action of probiotics is barrier effect also called resistance to the colonization that prevents and limits the colonization of pathogenic bacteria [59]. Considerable evidences showed that probiotics can strengthen the intestinal barrier. Intestinal epithelium is the largest mucosal surface of the human body that is in constant contact with the contents of the duct and intestinal flora, which is variable and dynamic which is composed of a single layer of intestinal epithelial cells (IECs) [60]. The important function of intestinal epithelial is to appoint a biochemical and physical barrier between the external environment and the host immune system [61]. After invading the intestine, pathogenic microorganisms encounter the intestinal mucosal barrier, which is the first drug-resistant barrier. Several researches approve that probiotics improve the barrier function by promoting the secretion of mucus [62]. The intestinal barrier consists of various

physical, chemical, and biological factors that create tight junctions, and these factors include intestinal vessels, epithelial barrier, mucous layer, antimicrobial peptides, epithelial cells, and secretory IgA, which have a vital role in health and different disease. This intestinal barrier prevents the colonization of pathogenic agents, and as a result, any disturbance and change in it cause many intestinal infections and immune system disorders. Probiotics have crucial role in improvement of intestinal barrier function by producing various factors such as microbial peptides, antimicrobial proteins (AMPs) such as alpha-defensins, cathelicidins, beta-defensins, C-type lectins, and ribonucleases from epithelial cells, and also preventing the colonization of pathogenic microorganisms. Defensins show antimicrobial activity against a wide range of bacteria, fungi, and viruses, and Cathelicidins play a role in host defense against pathogens [63].

The inhibition of bacterial growth is due to the production of some bacteriocins, some metabolites such as biosurfactants with an antimicrobial activity, and short-chain fatty acids. Additionally, this inhibitory effect can also act through different mechanisms: competition for binding sites and inhibition of adhesion. Hence, probiotics are able to improve their physiological barrier function and act at the level of signaling pathways that result in an improvement of the mucosal layer or an increase in the production of defensins, also tight junction proteins [63]. Briefly probiotics maintain intestinal permeability and mucosal barrier integrity and strengthen physical barriers to maintaining the host's health (Figure 2) [55].

### 3.3 Competitive elimination of pathogens

According to some researches, probiotics by using different ways are able to prevent the growth of pathogens or eliminate them: By creating an antagonistic environment, they can physically block receptor sites of pathogenic microorganisms, and they also compete on crucial nutrients with pathogenic microorganisms to reduce their access to necessary nutrients and produce and secrete some antimicrobial metabolites and elements [64]. Reduction of essential nutrients

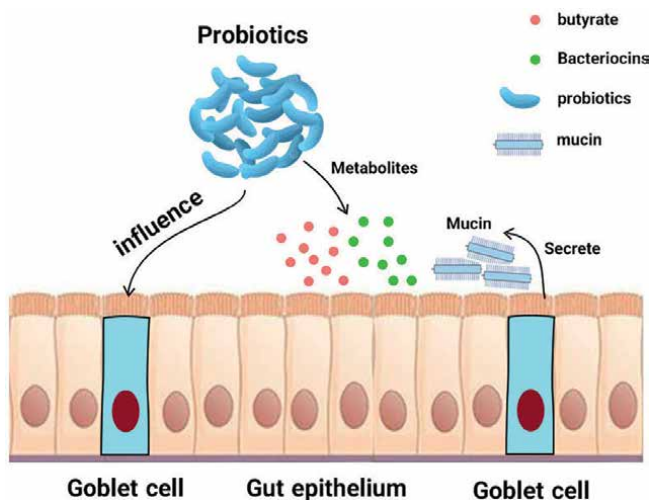


Figure 2.  
Probiotics maintain gut epithelial barrier [12].

leads to the prevention of pathogenic bacteria colonization [65]. *Lactobacilli* and *bifidobacteria* prevent the growth of many pathogens including *Escherichia coli*, *Salmonella*, *Helicobacter pylori*, *Listeria monocytogenes*, and *rotavirus*. This prevention is the result of various mechanisms and attributes of probiotics that cause inhibition of pathogen adherence, by producing some substances and stimulating of IECs [66]. Competitive elimination by probiotics is the result of interaction between bacteria, which is correlated to the competition of microorganisms for nutrients and mucosal adhesion sites [67]. To win this competition, bacteria change their environment to not favorable one for their competitors. For modifying the environments, probiotics produce some antimicrobial substances like lactic acid and acetic acid. In general, probiotic strains are able to inhibit the binding of pathogenic bacteria using the steric barrier in enterocyte pathogen receptors [50]. Organic acids such as butyric acid and propionic acid, which are produced by probiotics during their growth in the intestine and fermentation of carbohydrates, are one of the main factors that inhibit the growth of pathogenic microorganisms and are actually antimicrobial compounds of probiotics [68]. Several studies reported that some probiotics such as *Clostridium* and *Eubacterium* produce butyric acid that integrates intestinal epithelial layers [69].

### 3.4 The modulation of the immune system

Probiotic bacteria can act on various types of cells involved in innate and adaptive immune responses and interact with vary immune cells like epithelial cells, dendritic cells, monocytes/macrophages, B cells, T cells, regulatory T cells, and NK cells, thereby exerting their immunomodulatory effect [70]. Products synthesized by the metabolic activities of probiotics have a strong link in modulating host immune responses [71]. When *E. coli* penetrates host cells as a pathogen a probiotic strain of *L. rhamnosus* GG. It promotes the growth of intestinal cells by providing a special healing protein against *E. coli* to prevent its pathogenesis [72]. Probiotics by producing some substance including various metabolites, DNA, and cell wall components are able to effect on immune system. Obviously, immunomodulatory effects can even be obtained with dead probiotic or only some components derived by probiotics such as peptidoglycan or DNA fragments [73]. Intestinal epithelial cells and immune system cells related to the intestine can recognize different compounds from probiotics through special recognition receptors on them. This interaction between host cells and probiotics through the strength of adhesion of probiotics to the epithelium would initiate a signaling cascade and ultimately modulate the immune system. On the other hand, the release of soluble factors can trigger signaling cascades in immune cells or epithelial cells that subsequently affect immune cells [73]. Dendritic cells (DCs) are antigen-presenting cells and can induce both innate and the adaptive immune as immune cells [74], they have capacity of inducing regulatory T cells (T-reg) as well and are involved in recognition and elimination of pathogens in immune system [75]. DCs can be stimulated by some antigen-representing cells or antigen-dependent signals which lead to the production of anti-inflammatory cytokines [76]. It has been demonstrated that probiotics have immunomodulatory effect by inducing production of DCs and cytokines in the intestine and regulate innate and adaptive immune system [77]. Studies indicated that probiotics can activate macrophages [78], stimulate IgA production by elicit B-cells, and lead to the release of secretory IgAs [79]. As beneficial microorganism probiotics are able to enhance the NK cell activities and increase the production of L-2, IL-4, and IL-10 [80, 81].

#### **4. Pathways of microbiota-gut-brain connection**

From 10 years scientists highlighted the value of brain-gut interaction. There is a bidirectional communication between brain and gut microbiota that known as gut-brain axis which is vital for host homeostasis and dysfunction of it to various psychiatric and nonpsychiatric disorders. The gut-brain axis network includes central nervous system (CNS), neuroimmune systems, autonomic nervous system (ANS), the enteric nervous system (ENS), hypothalamic-pituitary-adrenal (HPA) axis [82], as well as endocrine, humoral, metabolic, and immune routes of communication and noticeably the intestinal microbiota [83]. This network allows the brain to control activities of intestinal such as immune effector cells function and has a potential impact on mental health, mood, and cognition. This interaction between gut and brain results in sending signals from brain to influence sensory, secretory, and absorption part of the gut and conversely some visceral signals from the intestine that have an effect on brain function [84]. Furthermore, modulation of the gut-brain axis is related to stress response, and microbiota has a significant role in gut-brain axis connection. However, the exact mechanism of action of intestinal microbes on brain function is not clear yet [85]. It has been shown that any deregulation and hemostasis in gut-brain function could result in immunological, neurological, and psychiatric disorders [86]. Gut microbiota regulates CNS through three main pathways including brain and gut interact through immunoregulation, via vagus nerve, and neuroendocrine pathway [86]. There is an intricate bidirectional network between gut microbiota and brain: hypothalamic-pituitary-adrenal (HPA) axis, CNS, and ENS that are known as descending pathways, while sensory vagal and dorsal root ganglion pathways, cytokines, immune mediators, and microbial and intestinal metabolites are known as ascending pathways [87]. The GI tract's enteroendocrine cells (EECs) have crucial role in descending and ascending pathways. Vagus-dependent and independent mechanisms are required in the gut-brain axis, both of these mechanisms can be activated directly or indirectly [88]. One of the predominant metabolites of bacterial fermentation in the colon and small intestine is short-chain fatty acids (SCFAs) (e.g., acetate, propionate, and butyrate), which are regulators of many physiological processes in body, such as lipid, glucose, and cholesterol metabolism in various tissues and adipolysis (acetate and propionate) histone acetylation (acetate) [89]. Also, these fatty acids control the secretion of some important hormones such as peptide YY (PYY), glycogen-like protein (GLP-1), and cholecystokinin (CCK) [1]. As explained above, gut flora has significant role in immune system modulation. Intestinal microbiota induces its effects on CNS by altering the circulating levels of pro- and anti-inflammatory cytokines, and on the other hand, CNS influences the direct or indirect liberation of different signaling molecules, antimicrobial peptides, and cytokines into the gut lumen [1]. Another system that plays a vital role in maintaining the balance of the immune response between beneficial intestinal microorganisms and pathogens is the mononuclear phagocyte system, which is located below the mucosal epithelium in the intestine [1]. Furthermore, there are some receptors on the intestine epithelial cells, which are key part of the intestinal immune system. These receptors receive signals from gut flora and respond them to keep homeostasis in the intestine. Moreover, intestinal microbiota is connected to manifold immune cells like T helper 17 (TH17) cells, gut-specific B cells, and regulatory T (TReg) cells, and they are also able to stimulate human intestinal macrophages to express toll-like receptors (TLRs) [38] which decrease the expression of nuclear factor- $\kappa$ B (NF- $\kappa$ B) in the gut epithelium that leads to stop unwanted and uncontrolled hyperinflammation [90]. Human intestine

macrophages are able to express diverse other receptors such as CD68 and CD14, IL-1, and interleukin-1 receptor (IL-1R), that have the same structure and function as TLRs to keep immune homeostasis [91]. Serotonin is a vital hormone that plays a very important role as a neurotransmitter in both the CNS and the human gut. Studies have shown that 90% of serotonin is in the digestive system. The precursor of this hormone is tryptophan, which is an essential amino acid must be present in the diet in sufficient quantity. Intestinal microbial flora has an important effect on serotonin precursor metabolism and serotonin synthesis and absorption [92]. Neuropeptide galanin is involved in plenty of critical neurobiological functions including sleep and wake cycle, mood, blood pressure regulation, pain, behavior, and neurotrophic functions. Intestine microbiota also is able to regulate to influence the salvation of biologically active peptides from enteroendocrine cells by alerting available nutrients in gut so that it can affect the gut-brain axis. Galanin enhances glucocorticoid secretion from the adrenal cortex and further norepinephrine release from the adrenal medulla by stimulating activity of the central branch of the HPA axis [93]. The other pathway that gut microbiota can influence the gut-brain axis is bacterial metabolites which are decisive humoral influencers. The most important of these metabolites are SCFAs and lipopolysaccharide (LPS) [94, 95]. SCFAs can affect gut-brain in several ways, they are able to regulate microglia homeostasis by passing blood-brain barrier

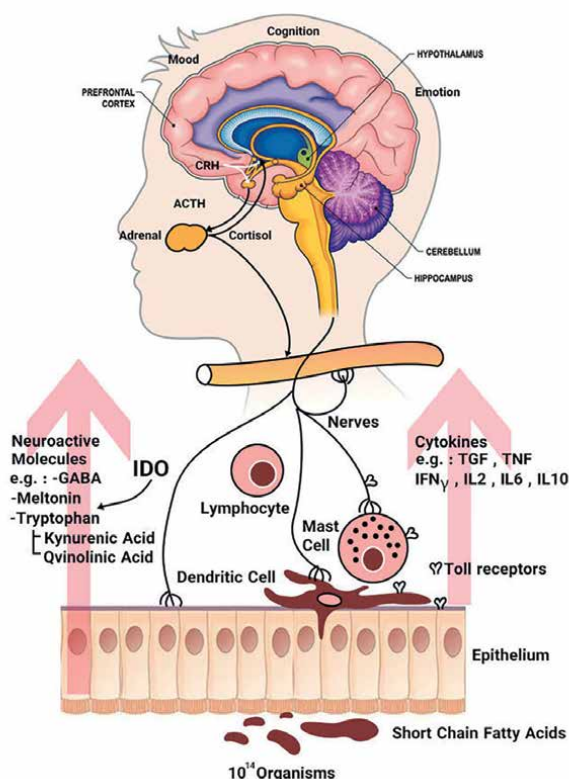


**Figure 3.**  
 Bidirectional interaction between gut microbiota and central nervous system. Intestinal flora can send signals to central nervous system by producing neurotransmitters or bioactive compositions/directly interact with immune cells and epithelial cells [101].

which modulates the behavior and are essential for suitable brain development and brain tissue homeostasis, and furthermore, they have hormone-like activity as well as have immunomodulatory properties, stimulate the sympathetic branch of the autonomic nervous system by interacting with nerve cells [96]. Researches shown that SCFAs can adjust the synthesis of gut-derived serotonin from enterochromaffin cells and production of gut peptides from enteroendocrine cells that can regulate gut-brain hormonal communication. Disruptions in the SCFAs metabolism can result in the development of autism [97]. Due to all the mechanisms and properties of gut microbiota which are discussed above, the dysbiosis and any disturbance of the complex of intestinal microbiota can lead to many diseases of the nervous system, neuroimmune disorders, and neurodegenerative and behavioral disorders such as anxiety, autism spectrum disorders (ASD), [98], depression [99], Alzheimer's disease (AD), and Parkinson's disease (PD) [100]. Intestinal inflammation is correlated with changes in bowel-brain interactions which is also related with different anxiety and inflammatory bowel disorder (**Figure 3**) [102].

## 5. Probiotics/psychobiotics and gut-brain axis disorders

As discussed above, the intestinal microbiome and its microbial balance and comprehensive health play a very important and vital role in the functioning of the digestive system, maintaining immune homeostasis, host metabolism, and brain function under the name gut-brain axis [103] and any disruption in the composition of these microbe communities which is called dysbiosis leads to important disorders in its function [104]. There is a considerable difference in gut microbiota of Parkinson's disease and Schizophrenia patients with healthy ones [105], and additionally, it has been demonstrated that the intestine flora of Alzheimer's patients has reduction in number of useful bacteria like *firmicutes* and *Bacteroidetes* [106]. Electrophysiological studies demonstrated that probiotics help the maintenance of intestinal microbial communities and improve their function [107]. Some researches validated that administration of some probiotic strains such as *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 can restore the intestinal barrier and be used in the treatment of CNS disorders [108, 109]. Furthermore, studies showed that treatment with *Lactobacillus rhamnosus* and *Lactobacillus helveticus* in rats is able to prevent bacterial translocation and gut barrier function in chronic psychological stress [110]. Different strains of probiotics could be used in various disorders that have led to new interests in developing psychobiotics [111]. Intestinal bacteria and probiotics have the ability to produce various neurohormones including melatonin, serotonin, catecholamines, histamine, acetylcholine, SCFA, and  $\gamma$ -aminobutyric acid (GABA) that can regulate brain function and behavior by participation in gut-brain communication [112]. Most important of these neurohormones is GABA which is the key inhibitory neurotransmitter in the CNS and has the significant duty in physiological processes within microbiota-gut-brain axis (MGBA), and any changes in expression of GABA receptors could be result in anxiety and depression. Applying *L. rhamnosus* (JB-1) and other probiotics in the animal model has modulated expression of GABA receptors in the CNS and reduced expression of corticosterone resulted in restriction of depression and anxiety; in consequence of probiotic effects on CNS the term psychobiotic has been applied [113]. Psychobiotic first defined by Dinan et al. is the sub-class of probiotics that have positive psychotropic effects on CNS and neuronal functions by anxiolytic and antidepressant activities when colonized in intestine

in adequate amount which interact with brain through different ways such as endocrine, metabolic, and secreting neuroactive metabolites, GABA and SCFAs [114, 115]. *Lactobacillus* and *Bifidobacterium* are the main psychobiotic that secrete GABA that affect gut-brain axis and regulate physiological and psychological processes [116]. Colica et al. in 2017 in a clinical trial on humans showed that psychobiotic significantly reduced anxiety parameters in whom had depressive symptoms [117]. A study on mice applying probiotic *Lactobacillus reuteri* showed that it can modulate immune system as well as decrease anxiety by reducing corticosterone which is a stress factor [118]. Additionally, as important metabolites produced by intestinal bacteria and especially probiotics, SCFAs play an important role in the health of the human digestive system. Butyrate has anti-depressant properties and plays an important role in reducing stress, which can enhance the treatment with psychobiotics [118].

## 5.1 Alzheimer's disease

Alzheimer's disease (AD) is the most common and progressive neurodegenerative disease that is the main cause of 80% of dementia in the world especially in people over 60 years [119]. AD is characterized by memory problem and cognitive decline that can lead to progressive loss of mental ability and affect speech, behavior, and motor system. Amyloid precursor protein (APP) is the main factor involved in Alzheimer's disease [120]. Accumulated clinical studies implicated that probiotics have various therapeutic accepts in AD through different mechanisms, and they can regulate the homeostasis of gut microbiota, reduce, delay the progression of AD, especially oxidative stress and inflammatory reaction, and as a result improve cognitive decline [121]. The most important mechanisms that probiotics can influence brain function are neuronal regulation, immune modulation, and endocrine pathways. Probiotics can incite adrenal release of cortisol that is a vigorous anti-inflammatory hormone and activate the hypothalamic-pituitary-adrenal (HPA) axis via endocrine pathways, stimulate the production of glucagon-like-peptide-1 (GLP-1) and peptide YY (PYY) hormones, secrete glutamate (GLU), and regulate secretion of neurotransmitters via serotonin. All of these neurotransmitters and neuroactive metabolites lead to preventing neuronal apoptosis [122]. *Lactobacillus* and *Bifidobacterium* genera are the most used probiotics in AD treatment [123].

## 5.2 Parkinson's disease

Parkinson's disease (PD) is the most common neurological disorder after Alzheimer's disease, which affects a large number of people every year [124]. The age of contracting this disease is 65 years and above, and the possibility of contracting it is more in men than in women [125]. This disease mainly begins with the loss of motor neurons, and neurons in different parts of the brain lead to the formation of abnormal intracellular deposits called Lewy bodies. The complications of this disease are movement disorders such as resting body tremors, muscle stiffness, balance problems, and non-movement disorders, including gastrointestinal disorders, olfactory, sensory, pain, and sleep disorders [126, 127]. Scientists suggested that gut microbiota can be related to PD, and changes in intestine flora composition could be correlated with a motor phenotype in PD which can result in  $\alpha$ -synuclein aggregation, inflammation, and Lewy body (LB) generation [128]. Various clinical studies approved that the consumption of probiotics supplements can reduce and modulate some symptoms in PD patients [129]. Administration of some probiotics such as

*Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, and *Lactobacillus reuteri* can improve certain symptoms in PD patients [130], including altering gut microbiota composition in PD, enhance GI function such as constipation, increasing the movement of spontaneous bowel, alleviate abdominal pain and bloating, and reduce gut leakiness [131]. Some other studies have shown that treatment with probiotics in some animal models with PD could reduce the number of apomorphine-induced spins by inducing 6-OHDA [129]. Other studies approved that administration of *Clostridium butyricum*, *Lactobacillus plantarum* CRL 2130, *Streptococcus thermophilus* CRL 808, and *Streptococcus thermophilus* CRL 807 improve motor skills [132].

### 5.3 Anxiety and depression

Depression and anxiety disorders are considered as most common and frequent mental health conditions and are complex heterogeneous psychiatric disorders that are in the top five mental disorders in USA, and about 4.4% of the world's population suffer from them and are extremely comorbid with other mental disorders [133]. Depression and anxiety disorders are related to CNS non-immune-mediated disorders. Evidences showed that modulation of neuroimmune by gut microbiota, its composition, and metabolites has a significant role in CNS disorders [134]. There is significant co-morbidity between anxiety and GI disorders such as irritable bowel disorder (IBS) and inflammatory bowel disorder (IBD) that show the importance of gut-brain axis in psychiatric disorders [135]. Current pharmacological treatments for anxiety disorders can cause consuetude problems and not be so effective in the treatment of it. Consequently, scientists suggested other novel treatment approaches such as applying probiotics and psychobiotics [136]. Firstly, Logan and Katzman suggested the administration of psychobiotics in the treatment of depression and anxiety disorders [136]. Probiotics can influence mood and stress symptoms by regulating cytokines production and ameliorating lactose maldigestion which is correlated with depression symptoms. Also, consumption of probiotics has significant role in IBD treatment and, as a result reduction of anxiety and depression signs. The most effective probiotic strains in depression treatments are *L. helveticus*, *L. rhamnosus*, and *L. casei*, as well as *B. longum*, *B. breve*, and *B. infantis* [137]. Despite numerous clinical studies on the effectiveness of probiotics in the treatment of neurological diseases and psychiatric disorders, further studies are needed to prove the probiotics and psychobiotics administration and effectiveness on these diseases [138].

## 6. Conclusion

The gut-brain axis is a bidirectional communication network between intestinal and the central nervous system (CNS) through different pathways including endocrine, humoral, metabolic, and immune known as gut-brain axis. Moreover, changes in gut microbiota composition may be associated with many psychiatric disorders such as Alzheimer's disease, Parkinson's disease, anxiety, and depression. In addition, probiotics as living bacteria have an essential role in gut microbiota function; furthermore, different studies suggested the administration of probiotics could improve the maintenance of intestinal microbial communities and their function. Nowadays, scientists recommended that the application of probiotics strains could be effective in neurological disorder treatment.

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# Exploring *Enterococcus* Species for their Next-Generation Probiotics Potential

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## Abstract

The genus *Enterococcus* is the third largest genus in the group lactic acid bacteria and has ubiquitous distributions with plenty of biomedical as well as other industrial applications. Tolerance to harsh conditions, genome plasticity, antimicrobial potential, enterocins production, and greater survivability are the key properties of enterococcal species that make them a suitable probiotic agent. Likewise, the presence of dozens of virulence traits, antibiotic resistance, and opportunistic pathogenic nature raises a serious concern regarding their safety. Still, it is a debate whether enterococcal species are used as probiotics or not, but their current industrial applications and preliminary positive attributes indicate their next-generation probiotic potential. Recent advancements in molecular techniques and genomic elucidation studies have increased the number of enterococcal species to more than 80, dominated by *Enterococcus faecium* and *Enterococcus faecalis*. A greater number of enterococcal species are identified in the twenty-first century, and thus, their next-generation probiotic potential is not defined yet. Many of the recently identified species are targeted for different applications and they showed promising results indicating the need to investigate their NGP potential. Hence, this chapter aims to provide the recent and updated literature about the common enterococcal species, their distinguishing characteristics, and the available data that revealed or directed their next-generation probiotic potential.

**Keywords:** *enterococcus*, probiotics, next-generation probiotics, enterocins, *E. faecium*

## 1. Introduction

The term probiotic is derived from a Greek word that means for life, and this concept was developed by Elie Metchnikoff (a Nobel laureate) in the early twentieth century. He proposed that changing gut microbiota with friendly bacteria can help to extend the organism's life. Since then, researchers around the globe have been dedicated to establishing their scientific basis and trying to identify new probiotic strains. Back in the 1960s, Lilly and Stillwell coined the term probiotics and defined that probiotics have the immunomodulatory potential, restoring gut microbiota and enhancing intestinal functions [1]. At the beginning of the twenty-first century (2001), the World Health Organization (WHO) and Food and Agriculture Organization (FAO)

proposed a concise definition of probiotics that was then updated by the panel of the International Scientific Association for Probiotics and Prebiotics (ISAPP). According to the ISAPP probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host [2, 3]. Probiotics have certain selection criteria that must be followed, while claiming the strain's probiotic potential. These selection properties include a safe origin, free from virulence factors, pathogen-killing potential, no antibiotic resistance, adherence properties, survivability in the gut conditions, potential to produce antimicrobial substances, tolerance to harsh conditions, and the ability to be viable after getting consumed [1, 4–10]. The pathogenicity, virulence factors and antibiotic resistance, toxicity, and the strain's intrinsic properties are considered during the probiotic safety [11]. Additionally, various health benefits and different functional properties are also considered during probiotic strain selection. The probiotic mechanism of action largely depends on their effectiveness in gut conditions, the expression of surface molecules, and their potential to compete with pathogenic microbes while producing antimicrobial substances [6, 7, 12–14]. Probiotics have a vast spectrum of health benefits that include the strengthening of the immune system and intestinal functions, reducing allergic reactions and metabolic disorders, enhancing food digestion, and restoring the gut microbiota [3, 8, 9, 15–17]. Probiotics also help in the prevention and treatment of psychological disorders, combating the aging process, and have good therapeutic potential [18–21].

Currently, seven genera of microorganisms are used as probiotics. *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* are widely used probiotic microorganisms [22, 23], despite there are unaccountable microbes (approximately 1159,000 species) in our environment that affect our life activities in different ways [24]. To explore the probiotic potential of microorganisms, they are divided into safe strains, doubtful strains, and risky strains indicating their wide usage, usage with extensive investigation, and not recommended for usage, respectively [1]. The genus *Enterococcus*, which is the third largest genus of LAB, falls into the doubtful categories as they have an opportunistic pathogenic nature. Additionally, this genus is still not included in the Generally Recognized As Safe (GRAS) and Qualified Presumptions of Safety (QPS) status microorganisms [2]. Their pathogenic nature is attributed to the presence of both intrinsic and acquired antibiotic resistances and more than a dozen virulence traits. These virulence factors, that is, hemolysin production, aggregation substances, gelatinase and DNase production, are the causative agents of multiple diseases [1]. These enterococcal diseases include bacteremia, endocarditis, abdominal pain, urinary tract infection, etc. and thus enhance the pathogenic nature of the genus [22, 25–27]. Besides its pathogenic nature, the genus also has various applications in different industries and also has proven probiotic strains. It is used in the pharmaceutical, food, and cheese industries, has a role in medicine (human and veterinary medicines), and can be applied as co-cultures, starter cultures, protective cultures, etc. [1, 27–30]. Thus, a controversial debate still exists whether enterococcal species are used as probiotics or not [31–34]. It is important that the current available enterococcal probiotics have not been associated with any kind of complications or side reactions, since using them for decades. For instance, *Enterococcus faecium* NCIMB 10415 and Symbioflor® 1 are used without any adverse reactions [2, 30, 35]. The main player in their probiotic potential is the production of enterocins, ubiquitous distribution, tolerance to harsh conditions, genome plasticity, and other positive potential [2, 36].

The genus *Enterococcus* has ubiquitous distribution and is found in different environments, representing the third largest genus of LAB with more than 80 well-identified species [2, 37, 38]. Genus *Enterococcus* is one of the most primitive

Gram-positive bacteria that originated approximately 500 million years ago and is found in plenty of environments, including the guts of humans and other animals [39, 40]. Phenotypically, it is ovoid in shape, Gram-positive, catalase and oxidase-negative, aero-tolerant, homofermentative, and non-motile microbes that have the potential to be used in different industries [25, 40–44]. Due to its excellent survivability in different environmental niches, it is also considered fecal-indicating bacteria (FIB) [37]. Its genome ranges from 2.3 to 5.4 Mbp with 34–45% GC contents and approximately 2154–5107 prescribed genes [27, 42]. The general spectrum properties of the genus *Enterococcus* are summarized in **Figure 1**.

Among the enterococcal species, *E. faecium*, *E. faecalis*, *E. hirae*, *E. casseliflavus*, *E. mundtii*, etc., have more prevalence. Both *E. faecium* and *E. faecalis* constitute a high percentage of the total species prevalence, while *E. faecium* is considered more virulent and *E. faecalis* is highly resistant [37]. Being isolated from hospital settings and foodstuffs, it was observed that high-virulent species are mostly present in hospital sources, while safe species or industrially used species are greatly isolated from food sources [22, 26, 45]. The potential of enterococci to be used as probiotics is currently being investigated, predominated by *E. faecium*, among other investigated species. Recently, the concept of next-generation probiotics was developed, which aims to identify novel bacterial species with probiotic properties despite not being used for this potential. This NGP concept is applied to many species, including those of the genus *Enterococcus*. The common enterococcal species are well-studied for their probiotic potential, but with more than 80 well-identified species, it is worth exploring the remaining species with similar potential.

Hence, this chapter aimed to provide recent, up-to-date, and relevant literature about the potential use of enterococcal species as next-generation probiotics. This will also shed light briefly on probiotics, the genus *Enterococcus*, and next-generation probiotics. In this chapter, the less common enterococcal species are explored and

| The general spectrum of genus <i>Enterococcus</i> |                                |                           |                           |                                  |                            |
|---------------------------------------------------|--------------------------------|---------------------------|---------------------------|----------------------------------|----------------------------|
| Prevalence<br>Ubiquitous<br>distributions         | Classification<br>Seven groups | Properties<br>General     | Resistance<br>Antibiotics | Virulence<br>Traits              | Applications<br>Industries |
| Air                                               | <i>E. faecium</i>              | Enterocins<br>productions | β-lactams                 | Gelatinase                       | Food industry              |
| Soil                                              | <i>E. faecalis</i>             | Tolerance                 | Aminoglycosides           | Enterococcal<br>surface proteins | Pharmaceuticals            |
| Water                                             | <i>E. gallinarum</i>           | Aggregation               | Glycopeptide              | Hemolysin                        | Meat industry              |
| Food                                              | <i>E. avium</i>                | Survivability             | Quinolones                | Lipases                          | Cheese<br>productions      |
| Animals                                           | <i>E. cecorum</i>              | Adherence                 | Macrolides                | Ureases                          | Flavor<br>development      |
| Birds                                             | <i>E. dispar</i>               | Viability                 | Linezolid                 | DNAse                            | Dairy products             |
| Humans                                            | <i>E. saccharolyticus</i>      | Genome<br>plasticity      | Lincosamides              | Sex pheromones                   | Probiotics                 |

**Figure 1.**  
The general aspects of genus *Enterococcus* indicate environmental distribution, group classification (each group contains different species), distinguishing properties, health profile (antibiotic resistance and virulence traits), and important applications.

documented for their NGP potential, either already described in the literature or anticipated after the species' properties exploration. Thus, approximately 30 enterococcal species are explored, highlighting their NGP potential.

## 2. Next-generation probiotics

The commonly used probiotics belong to the genus *Lactobacillus* and *Bifidobacterium*, followed by the genus *Enterococcus*. Species of these genera are explored and commercialized as probiotics. The *Lactobacilli* species are even more studied for their different potentials, including their potential as anti-aging anti-depressant and anti-neurodegenerative disorders. Over the period of time and with the advancement of technologies, the scientists have tried to elucidate other microbes that are not commonly used as probiotics. This switch to identify new species was vastly based on the strain-dependent phenomena of the probiotics and their intrinsic properties. Thus, the identification and use of probiotics with enhanced functions, greater potency, and disease-specific potential in the area of next-generation probiotics is considered a potential and emerging area of research [46–48].

Next-generation probiotics (NGP), or live biotherapeutics products, denote those microorganisms and species that have never been used before. NGPs are beneficial microbes that are not purely proposed, like traditional probiotics as foods or dietary supplements, but have pharmaceutical applications [49]. Many previously unrecognized probiotic strains have been isolated and identified from the intestinal microbiota using modern next-generation sequencing techniques, and these next-generation probiotics have become potential sources for novel drugs as therapeutics for different diseases [48]. Currently, candidates have been identified from the gut microbiota that are associated with health benefits, like species of the genera *Bacteroides*, *Clostridium*, *Faecalibacterium*, and *Akkermansia*, as well as some genetically modified (GM) strains [49]. All the investigated or experimentally validated strains have advanced properties compared to conventional probiotics, and numerous metabolites like folate, indoles, serotonin, gamma-aminobutyric acid (GABA), short-chain fatty acids (SCFAs), etc. have been identified, which play an important role in the host physiology [46]. The human GIT contains approximately 100 trillion microorganisms (including bacteria, fungi, archaea, viruses, and protozoa), as elucidated during the human microbiome project. Each species has its own nutritional requirements, which makes it difficult to properly recognize them. It is the glory of genome sequence that allows to identify such a large number of microbes with detailed properties. The presence of such a large number of microbes in the human gut can be targeted as probiotics if identified properly in terms of their nutrition, growth properties, and genomic construct [47]. Fortunately, with the advancement of technologies, different species are identified and targeted for next-generation probiotic potential. The general properties of NGPs are illustrated in **Figure 2**.

To ensure the safety and ethical consideration of NGPs or other biotherapeutics are guaranteed, while following different guidelines, different countries developed their own safety assessment measures, which helped them properly regulate and ensure the safety of NGPs. For instance, in the EU, the European Food Safety Authority (EFSA) is responsible for the safety assessment of novel foods, including NGPs. The species taxonomic classification, complete genome sequencing, probiogenomic analysis, and investigation of virulence and antibiotic resistance traits, etc. are the prime elements that should be properly documented for NGPs [50].

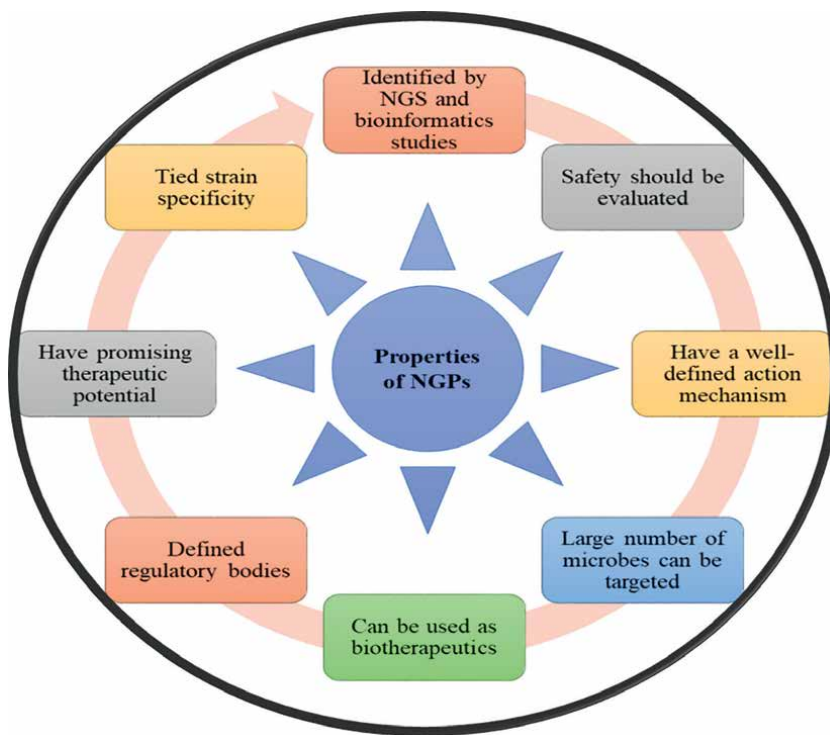


**Figure 2.**  
 The general characteristics of next-generation probiotics.

## 2.1 Prospects of next-generation probiotics as biotherapeutics

Probiotics have been used as fermented foods by humans for a long time, but the beneficial effects of these bacteria were not well understood until the development of new and emerging techniques. Probiotics of the current generation are demonstrated to have a role in the maintenance of the gut microbiota and can reduce inflammatory responses, allergic illnesses, and autoimmune diseases [51]. New NGPs with potential beneficial characteristics for human health expand this traditional probiotic spectrum and contribute to the development and elaboration of new food products that respond to the population's growing interest in health and quality of life, again making the NGPs an important area in the food industry. The biotherapeutics potential of NGPs should be stated after passing the three phases of clinical trials, that is, preclinical, toxicological studies, and pharmacodynamics [46, 52–54].

The availability of microbe-free animal models will aid in the identification of safe NGPs that meet regulatory parameters such as the US-LBP FDA's program (US Live Biotherapeutic Products, Food and Drug Administration). The efficacy and safety of the next generation of probiotics and the technological elements of using these microbes in food preparation are considered the most important concerns in processing. The majority of these microorganisms have metabolic properties that make them challenging to utilize as sustainable methods in the development of novel products, particularly in large-scale food production [53]. However, limited studies have been conducted to establish the effectiveness and safety of these microorganisms, necessitating more research in *in vivo* systems and clinical trials. In the food industry, there

must be studies that analyze the potential applications of next-generation probiotics in food matrices and their impacts on the intrinsic technical and sensory properties of food. In the future, these microbes may be employed as bio-therapeutic items and sold mostly as nutritional supplements [46].

## 2.2 The next-generation probiotics microorganisms

As described, the NGPs have advanced functions over traditional probiotics and are used in a specific application. A number of species, mostly from the gut microbiota, are targeted to be screened for their next-generation probiotic potential. These include *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Bacteroides fragilis*, *Eubacterium hallii*, *Bifidobacterium* spp., *Prevotella copri*, *Christensenella minuta*, and *Roseburia* spp. [48, 55, 56]. It is important to mention here that the NGPs include commensal species that have no previous history of safe use. Likewise, the conventional probiotics, with the beneficial effects of NGP, are also strain-dependent [47]. The NGPs of various bacterial species are previously documented [46, 57, 58].

## 3. The next-generation probiotic potential of enterococcal species

The NGPs potential of enterococcal species is also evaluated, along with that of other microbes. This potential is solely dependent on the genus intrinsic, chemical, biochemical, and genomic properties. Currently, well-defined and characterized enterococcal species such as *E. faecium*, *E. faecalis*, *E. mundtii*, *E. hirae*, and *E. lactis* are targeted for their next-generation probiotic potential and have been identified in a good number of strains. At the same time, both the newly identified and less explored species of enterococci can also be targeted for this NGPs potential in this advanced period. The development of advanced molecular techniques, like genome sequencing, can even make this potential easier. The genus properties, such as tolerance, aggregation, environmental prevalence, metabolite production, give additional attraction to the researchers to be targeted for NGPs. Likewise, the disease treatment potential, industrial applications, and as fecal indicator bacteria, the enterococcal species provide an excellent opportunity to be targeted. The presence of general NGP properties, the proposed enterococcal species, and the potential of next-generation probiotics are summarized in **Figure 3**.

The genus *Enterococcus* has unique features that make it a suitable agent to be targeted for next-generation probiotic potential. Although more than 80 species are identified so far as enterococcal species, very few are investigated for their probiotic potential. Certain species such as *E. faecium*, *E. faecalis*, and *E. mundtii* have been used for a long time, while strains from other species such as *E. italic*, *E. hirae*, and *E. gallinarum* are also elucidated as having good probiotic properties. The different enterococcal species and their next-generation probiotic potential are described below.

### 3.1 *Enterococcus faecium*

*E. faecium* is one of the most prevalent enterococcal species in the environment and has received exceptional attention for its probiotic potential. It is generally considered a safe species and is hence widely used in different industries. It has a small genome size (2.23 to 3.72 Mbp) and a short generation time, which make it suitable for its NGP potential. The production of multiple enterocins also aids in their NGP potential [1, 39]. *E. faecium* also showed greater environmental persistence and became dormant under stress conditions [39]. Currently, a good number of probiotic strains

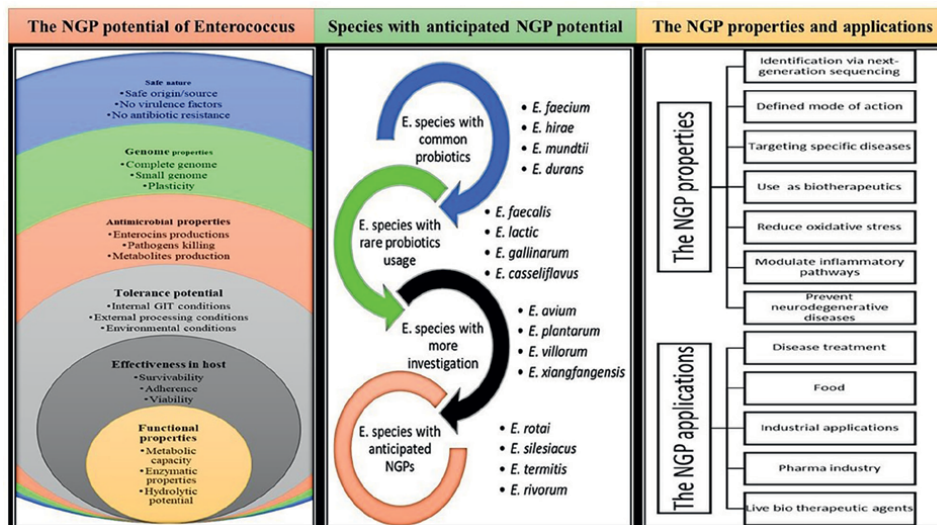


**Figure 3.** The next-generation probiotic properties of Enterococcus, the proposed species of enterococci for NGPs, the properties of NGPs, and their potential applications.

from *E. faecium* have been identified and developed commercially, indicating its excellent NGP potential. These developed probiotics have good potential to treat different diseases and aid in intestinal microflora restoration probably due to their short lag phase and generation time. Its strains can hinder the growth of *E. coli*, *Salmonella* Serovars, *Shigella* spp., and *Enterobacter* spp. *in vitro*, while also showing resistance to low pH and bile. Some strains showed anti-inflammatory and immunomodulatory potential, which is because of their inhibition of nuclear factor- $\kappa$ B [59–61].

There are vast areas in which *E. faecium* probiotics are used. For instance, they are used in the treatment of diseases, the restoration of gut flora, and as food and feed supplements for pets [29]. Some strains also decrease cholesterol and help in the production of cheese as starter culture [62]. *E. faecium* probiotics are taken *via* different routes, like oral and inhalation, depending on the specific applications [63]. The commercially available *E. faecium* probiotics, such as *E. faecium* SF-68 and ECOFLOR, have proven benefits and are not associated with any potential side effects [62]. *E. faecium* M74 strains in boilers increase serum calcium contents and antioxidant status [64]. Likewise, the heat-killed *E. faecium* NHRD IHARA is suggested to be used as feed additives [65]. Its potential in veterinary medicine is also elucidated and found to have promising results [66]. Thus, highlighting these probiotics, it is concluded that *E. faecium* has excellent NGP potential and can be explored for more applications.

### 3.2 Enterococcus faecalis

*E. faecalis* has the highest prevalence in hospital settings and is considered one of the virulent enterococcal species. Generally, *E. faecalis* causes 75–80% of the enterococcal infections. Due to its widespread distribution and the ability to tolerate harsh conditions, it is often isolated from medical settings, while a low proportion is also collected from other environmental niches. It has a larger genome size (2.7 Mb) than *E. faecium* and generally shows more virulence characters instead of resistance [1, 37].

Unlike *E. faecium*, the NGP potential of *E. faecalis* is less investigated due to its apparent virulence profile, but still, few strains of it are commercialized as probiotics. For example, Symbioflor® 1 is a leading *E. faecalis* probiotic that has the properties of immune regulation and combats chronic sinusitis and bronchitis [40]. In combinatorial treatment, the effectiveness of Symbioflor® 1 was found to enhance the treatment potential of respiratory diseases [67, 68]. Upon extensive investigation, the *E. faecalis* strains can be used in different applications, including medicinal aspects and food supplements. *E. faecalis* strains are generally considered in that category, which requires extensive characterization, particularly their genomic analysis.

### 3.3 *Enterococcus hirae*

*E. hirae* is a non-motile enterococcal species that was identified in 1985 and has lower environmental prevalence. It is frequently isolated from the intestinal flora of different domestic animals [69]. It is associated with different diseases, like septicemia and focal necrosis. *E. hirae* has the potential to produce acid from melibiose and sucrose and shows close resemblance to *E. durans* [69]. Its NGP potential is explored due to its anti-inflammatory and antibacterial potential. *E. hirae* strains also have tolerance potential, good hydrophobicity, and no antibiotic resistance. The ability to produce enterocins and their antibacterial activity against *Micrococcus luteus*, *Listeria monocytogenes*, *Salmonella* Typhi, *Staphylococcus aureus*, *Shigella flexneri*, *Pseudomonas fluorescens*, *E. coli* O157:H7, etc., can aid in their NGP potential [70]. Its major enterocin is Hiracin JM79, which belongs to Class IIa antilisteria-pediocin-like bacteriocins [71].

The currently available *E. hirae* probiotics not only confirmed their NGP potential but can also indicate their widespread applications. These NGPs show the therapeutic potential and have immunomodulatory effects without any documented side effects [72]. Adnan et al. documented the NGP potential of *E. hirae* F2 strain and identified its antimicrobial and antioxidant activities, while suggesting its promising probiotic potential [73].

### 3.4 *Enterococcus durans*

*E. durans* has close similarities with *E. hirae* and has a low environmental prevalence [74]. It is found in the gut in rare cases and is responsible for different animals' diseases like enteritis [69]. Characteristically, it is positive for the arginine test and shows negative tests against arabinose, raffinose, pyruvate, and sorbose [74]. Like other enterococcal probiotics, *E. durans* also has the tolerance potential, adherence capacity, and ability to colonize mucosal surfaces, have no virulence traits, show antibacterial potential, and have immune modulatory effects. It also has an effect on cytokine production and enhances IgA secretion [75]. These properties enable *E. durans* strains to be targeted as NGP and, hence, play a role in wide applications.

Different *E. durans* strains are identified as having good NGP potential. For instance, *E. durans* M4 was found to have anti-inflammatory effects and preserve intestinal epithelial integrity, indicating its prophylactic potential [76]. Another strain, *E. durans* VJI19, was found to have antifungal and pro-apoptotic activities based on its palmitic acid and 2,3-dihydro-3,5-dihydroxy-6-methyl 4H pyran-4-one.

Additional properties such as the presence of hydrophobicity, tolerance, and colonization potential suggest its NGP potential [77]. Likewise, the potential of *E. durans* 6H and *E. durans* LAB18s was also screened and identified its NGP potential [60, 78]. Human serum cholesterol lowering strain *E. durans* KLDS 6.0930 is also documented as a potential probiotic strain [8].

### 3.5 *Enterococcus lactis*

*E. lactis* has a close resemblance to *E. faecium* and was first isolated in 2012 from milk. It has distinct genomic and carbohydrate metabolism properties and is thus considered a separate species [79, 80]. The absence of antibiotic resistance genes and virulence traits makes the species more suitable for NGP potential even than *E. faecium* with applications in the food and health industries [80]. The disease-causing potential of *E. lactis* is much less than that of *E. faecium*. Due to their close resemblance with *E. faecium* some probiotics that are claimed to contain *E. faecium* actually contain *E. lactis* [81]. *E. lactis* shows gelatinase negative potential and carries enterocin-producing genes [79]. The existing *E. lactis* probiotic strains mostly have the cholesterol-lowering potential and susceptibility to antibiotics, besides other probiotic properties [79].

Once the *E. lactis* is recognized as a separate species, it attracts researchers to identify its NGP potential because of its apparent safe profile and enterocins production capacity [79]. The NGP potential of *E. lactis* makes it a suitable candidate to prevent and help in the treatment of different human diseases, like malignant tumors. It prevents the tumor in different ways, such as reducing the expression of pro-carcinogenic enzymes, improving normal cell proliferation, and hindering inflammation-associated cell apoptosis. For instance, *E. lactis* BT 16 and *E. lactis* MTCC 25438 strains, which are isolated from cheese and goat milk, respectively, have the cholesterol-lowering potential and thus indicate their NGP potential [68, 82]. Other strains, such as *E. lactis* YHC20, *E. lactis* FM11-1, and *E. lactis* IW5, showed good cellular hydrophobicity and carbohydrate and bile salt hydrolase (BSH) activity [83–85]. The NGP potential of strain *E. lactis* PMD74 was investigated, and it was observed that, besides other probiotic properties, this strain shows greater survivability in fermented milk [86].

### 3.6 *Enterococcus gallinarum*

*E. gallinarum*, a motile microbe, besides other enterococcal species, is part of the normal stool flora of chickens and cattle but was also isolated from clinical samples. It is considered less pathogenic than other clinically isolated species [87–90]. It shows 99.8% homology in 16S rDNA similarities with *E. casseliflavus*, making it difficult to distinguish [91]. It causes common enterococcal infections such as bacteremia, endocarditis, and meningitis. A rare effect on the central nervous system is also identified [92, 93]. It is separated from its close partner, *E. casseliflavus*, as it does not produce the yellow pigment [89].

The next-generation probiotic potential of *E. gallinarum*, though it is not authorized for probiotics, is still established and some strains have been found that have the NGP potential [94]. The NGP potential is mostly dependent on the strains antimicrobial potential; for example, *E. gallinarum* T71 and W21 were found to have broad-spectrum activity and hence can be investigated for use as starter cultures in

foods [95]. Another strain, *E. gallinarum* L-1, was evaluated on the cellular immune systems of four different fish species and found to stimulate the leucocytes of the tested fishes [96].

### 3.7 *Enterococcus casseliflavus*

*E. casseliflavus* is the second motile specie of the genus *Enterococcus* that has the capacity to produce a yellow pigment, and hence, it is separated from other motile specie, that is, *E. gallinarum* [89, 97]. This species shows an environmental adaptation to aquatic vegetation [97]. Its inulin fermentation property makes it a distinguishing species and has the potential of environmental persistence, facilitated by cell-associated carotenoid pigments that also protect it against photooxidation [89]. Another unique feature of this specie is the presence of acetoin dehydrogenase genes (ECAG\_02019 to ECAG\_02022), which helps in the conversion of acetoin to acetaldehyde and acetyl coenzyme A [89]. Despite enterococcal species, it is considered a less virulent species [94].

Unlike other enterococcal species, *E. casseliflavus* and *E. gallinarum* are not authorized for probiotic potential, but recently they were found in two probiotic products indicating their NGP potential [94]. This potential is mostly because of its antimicrobial potential, bacteriocins production, improving growth performance, immune modulation, and resistance against pathogens [98]. Studies also showed the tolerance potential of *E. casseliflavus*, particularly in its fermentation profile [99]. Deng et al. [94], identifying the new insight of antibiotic resistance in enterococcal probiotics, declared that both *E. casseliflavus* and *E. gallinarum* are free from antibiotic resistance genes but can carry low-level vancomycin resistance genes (i.e., *vanC*), thus influencing the host immunity [23, 94, 100]. Recently, a study was conducted to identify the response of cancer patients to chemotherapy treatment, and it was identified that *E. casseliflavus* is more abundant in responding compared to non-responding patients, thus indicating its potential to be used as a biological biomarker for chemotherapy response in lung cancer patients [101].

### 3.8 *Enterococcus avium*

*E. avium* is a member of the genus *Enterococcus* that is commonly isolated from bird droppings [87] and was previously included in group Q *Streptococcus* [74]. It is also isolated from hospital settings and identified as showing similar resistance to *E. raffinosus* [74], but being negative to raffinose metabolism, it is considered a separate species [102]. The pathogenic nature of this specie is less understood but still involved in different enterococcal infections, including peritonitis [74]. *E. avium* also has the potential to produce enterocins like Avicin A, a double-glycine lead bacteriocin belonging to the class IIa anti-listeria-pediocin-like bacteriocins [71].

The next-generation probiotic potential of *E. avium* is rare, but there are some positive attributes that might be helpful in its NGP status. For instance, the production of Avicin A, which is isolated from babies' feces, indicates its distribution in the GIT and helps in the maintenance of normal commensal flora [71]. The results of another study showed that *E. avium* has no virulence factors, antibiotic resistance, inhibits the growth of *L. monocytogenes*, shows adhesion to epithelial cells, and has greater cholesterol assimilation potential. These properties indicate the NGPs of *E. avium* and could be used in functional foods, particularly in broiler chicks [103, 104].

In contrast, a sample isolated from animals in Tunisia indicated the presence of vancomycin resistance in *E. avium* isolate [105].

### 3.9 *Enterococcus mundtii*

*E. mundtii* belongs to the group *E. faecium*, has ubiquitous distribution, and is found in plants, the gut of animals, and raw milk with low GC contents of 38–39% [106]. Functionally, *E. mundtii* lacks catalase and cytochrome C oxidase enzymes but can perform carbohydrate fermentations. It has the potential to produce enterocins, that is, Bacteriocin ST15, and thus has antibacterial activity against *Pseudomonas*, *Clostridium*, *Klebsiella*, *Lactobacillus*, *Acinetobacter* [106].

The NGP potential of *E. mundtii* is greatly investigated and identified, as many strains have the NGP potential. Tolerance, hydrophobicity, higher auto-aggregation, and no hemolytic activity, besides no antibiotic resistance genes, are the major probiotic attributes [107]. The NGPs potential is mostly because of its bacteriocin and enzyme production abilities, anti-pathogenic capacity, and tolerance potential. Nawaz et al. documented the different enzymatic activities (i.e., proteolytic, lipolytic, amylolytic, and cellulolytic) of *E. mundtii* QAUEM2808, suggesting its role in aroma and flavor development in food [106]. Likewise, another strain, *E. mundtii* ST4SA, is also documented as a potential probiotic strain [8]. Grau et al. documented that administration of the *E. mundtii* strain in *Tribolium castaneum* (the red flour beetle) indicated the desired probiotic properties, increasing the researcher's interest in using this microbe for *in vitro* probiotic characterization [107]. Similarly, experiments conducted to check the strain's survivability in the presence of antibiotics indicated that *E. mundtii* ST4V has the potential to survive in the presence of most of the administered antibiotics, thus, suggesting its potential to be used as a probiotic in those individuals who are receiving medical treatment [108].

### 3.10 *Enterococcus raffinosus*

*E. raffinosus* is a non-motile, enterococcal species, first identified in 1989, and shows negative tests for tellurite and arginine but positive tests for mannitol, sorbose, arabinose, raffinose, and pyruvate [74, 109–111]. In recent years, the pathogenesis in humans has been identified but it is considered a less pathogenic species, causing infections with heterogenicity like sinusitis, urinary tract infection, vaginal infection, and decubital ulcer [74, 102, 109]. This bacterium also harbors the *VanA* genes, thus creating the glycopeptide resistance [74]. The complete genome of *E. raffinosus* CX012922 was conducted by Zhao et al., which showed a close relationship with *E. gilvus* and *E. avium*, and had pathogenicity-encoding genes in its genome. The presence of giant mega-plasmids (~1 Mb) in its genome is the key feature of this species, which provides it with different functions including host adaptation [110]. The *E. raffinosus* less pathogenic nature and good sugar metabolic potential can be targeted for its next-generation probiotic potential. However, to the best of our knowledge, there are no current reports showing the NGP of this species.

### 3.11 *Enterococcus villorum*

*E. villorum* belongs to the *E. faecium* group and is mostly isolated from domestic animals [69]. It is separated from other enterococcal species using methyl- $\beta$ -D-glucopyranoside and sucrose tests, while a positive D-xylose test is used occasionally

[69]. Due to the limited information, it is difficult to determine the exact nature, different potentials, and applications of this species. The sequence of *E. villorum* strain LMG 12287 T was found identical to *E. porcinius* strain ATCC 700913 T, hence creating confusion but the name *E. villorum* was used earlier than *E. porcinius*, and hence, the latter is considered a junior synonym of *E. villorum* [112]. A study conducted on the comparative genomics of enterococcal species showed tetracycline resistance genes in *E. villorum* [113]. Likewise, prophages from the Phycodnaviridae family and type II CRISPR-Cas system genes were also identified in *E. villorum* [113].

Due to the scarcity of available data on the different aspects of *E. villorum*, it is difficult to explore the NGP potential of this species. To the best of our knowledge, there are no data touching its NGP potential. But we anticipate that the presence of CRISPR-Cas genes and sugar metabolism can be used to identify its next-generation probiotic potential.

### 3.12 *Enterococcus viikkiensis*

*E. viikkiensis* is a member of the genus *Enterococcus*, which shows light gray translucent colonies on its growth media. It was isolated from broiler and broiler production lines [114, 115]. Characteristically, this species grows on bile-esculin agar and temperatures ranging from 10 to 37°C but cannot grow at 4 and 45°C, but shows tolerance to 6.5% NaCl [116]. Li et al. isolated a new enterococcal species, that is, *Enterococcus xiangfangensis* that has similarities with the *E. viikkiensis* strain [117, 118]. In the study by Glaeser et al., it was also identified that *E. viikkiensis* strains harbor the VanA gene besides other *E. lemanii* clusters [115].

The health profiles, such as pathogenesis, enterocin production, probiotic potential, are still undefined for this species; hence, it is difficult to elaborate on its next-generation probiotic potential. In a broad sense, one study has shown that it harbors the vancomycin resistance genes, hence making it unfit for next-generation probiotic potential. But as its brighter side is not yet conclusively established, further studies are required that target its NGP potential.

### 3.13 *Enterococcus italicus*

*E. italicus* is a newly identified enterococcal species that belongs to group V and has now been isolated from a variety of cheeses around the globe [119–123]. Besides other sources, it is also isolated from dairy products [124]. It shows a close relationship with *E. sulfureus* and *E. saccharolyticus*, which have 96 and 97% 16S rDNA sequence similarity, respectively [114]. Its unique properties include no growth at 6.5% NaCl and the lack of acid production from L-arabinose, melibiose, raffinose, and ribose [114, 125].

The safety profile of *E. lactis* is good as there are no potential virulence factors or antibiotic resistance genes identified, thus representing its promising role in adjunct culture and use in different industries [121, 123, 126]. For instance, a study was conducted to evaluate the antimicrobial activity and safety of *E. italicus* GGN10, which was isolated from Tunisian bovine raw milk. The results showed its antimicrobial activity, stability over a wide range of pH, the production of enterocins (e.g., EntA, EntB), the absence of pathogenic islands, and the production of enzymes such as acid phosphatase and aminopeptidase. These positive attributes of this strain make them an important agent to use in flavor and texture development [122, 127, 128]. Likewise, the genomic analysis of the *E. italicus* DSM 15952 T-type strain (Accession PRJNA61487, ID

61487) showed the presence of genes responsible for the bifunctional enzyme, which is involved in glutathione production [121]. The aforementioned beneficial properties and its tolerance potential make it a promising agent for NGP potential. In contrast, the antibiotic resistance genes such as *tetS* (which encodes for a ribosomal protection protein) and *tetK* tetracycline-resistance were also determined in *E. italicus* [119].

### 3.14 *Enterococcus xiangfangensis*

*E. xiangfangensis* (named from the China district where it was isolated) is a non-motile species of *Enterococcus*, having a 1–1.5 mm size and giving circular and smooth colonies on the growth medium, that is, MRS agar [117, 118]. Its physiological properties include growth at 37 to 45°C, growing best at a basic pH of 11.0, tolerating a maximum of 5% NaCl, no ammonia being produced from arginine, and having the highest production of L-lactate, catalase negative, and homofermentative potential. It gives positive tests for pyrrolidonyl arylamidase and leucine arylamidase, while giving negative tests for  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase, alkaline phosphatase, and arginine dihydrolase [117].

*E. xiangfangensis* has a complex sugar profile and showed both positive and negative tests against different metabolites. Despite being positive against different sugar metabolites, its tolerance potential, particularly against low pH and high salinity, is not good. Hence, it is ambiguous whether its NGP potential is really targeted or not. There are no data available to explore its NGP potential, so its genomic analysis may help identify its correct position from the perspective of NGPs.

### 3.15 *Enterococcus phoeniculicola*

*E. phoeniculicola* was first isolated by Law-Brown and Meyers from the uropygial gland of wild red-billed wood hoopoes (*Phoeniculus purpureus*). Sequence analysis of the 16S rDNA showed 97.3 and 97.5% sequence similarities with *E. faecium* and *E. avium*, respectively [114]. It is separated from these species by its metabolic profile (i.e., it is unable to form acid from lactose, D-mannitol, and D-sorbitol) and its lower tolerance potential against 40% bile or 6.5% NaCl [114].

*E. phoeniculicola* positive attributes are not well known. Its next-generation probiotic potential is not elucidated yet. Highlighting its metabolic profile indicates positive potential, but the tolerance potential creates a difficult dilemma. The investigation of its safety and genome analysis will help to identify its positions in the NGP.

### 3.16 *Enterococcus plantarum*

*E. plantarum* was isolated from plants as non-motile, ovoid-shaped cocci during enterococcal identification and characterization from plant sources. Its 16S rDNA sequence shows similarities to the *E. faecalis* group while separated from its close species via phenylalanyl-tRNA synthase alpha subunit (*pheS*) and RNA polymerase alpha subunit (*rpoA*) gene sequence analysis. This species is unpigmented despite being showing a yellowish color on an agar plate [114, 129]. The biochemical properties include growth at different temperatures and pH, tolerance to 6.5% NaCl, and positive for Group-D antigen, and produce acetoin, leucine arylamidase, pyrrolidonyl arylamidase, urease,  $\beta$ -mannosidase, and  $\beta$ -glucosidase [129].

Till date, enterococci from plant sources are not very well investigated, and limited studies have shown their insight [130]. Luckily, the draft genome sequence of strain *E. plantarum* TRW2 (NCBI GenBank number SAMN07453839) was recently performed,

which revealed 132 contigs ( $\geq 500$  bp), 3,383,441 bp with a G + C content of 35.8% and N50 length of 89,597 bp [130]. The strains harbored no antibiotic resistance genes and neither any virulence traits [130]. So far, the strain is not investigated for its next-generation probiotic potential, but it is anticipated that it has NGP potential, as shown by its apparent safety profile.

### 3.17 *Enterococcus quebecensis*

*E. quebecensis* was recently identified by Sistek and his colleagues from a well water sample in Canada [131]. It is separated from other enterococcal species *via* sequences of a set of genes (i.e., 16S rRNA, *rpoA*, *pheS*, *tufA*, and *atpD*), DNA fingerprinting, and DNA–DNA hybridization [132]. It belongs to the *E. faecalis* group and has a close relationship with *E. moraviensis* [133]. Its biochemical characteristics include a negative test for the Voges-Proskauer test (no acetoin), peculiar temperature ranges for growth, catalase-negative, and not being grown in the presence of 6.5% NaCl. It hydrolyzes aesculin, leucine  $\beta$ -naphthylamide, L-pyrrolidonyl  $\beta$ -naphthylamide, and deaminated arginine [114, 132–134].

The next-generation probiotic potential of this species has not been described so far. However, the biochemical properties of the species give a positive insight into their use as probiotics, but their low tolerance to salts and temperatures, etc., can limit the NGP potential. Hence, it is important to investigate the genomic properties of the strains and explore their NGP potential.

### 3.18 *Enterococcus rivorum*

*E. rivorum* was identified by Niemi and collaborators in 2012 from pristine waters in Finland. This species shows a close relationship to the *E. faecalis* group [135]. *E. rivorum* showed less growth at high temperature (45°C) in the presence of 6.5% NaCl and has variable potential to produce Group-D antigen [114]. Although it can also tolerate high temperatures of 60°C for 30 minutes and 40% bile [135], it also showed positive tests for acid production from N-acetylglucosamine and the Voges-Proskauer test, and can produce acid from glycerol, ribose, galactose, D-glucose, D-fructose, methyl  $\alpha$ -D-glucoside, cellobiose, maltose, sucrose, trehalose, and starch [135].

The next-generation probiotic potential of *E. rivorum* is little explored. A study conducted by Bhagwat et al. showed that several other enterococcal species, besides *E. rivorum*, have the potential to be used as probiotics. Their results indicated that *E. rivorum* strains have adherence potential, tolerance to pancreatic enzymes, produce hydrogen peroxide, and possess antimicrobial potential [136].

### 3.19 *Enterococcus rotai*

*E. rotai* is a yellow-pigmented, urease-producing enterococcal species that is isolated from different environmental sources such as water and plants and placed in the *E. faecalis* group [114, 134, 137]. It shows a close relationship with *E. lemimoi* [138]. Its biochemical properties include growth at pH 9.6 and 42°C (not grown on 45°C), no gas production, and the production acid phosphatase, chymotrypsin esterase,  $\beta$ -glucosidase, and  $\beta$ -mannosidase [133, 137].

*Enterococcus rotai* strains show resistance to clindamycin, chloramphenicol, oxacillin, and ofloxacin but show susceptibility to imipenem and gentamicin [114, 137]. The

complete genome sequence of *E. rotai* LMG 26678 T was performed by Lauer et al. and submitted to GenBank under the accession number CP013655 [139]. Resistance to antibiotics makes the species unfit for next-generation probiotic potential, but its tolerance and hydrolytic potential, and the complete known genome can help explore in the race of NGP potential enterococci.

### 3.20 *Enterococcus silesiacus*

*E. silesiacus* was identified by Švec and collaborators from water in the Czech Republic and placed in the group *E. faecalis* [140]. This enterococcal species gives non-pigmented, shiny, and round colonies on an agar plate that can elongate in all directions. It is a non-motile species with best growth observed in Todd-Hewitt agar and brain heart infusion (BHI) agar in comparison with MRS medium [114, 140]. It produces arginine dihydrolase, pyrrolidonyl arylamidase, acetoin, and  $\beta$ -galactosidase, while hydrolyzing glycerol, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-maltose, D-lactose, and D-trehalose [140].

Its next-generation probiotic potential has not been elucidated, so far. However, its hydrolytic potential and tolerance capacity may help to select it as a next-generation probiotic candidate. Lauer et al. performed the complete genome sequence of the type strain *E. silesiacus* LMG 23085 T and submitted it to the GenBank under the accession number CP013614 [139].

### 3.21 *Enterococcus termitis*

*E. termitis* was isolated by Švec from the gut of the termites and placed in the *E. faecalis* group, which shows a close association with *E. dunnyi* [129, 134, 138, 140]. Besides basic enterococcal characteristics, it gives shiny and circular colonies that are smooth, non-motile, and non-pigmented. It shows more similar biochemical properties to *E. silesiacus* and has a genome size of 4.7 Mb [138]. It does not produce pyrrolidonyl arylamidase, arginine  $\alpha$ -galactosidase,  $\beta$ -galactosidase, and  $\beta$ -glucuronidase, while hydrolyzing glycerol, ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-cellobiose, D-maltose, D-lactose, and D-trehalose [134, 140]. *E. termitis* LMG 8895 T is considered a type strain that is obtained from a termite's gut [114].

The next-generation probiotic potential of *E. termitis* is not fully known yet, but Hamad et al. identified the incidence of this species in cheese whey, indicating its potential applications in the cheese industry. The strain *E. termitis* LMG 8895 showed greater proteolytic activity, produced exopolysaccharides, and tolerated high nisin concentrations. There was also no hemolytic activity or vancomycin resistance observed [141]. These potential probiotic properties indicate the NGP potential of this species.

### 3.22 *Enterococcus thailandicus*/*E. Sanguinicola*

*E. thailandicus* was isolated in Thailand from sausage in 2008. The 16S rRNA and *rpoA* gene sequence analysis show more similarities to the *E. faecium* group [114, 142]. *E. thailandicus* is also described as *E. sanguinicola* in the literature [143, 144]. Characteristically, it is a non-motile, ovoid, non-spore-forming enterococcal species that gives red colonies on Slanetz-Bartley agar, while being considered a non-pigmented microbe [142].

The next-generation probiotic potential of this species has been elucidated in some studies, which showed promising results. Li et al. for the first time identified the antimicrobial potential of *E. thailandicus* against *C. difficile* infections (CDI) [145, 146]. *E. thailandicus* d5B, which is isolated from Chinese children's feces, also showed enhanced colonization ability and had the basics probiotic properties, like free from virulence traits and antibiotic resistance [145]. In the study by Beukers et al. no intact prophages were detected, while an array of CRISPR-Cas was identified [113]. Likewise, strain *E. thailandicus* TC1 was also declared a potential probiotic strain as described [143]. Hence, these data showed that *E. thailandicus* has a promising NGP potential that needs to be explored in more detail.

### 3.23 *Enterococcus ureasiticus*

*E. ureasiticus* was first isolated from a well water sample and shows positive urease activity hence, its name. Sequence analysis showed its relationship with the *E. faecalis* group but can harbor variable *rpoA*, *pheS*, *tufA*, and *atpD* gene sequences than 16S rDNA analysis alone [114, 132]. It grows in the presence of 5% CO<sub>2</sub> and a wide range of temperatures, while no known strain is grown in a high salinity of 6.5% NaCl. Likewise, no extracellular polysaccharide production was observed. It produces acids from ribose, lactose, trehalose, sucrose, maltose, methyl, and tagatose, while no acid is produced from L-arabinose, D-arabitol, raffinose, and melibiose. It shows the potential to produce different enzymes and can hydrolyze different substances [132–134].

The next-generation probiotic potential of *E. ureasiticus* has not been explored yet. Its biochemical properties indicate a positive indication for probiotic usage, but its NGP potential needs to be identified.

### 3.24 *Enterococcus ureilyticus*

*E. ureilyticus* which is isolated from plants and water, is a yellow-pigmented, urease-producing, non-motile, non-spore-forming, ovoid shaped, facultative anaerobic enterococcal specie that is placed in the *E. faecalis* group on the basis of its 16S rDNA sequence [137, 147]. In contrast, it shows distinct characteristics based on *pheS* and *rpoA* gene sequence analysis, whole-cell protein electrophoresis, and DNA-DNA hybridization techniques [137]. It shows tolerance to high pH and salts and is able to grow in wide temperature ranges, but growth is limited at 45°C. It produces pyrrolidonyl arylamidase, leucine arylamidase, acetoin, and acid phosphatase, but not produces catalase, NH<sub>3</sub>, and arginine dihydrolase. It also has the potential to hydrolyze different sugars such as ribose, galactose, D-glucose, D-fructose, D-mannose, sorbose, cellobiose, maltose, sucrose, and trehalose [114, 133, 134, 137].

The next-generation probiotic potential of this species has not been explored yet. Some strains of this specie show resistance to chloramphenicol, oxacillin, and tetracycline, and susceptibility to imipenem [137], indicating the presence of antibiotic resistance, which indirectly lowers the NGP potential. However, its NGP potential will be explored based on *in vitro* assays and probiogenomic analysis.

### 3.25 Miscellaneous enterococcal species

The following are some enterococcal species that are less known and have not yet been explored for their next-generation probiotic potential.

*Enterococcus caccae* was isolated from a human stool sample and considered a separate specie in the *E. faecalis* group based on 16S rDNA sequence analysis, DNA-DNA hybridization, and whole-cell protein analysis. It also shows high similarities with *E. moraviensis* [97, 148]. Its peculiar behavior comprises its non-motile and non-pigmented nature, catalase-negative, ability to hydrolyze arabinose, lactose and mannitol, low G + C contents (32.5%), and tolerance to bile, NaCl, and temperature [114, 133, 148]. There is no such study available showing the next-generation probiotic potential of *E. caccae*. Firman et al. studied the effect of quercetin (a polyphenol present in fruits) on *E. caccae*, and it was identified that quercetin can inhibit the growth of *E. caccae* [149].

*Enterococcus aquimarinus* was identified by Švec et al. [150, 151] from sea water [131]. 16S rDNA sequence analysis shows the closest relationship with *E. saccharolyticus*, *E. sulfureus*, and *E. italicus* [133, 150]. Its distinguishing features include polymorphism in the *pheS* gene, being unable to hydrolyze ribose, good growth at 42°C, and able to digest L-arabinose [114, 133, 150]. Its next-generation probiotic potential is not described.

*Enterococcus camelliae* was isolated in Thailand from fermented tea leaves and considered a separate specie based on its 16S rDNA genome sequence, *rpoA* genes, and DNA-DNA hybridization analysis. It showed a close relationship with *E. italicus* [99, 131, 152]. It is a non-pigmented, non-motile, catalase-negative species that has the capacity to produce acid from D-galactose and lactose, grows at a wide range of pH and temperatures, and shows no hydrolysis of arginine and gelatin [152]. It causes no acidification, coagulation, reduction, or liquefaction of litmus milk, and has a mean G + C content of 37.8% [114, 133, 152]. Currently, there are no such data found that show its next-generation probiotic potential.

*Enterococcus canis* was first identified by De Graef and colleagues from rectal swabs and chronic otitis in dogs that showed 99% similarity with *E. faecium* [97, 112, 124]. The distinguishing features include arginine hydrolysis, non-motile and non-pigmented nature, growing at 6.5%, no hydrolysis of starch, being unaffected by the presence of CO<sub>2</sub>, and hydrolyzing some sugars [112, 114]. To the best of our knowledge, there are no data focusing on the next-generation probiotic potential of *E. canis*.

*Enterococcus devriesei*, an animal origin novel enterococcal specie first identified by Švec et al., has a close relationship (99% sequence identity) with *E. pseudoavium* [116, 151]. It belongs to the *E. avium* group and besides the groups characteristics, its distinguishing properties include hydrolysis of lactose, non-motile and non-pigmented nature, no growth at 45°C, tolerate to 8% NaCl, and hydrolysis of common sugars. The G + C contents are 40% [114, 117, 151]. It was also isolated from Spanish goats and characterized by safety measurements. The results showed antimicrobial activity against common pathogens and were not involved in any human infections, hence indicating its positive potential in different applications [153]. The next-generation probiotic potential is not attempted, but we anticipate this species based on its safety profile and potential antimicrobial value.

*Enterococcus hermannienseis* was first reported by Koort et al. from broiler meat and tonsils that have close phylogeny with *E. pallens* (99%) and was placed in the *E. avium* group [116, 133, 154]. It was distinguished from related species by the analysis of its 16S rDNA sequence, whole-cell protein, and DNA-DNA hybridization techniques. Other silent features include non-production of acids from common substrates, non-motile, non-pigmented, and catalase-negative nature, tolerance to 4% NaCl solution, no growth at 45°C, no production of Group-D antigen, acid production from

mannitol, and mean G + C contents of 37.1% [114, 154]. To the best of our knowledge, there are no data available that describe the next-generation probiotic potential of this species.

#### 4. Discussion

The enterococcal species are Gram-positive, catalase-negative, non-spore-forming, facultative anaerobic cocci that produce lactic acid when metabolizing carbohydrates, thus being considered members of LAB. There has been growing attention to the benefits of LAB, from traditional foods to current nutritious and healthy foods. Understanding that probiotics restore the beneficial bacterial populations of the gut flora, an increasing effort has been made to utilize probiotics with their maximum potential, from source of isolation to different applications. With this concept, LAB species are extensively targeted for probiotic potential, that is, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* species. These species have proven health benefits and contribute to the wellbeing of humans and animals alike. Probiotics are selected from the ocean of microbes on the basis of certain properties such as tolerance potential, aggregation properties, safe nature, no antibiotic resistance or virulence traits, and antimicrobial potential. Different *in vitro*, *in vivo*, and genomic studies are required to explore these probiotic properties, and hence, new beneficial species could be identified.

In the search of new probiotic development with enhanced functionalities, the concept of “next-generation probiotics” has been developed, which describes the probiotic potential of those microbes that are not usually explored, used, and targeted for probiotic potential. Following field development, different species (as aforementioned) are identified as next-generation probiotic species. Enterococci, although greatly used in different industries, are still not counted as GRAS and QPS status microbes, thus raising a question about their probiotic potential. On the other side, there are a lot of literature available focusing on the probiotic potential of enterococcal species, and many enterococcal strains are available as commercial probiotics. For instance, *E. faecium* NCIMB 10415 and Symbioflor® 1 have been used for decades without any human infections [40, 155, 156]. *E. faecium* and *E. faecalis* are two well-known and extensively studied enterococcal species that are elucidated for different properties, including their probiotic potential. But there are many other enterococcal species that have preliminary probiotic properties but are not explored for this potential. Herein, we documented the common enterococcal species (approximately 31 of the total species) and explored their basic properties, distinguishing features, and the next-generation probiotic potential.

The enterococcal probiotics safety can be tailored with respect to their pathogenesis, antibiotic resistance, virulence factors, and the transfer potential of detrimental properties to others (i.e., horizontal gene transfer). Extensive investigations in terms of safety, productions, and applications are required to optimally utilize the enterococcal probiotics. Such studies should help to develop guidelines that help in the safety assessment and regulations of enterococcal probiotics. Due to the opportunistic pathogenic nature of enterococci, it is advised to monitor the currently available enterococcal probiotics for any emergence, transfer, and acquisition of virulence traits or antibiotic resistance. Following such monitoring not only helps the industrialists to use enterococcal probiotics but also gives a consumer's satisfaction [157]. It is also important to characterize probiotic strains for their safety by ensuring that they do not harbor any transferable antibiotic resistance genes that may be transferred to

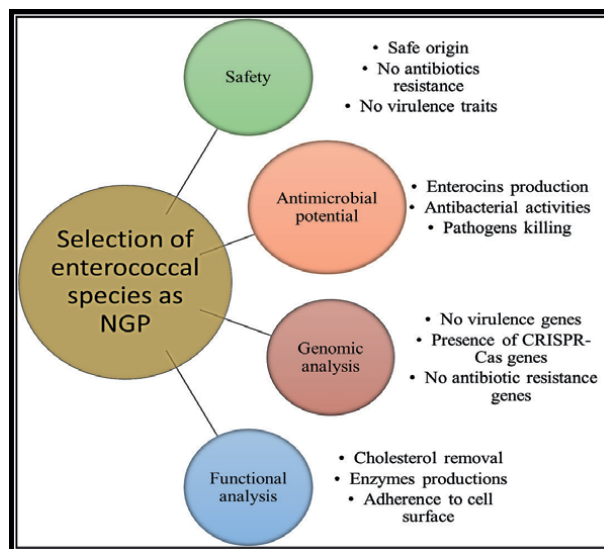


**Figure 4.**  
 The properties that should be considered in a strains to be use as next-generation probiotics.

other pathogenic bacteria. Certain enterococcal strains are recognized as opportunistic pathogens that cause urinary tract infections and other infections [62]. Resistance to different antibiotics is also a major contributing factor to the pathogenesis of *Enterococcus*. Thus, it is important to screen enterococci for pathogenesis and antibiotic resistance before testing them for animal and human clinical trials. **Figure 4** summarizes the basic properties that should be considered while selecting enterococcal strains as NGPs.

The literature reviewed in persuasion showed the next-generation probiotic potential of different enterococcal species isolated from different environmental niches and having different characteristics. Some species, such as *E. faecium*, *E. faecalis*, *E. hirae*, *E. mundtii*, have proven probiotics; others, such as *E. casseliflavus*, *E. durans*, and *E. gallinarum*, required more care while investigating their NGP potential. Other newly identified enterococcal species, such as *E. raffinosus*, *E. italicus*, and *E. villorum*, are anticipated to have NGP potential.

## 5. Conclusion

The genus *Enterococcus* belongs to group LAB and has a ubiquitous distribution with more than 80 identified species. It is the third largest genus that is used for probiotic potential. The intrinsic positive attributes of enterococcal species in many industries make them an important agent to be used as next-generation probiotics. The NGP potential of enterococcal species is also elucidated in those species that were recently identified but not explored well. The literature data suggested that the available enterococcal species have the potential to be used as NGPs, primarily because of the intrinsic probiotic properties of the genus *Enterococcus*. Although there is a scarcity of available data about the NGPs of newly identified enterococcal species, it may be anticipated that these species will also have NGP potential, particularly those that have already been screened for their safety measurements. In

conclusion, the different enterococcal species isolated from diverse environmental niches have NGP potential and thus enhance enterococcal industrial, food, and biotechnological applications.

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## **Conflict of interest**

The authors declare no conflict of interest.

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# Probiotic: Global Market, Mechanisms of Action, and Beneficial Effects on Health

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## Abstract

Probiotics are microorganisms used in the food and pharmaceutical industries to improve the nutritional quality of foods, preserve them, produce antibiotics and fight against certain pathogenic strains. Probiotics are also microorganisms with beneficial effects. The most commonly used are lactic acid bacteria (LAB). These have been the subjects of numerous studies in food and medicine. Recently, the advent of new technologies has enabled the selection, development, and demonstration of the probiotic aptitude of other microorganisms such as viruses (phagebiotics) and molds. The probiotic ability of the latter has been demonstrated by in-vitro and in-vivo tests. Phagebiotics are used for their functional properties, especially against multi-resistant bacteria, for the reduction of *Clostridium difficile* spores and *Pseudomonas aeruginosa*. Thus, certain molds, which are used as probiotics, are capable of reducing cholesterol. This chapter shows the possibility of using probiotic microorganisms as an alternative to antibiotics for the treatment of certain human and animal pathologies or the detoxification of foods contaminated by mycotoxins.

**Keywords:** probiotic, phagebiotics, antiviral effects, beneficial effects, health

## 1. Introduction

The word probiotic is composed of a Latin preposition pro “for” and a Greek adjective βιωτικός (biōtikos) “in favor of life or the living” which derives from the noun βίος (bios “life”). However, the word has a completely Greek etymology [1]. This term biotikos was used for the first time in 1953 by Kollath to designate the intestinal microbiota as having beneficial effects on health. This microbiota refers to all the microorganisms living in the intestinal microbiome. However, the probiotic concept dates back to the beginning of the twentieth century and was only introduced 50 years later [2]. This concept was originally based on observations by Elie Metchnikoff in 1907, who suggested that regular consumption of fermented dairy products contributed to improved health and longevity of Bulgarian peasants [3].

They are non-pathogenic and technologically adapted to industrial processes. They produce antimicrobial substances and are resistant to stomach acid and bile [4]. These microorganisms are generally recognized as safe (GRAS) due to their use for thousands of years as starter cultures in food fermentation. Currently, most probiotic bacteria belong to the genera *Lactobacillus* and *Bifidobacterium*, but other genera such as *Lactococcus*, *Enterococcus*, and *Saccharomyces* are also used. The beneficial effects exerted are specific to each microorganism and cannot be extrapolated to other microorganisms of the same species or subspecies.

The therapeutic potential of its strains has greatly expanded and constitutes a revolutionary approach in medicine. Currently, Universities and Research Centers are working on the development of new probiotic strains and the formulation of functional foods containing them. It would be very important to know the beneficial properties of microorganisms in order to resolve digestive disorders, and the production of different functional foods. The objectives of this chapter were to provide information on the global probiotic market, the microorganisms involved, and their mechanisms of action.

## **2. Methodology**

The databases searched were Google Scholar, Scopus, Science Direct, and Web of Science. Relevant articles found via search engines were cited in this review. Information has been selected based on its relevance to the manuscript. The key phrases used were Probiotics, Global Probiotics Market, Beneficial Effects of Probiotics, and Phagebiotics, Antiviral effects, Beneficial effects, Health, Mechanism of probiotics, Properties of Probiotic. All relevant documents were used without restriction on the year of publication and the language of the edition.

## **3. Results and discussion**

### **3.1 Global market and groups of probiotic microorganisms**

#### *3.1.1 Global probiotics market*

The global demand for functional foods and probiotics is growing increasingly due to consumer awareness of the positive impact of these products on health. The global market for functional foods grew from 33 billion USD in 2005 to 67 billion USD in 2010 and continues to grow according to Sharma et al. [5]. The probiotics market was worth 46.55 billion in 2020 and is expected to grow between 2020 and 2030. Data Bridge Market Research analyzes that the probiotics market will reach USD 128.38 billion by 2030, with a growth of 8.6%. This strong growth in the market for these products is due to investment in prebiotics and probiotics in 2013, coinciding with an increase in funding for the microbiome. These investments have quadrupled over the past decade. Currently, approximately 550 products containing probiotics are subject to approval by Health Canada [6]. The use of probiotics in functional foods offers numerous health benefits to consumers [7]. Probiotics are used in food formulation in the form of tablets, capsules, powders, and liquids in precise doses [8]. However, only *Lactobacillus johnsonii*, *Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* CLR2MD, *Lactobacillus acidophilus* CL1285MD, *Lactobacillus casei* LBC80RMD, and

*Saccharomyces boulardii* are strains approved for beneficial effects specific to probiotic claims [6]. Some strains of *Lactobacillus* are used as vaccine adjuvants or prophylactics in the treatment of human and animal diseases [9].

### 3.1.2 Groups of probiotic microorganisms

Among the microorganisms with beneficial effects, the most commonly used probiotics are lactic acid bacteria (LABs) [4]. These have been the subject of numerous research studies in food and medicine. Recently, the advent of new technologies has enabled the selection, development, and demonstration of the probiotic aptitude of other microorganisms such as molds and viruses (phagebiotics). However, the probiotic aptitude of the latter has been demonstrated by in-vitro and in-vivo tests [10–12]. The phagebiotics are used for their functional properties, especially against bacteria that are multi-resistant to conventional antibiotics. It is in this concept that phage therapy was born for the reduction of *Clostridium difficile* and *Pseudomonas aeruginosa* spores [13]. Thus, certain molds, used as probiotics, are capable of reducing cholesterol [14]. Probiotics can therefore be classified into three groups. The most widely studied and used probiotics are listed in **Table 1**.

### 3.2 Mechanisms of action of probiotic microorganisms

Probiotics exert their activities mainly through two mechanisms of action (**Table 2**). As for the indirect mechanism of action (**Table 2A**), the effects are linked to the multifactorial actions (neural, endocrine, and immunological) of the intestinal microbiota on the central nervous system, therefore bidirectional communication. These effects are achieved via the production of metabolites. For certain proven effects of probiotics, the mechanisms of action are still unknown [17]. The direct mechanism (**Table 2B**) of action allows probiotics and/or their metabolites to exert their beneficial effects following direct contact [15, 16]. This direct mechanism (**Table 2B**) of action brings together the essential characteristics for a microorganism to be considered probiotic (action on the intestinal microflora).

| Groups   | Types                | Examples                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|----------|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Bacteria | <i>Bacillus</i>      | <i>Bacillus clausii</i> ; <i>Bacillus subtilis</i> ; <i>Bacillus coagulans</i> Ganeden BC30, <i>Bacillus velezensis</i> , <i>Bacillus pumilus</i> , <i>Bacillus cereus</i> , <i>Bacillus circulans</i>                                                                                                                                                                                                                                              |
|          | Lactic acid bacteria | <i>Lactobacillus paracasei</i> , <i>Lactobacillus rhamnosus</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus bulgaricus</i> , <i>Lactobacillus sakei</i> , <i>Lactobacillus salivarius</i> spp <i>salcinius</i> , <i>Lactobacillus crispatus</i> , <i>Lactococcus Lactis</i> , <i>Leuconostoc Mesenteroides</i> , <i>Pediococcus acidilactici</i> , <i>Enterococcus Faecium</i> , <i>Streptococcus thermophilus</i> , <i>Oenococcus oeni</i> |
|          | Others               | <i>Escherichia coli</i> Nissle 1917, <i>Akkermansia muciniphila</i>                                                                                                                                                                                                                                                                                                                                                                                 |
| Fungal   | Yeasts               | <i>Saccharomyces boulardii</i> , <i>Saccharomyces Cerevisiae</i> , <i>Torulaspora delbrueckii</i>                                                                                                                                                                                                                                                                                                                                                   |
|          | Molds                | <i>Penicillium roqueforti</i> , <i>Penicillium camemberti</i> , <i>Aspergillus oryzae</i> , <i>Aspergillus niger</i> , <i>Candida pintolopesii</i>                                                                                                                                                                                                                                                                                                  |
| Virus    | Bacteriophages       | Phage $\phi$ Ef11, phage ECA2, phage KPO1K2, phage P3-CHA, phage T4, PhagoBioDerm, <i>Staphylococcus aureus</i> phage K                                                                                                                                                                                                                                                                                                                             |

**Table 1.**  
Groups of probiotics.

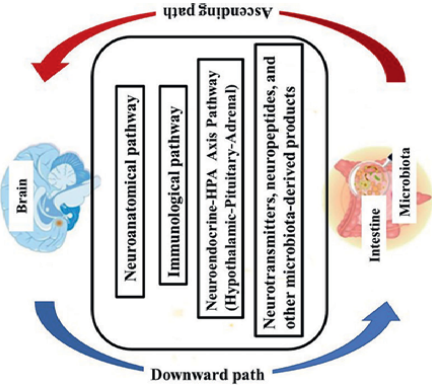
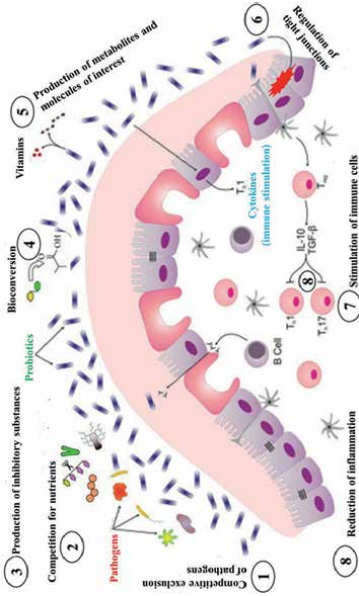
| Type of mechanism of action of probiotics      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (A) Indirect mechanism of action of probiotics | <div></div> <p>This is the general concept of the bidirectional axis of gut, brain, and microbiota. The brain regulates the intestine and intestinal microbiota via neuroanatomical, immunological, and neuroendocrine-HPA axis by communicating through neurotransmitters, neuropeptides, and other products derived from microbiota (psychobiotics and metabiotics), and in return intestinal microbiota influences the brain (mood and psychological health of the subject).</p> <p>Source: Adapted from Ghosh et al. [13], O'Toole et Cooney [15], and Zhao et al. [16].</p> |
| (B) Direct mechanism of action of probiotics   | <div></div> <p>These are the eight most well-known direct action mechanism probiotic properties by which live probiotic microorganisms could have a positive impact on the stability of the intestinal microbiota, the reduction of metabolic disorders, and the health of the host. These properties of the direct mechanism of action are the main properties attributed to probiotic microorganisms according to the guidelines and requirements of the FAO and WHO for the selection of potential probiotic candidates.</p>                                                    |

**Table 2.**  
Mechanisms of action of probiotic microorganisms.

### 3.3 Benefits of probiotic microorganisms

Probiotics are generally microorganisms promoting the balance of the intestinal microbiota and generating benefits on the health of the host following their administration in adequate doses. The beneficial effects of the latter are widely described in the literature and supported by in-vitro and in-vivo clinical studies on laboratory animals. Thus, modulation of the immune system, reduction of cholesterol levels, lactose intolerance, and prevention and treatment of diarrhea associated with ATBs are among the beneficial effects attributed to bacteriophages and microorganisms used as probiotics.

#### 3.3.1 Balance of intestinal flora or “eubiose”

The microbiota is all the bacteria, viruses, fungi, and archaea bacteria colonizing a food matrix, the digestive system, or all other parts of the body. During a change in diet, environment, psychological state, and antibiotic therapy, an imbalance in this microbiota called dysbiosis can occur in the host. Probiotics are used to compensate for dysbiosis [18]. They are currently attracting more and more interest from the scientific community which is studying their effectiveness in the context of various pathologies in adults and children [6]. Some of these probiotic strains have also proven effective in several pathologies such as diarrhea associated with taking antibiotics, rotavirus diarrhea, colic, constipation, and even atopic dermatitis. However, although probiotic strains have generally the same effects on human health, namely the strengthening of the intestinal barrier or the stimulation of the intestinal immune system essentially, each strain has its own effectiveness and must therefore be the subject of studies to confirm its interest [4].

#### 3.3.2 Reduction of mycotoxins

In addition to their ability to produce bioactive molecules of nutritional interest, certain microorganisms, such as *Bacillus cereus*, *Bacillus pumilus* E-1-1-1, *Bacillus subtilis* UTBSP1, *Flavobacterium aurantiacum*, *Lactobacillus casei*, *Lactobacillus plantarum* MON03, *Mycobacterium fluoranthenvivorans* sp. nov, *Pichia kudriavzevii*, *Rhodococcus erythropolis*, *Saccharomyces cerevisiae*, and *Stenotrophomonas maltophilia* are used in the agri-food industry to degrade mycotoxins and increase mineral availability by hydrolyzing phytates and tannins [19, 20]. Once ingested via fermented foods, these microorganisms exert beneficial effects on their hosts, such as degrading phytates and producing folates. They are able to survive the conditions of the gastrointestinal tract and adhere to intestinal cells. The use of these microorganisms in food fermentation in Africa would be an undeniable asset in reducing illnesses linked to the ingestion of food containing mycotoxins and the often fatal nutrient deficiencies [21].

#### 3.3.3 Antiviral activities of probiotics

The viruses cause infections in immunocompromised patients, infants, young children, and the elderly. Treatment of these infections with antivirals is often ineffective due to the resistance mechanisms developed by these viruses. In recent decades, the use of probiotic microorganisms has increased considerably in the prevention and treatment of viral infections [22]. Al-Kassaa et al. [23] and Abdelazez

et al. [24] reported that several viral infections such as Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), Transmissible Gastroenteritis Virus (TGEV), Respiratory Viral Infections (RVI), Rotavirus (RV), Respiratory Syncytial Virus (RSV), Vesicular Stomatitis Virus (VSV), Human Papillomavirus (HPV), Newcastle Disease Virus (NDV), and Influenza A (H1N1A) are relieved by the use of probiotics. Mao et al. [25] reported that lactic acid produced by *Lactococcus lactis* reduced the load of *Vibrio cholerae* in the intestines of infected mice, improving their survival. Similarly, *Lactobacillus plantarum* 299v exerts immunomodulatory and protective effects by significantly reducing the viral load in patients [26]. Thus, probiotics generally act by inactivating viruses, stimulating the immune system, the adsorption mechanism, the trapping system, and the production of protein or non-protein antiviral agents. The treatment of these viral infections is often accompanied by the use of anti-inflammatories, analgesics, and antipyretics, often causing serious side effects in some people. However, prevention and alternative treatment with probiotics are necessary, as they help limit the emergence of new infectious viruses and increase resistance to available antivirals [23].

### 3.3.4 Lactose metabolism

Lactose is a diholoside composed of glucose and galactose. It is absorbed in the small intestine following enzymatic hydrolysis by lactase in the enterocytes. Lactase activity declines from the early years in humans and ceases at the age of 6–8 years [27, 28]. This decrease is a normal physiological phenomenon and not a pathology, but rather an evolution. In individuals capable of digesting lactose, the persistence of lactase is due to a mutation (ACGA to ATGA) in the genome of these individuals. Lactose maldigestion is most often asymptomatic, but can manifest as digestive

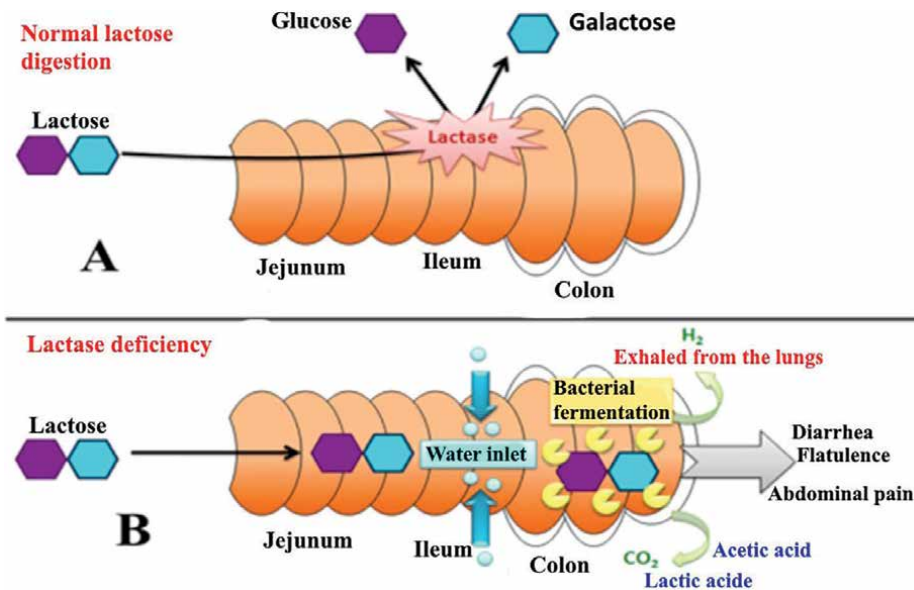


**Figure 1.**  
Mechanism of normal lactose digestion (A) and lactase deficiency (B) [27].

symptoms called lactose intolerance [29]. Undigested lactose is fermented and transformed into lactate, volatile fatty acids, and gases (H<sub>2</sub>, CO<sub>2</sub>) by colonic flora (**Figure 1**). However, strains such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* easily digest lactose [27].

### 3.3.5 Pharmacological properties

Probiotics are defined as microorganisms that, when ingested live, exert a positive influence on the health or physiology of the host. The use of probiotics in pharmacology is highly complex, as they contain numerous active ingredients such as components of their wall (lipoteichoic acid, peptidoglycans) and their DNA [30, 31]. These beneficial effects are due to a modulation of the intestinal microbiota directly or indirectly by enzymes that they synthesize, thus influencing the intestinal barrier (immune system, defensins, or mucus) [32]. However, this results in a reduction in the intestinal hyperpermeability observed during chronic inflammatory bowel disease and signs of inflammation such as the secretion of multiple cytokines and NFκB activation [31]. Thus, the beneficial effects exerted by a given probiotic microorganism cannot be extrapolated to another species or another identical genus. This is because probiotic activity is strain-dependent [33]. Since probiotic properties and active ingredients differ greatly from one another, it is difficult to compare the effects of these microorganisms during treatment. Similarly, during antibiotic therapy, diarrhea may occur and its impact on the intestinal microbiota depends on the spectrum and pharmacokinetics of the antibiotic used during treatment [33]. For example, *E. faecium* prevents the adhesion of pathogens such as K88 enterotoxinogenic *Escherichia coli* to the intestinal mucosa by occupying sites in the intestinal mucosa. Similar results were obtained in rabbits by a Hungarian team using *B. cereus* var. Toyoi (Toyocerin R) and *E. faecium* EK13 with a Slovenian team on pathogen exclusion [34]. However, from an immunological point of view, probiotic microorganisms help to boost immunity against intestinal diseases, by activating IgA production and stimulating macrophage or natural killer activity [32, 33].

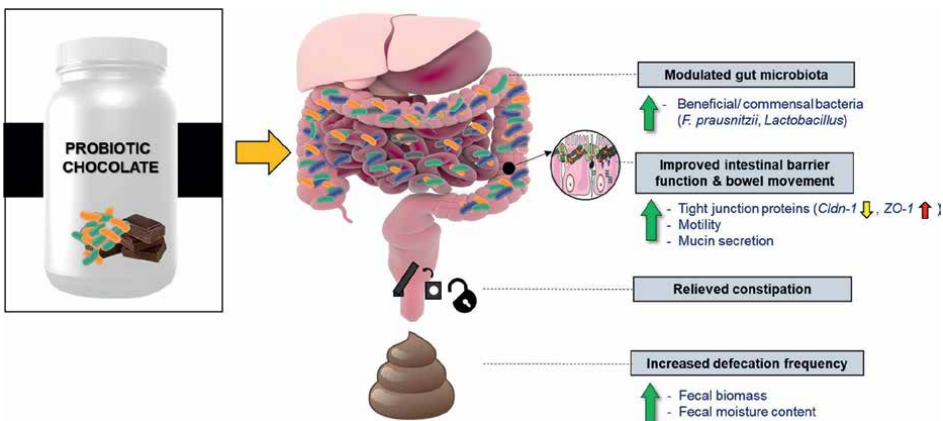


**Figure 2.**  
Mechanism of laxative effect of probiotics [35].

### 3.3.6 Probiotics as laxatives

Constipation is a functional gastrointestinal disorder characterized by intestinal symptoms due to a lack of defecation and/or incomplete and difficult evacuation of stools in a subject [35]. This digestive disorder affects all age groups. According to **Figure 2**, the administration of probiotic chocolate incorporating *S. thermophilus* MG510 and *L. plantarum* LRCC5193 modulates the intestinal microbiota, improves intestinal barrier function, relieves constipation, and increases defecation frequency in constipated subjects [35]. The synergistic action of these two microorganisms has led to the regulation of this gastrointestinal disorder. Thus, *L. plantarum* LRCC5193 used alone confers advantages in the treatment of constipation. However, further clinical trials are still required to evaluate this efficacy [35].

### 3.3.7 Predictive microbiology

Predictive microbiology is a multidisciplinary field combining mathematics, microbiology, engineering, and chemistry. It uses a diversity of microorganisms of interest with a view to their incorporation in the processing and/or formulation of new food matrices for the production of functional foods [36]. Thus, its purpose is the development and application of mathematical models predicting probiotic responses according to consumer requirements and specific environmental conditions. However, the qualitative and quantitative assessment of the nutritional profile of foods incorporating these microorganisms is based on applications for improving biomolecules of interest and reducing health risks [37]. Thus, the predictive model of food microbiology being a mathematical expression describing the growth, survival, and inactivation of the metabolic activities of food-borne microorganisms is defined by classification schemes allowing them to be grouped into primary, secondary, and tertiary models [38, 39]. The growth model of Baranyi and Roberts [40] is one of the most widely used models for describing the evolution of food microbiota as a function of time and specific environment.

### 3.3.8 Fecal transplantation

Fecal transplantation is a process for transferring intestinal microbiota from preparation of fecal matter from a healthy subject to a patient suffering from digestive pathologies linked to dysbiosis, with a view to exerting therapeutic effects [32–41]. This transfer from donor to recipient results in eubiosis of intestinal biodiversity in probiotic microorganisms to restore the broken dialog. It is currently considered an alternative for the treatment of recurrent *Clostridium difficile* infections, with success rates of 80–90% [42]. Similarly, in cases of Crohn's disease, chronic constipation and irritable bowel syndrome, the application of this practice via probiotics contained in the stool has been successful [43]. The use of the microbiota is therefore a therapeutic tool for many pathologies and the treatment of intestinal disorders following antibiotic therapy.

### 3.3.9 Autism prevention

Studies have shown that there is a link between dysbiosis and the onset of autism in children. This link is reflected in a reduction in the levels of probiotic bacteria such as *Akkermansia muciniphila* and *Bifidobacterium* spp. in the gastrointestinal tract and stools of these children. Similarly, a recent study conducted on intestinal biopsies

| Genre                  | Species and beneficial effects reported in the literature                                                                                                                                                                                                                                                                                                                                                                                                 |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Bifidobacterium</i> | <i>B. infantis</i> : Reduction in necrotizing enterocolitis in premature babies, symptoms of irritable bowel syndrome and bloating in people with functional intestinal disorders<br><i>B. animalis</i> subsp. <i>lactis</i> DN-173010: Treatment of functional constipation in adults, reduction in total microbial flora in dental plaque and febrile urinary tract infections in children, modulation of brain activity.                               |
| <i>Lactobacillus</i>   | <i>L. rhamnosus</i> GG: Prevention and reduction of atopic dermatitis in children.<br><i>L. plantarum</i> NRRL B-4496: Reduction in symptoms of irritable bowel syndrome.<br><i>L. johnsonii</i> MH-68: Reduction in gastroenteritis and risk of <i>Helicobacter pylori</i> infection.<br><i>L. fermentum</i> NCIMB 5221: Reduced insulin and cholesterol resistance.<br><i>L. reuteri</i> DSM 17938: Reduction in gastrointestinal disorders in infants. |
| <i>Lactococcus</i>     | <i>L. lactis</i> subsp. <i>lactis</i> KLDS4.0325: Treatment of antibiotic-associated diarrhea, adhesion to vaginal epithelial cells.<br><i>L. lactis</i> subsp. <i>lactis</i> ATCC 11454: Modulation of brain activity, antimicrobial activity against <i>Clostridium difficile</i> , and other probiotic properties.                                                                                                                                     |
| <i>Enterococcus</i>    | <i>E. durans</i> LAB18S: Antimicrobial and antioxidant activity, adhesion to intestinal cells, and anti-inflammatory activity.<br><i>E. faecium</i> : Treatment of antibiotic-associated diarrhea, use as a probiotic in animal feed.<br><i>E. faecium</i> SF68: Effective treatment of intestinal disorders in humans.                                                                                                                                   |
| <i>Streptococcus</i>   | <i>S. thermophilus</i> : Reduced symptoms of irritable bowel syndrome, improved lactose digestion, reduced necrotizing enterocolitis in premature babies, production and resistance to BLIS contained in yogurt.<br><i>S. salivarius</i> K12: Prophylactic treatment of pharyngo-tonsillitis.                                                                                                                                                             |
| <i>Pediococcus</i>     | <i>P. acidilactici</i> UL5: Production of pediocin, antibacterial and probiotic activities.<br><i>P. acidilactici</i> BA28: Production of bacteriocin, treatment of <i>Helicobacter pylori</i> infections.<br><i>P. pentosaceus</i> CRAG3: Antibacterial activity and glucansucrase production.                                                                                                                                                           |
| <i>Leuconostoc</i>     | <i>L. mesenteroides</i> B7: Production of Leuconia and probiotic properties.<br><i>L. mesenteroides</i> subsp. <i>cremoris</i> : Stimulation of Th1 cytokines, IL-12, IFN- $\gamma$ .<br><i>L. citreum</i> : Production of vitamin B2.<br><i>L. lactis</i> : Production of vitamins B11, K1 (phylloquinone), and K2 (menaquinone).                                                                                                                        |
| <i>Bacillus</i>        | <i>B. cereus</i> NVH75/95: Effective probiotic for animals.<br><i>B. subtilis</i> R0179: Treatment of diarrhea and <i>Helicobacter pylori</i> infections.<br><i>B. coagulans</i> Ganeden BC30: Immunological support, prevention of dental caries in children.                                                                                                                                                                                            |
| <i>Saccharomyces</i>   | <i>S. boulardii</i> : Treatment and reduction in the duration of diarrhea, treatment of irritable bowel syndrome, treatment and reduction of recurrent Pseudomembranous colitis infections caused by <i>Clostridium difficile</i> , treatment of acute infantile gastroenteritis.<br><i>S. cerevisiae</i> AKP1: Anti-inflammatory activity, stimulation of the immune system, inhibition of <i>E. coli</i> MTCC 118 and <i>Vibrio cholerae</i> MTCC 3906. |
| <i>Escherichia</i>     | <i>E. coli</i> Nissle 1917: Treatment of functional constipation in adults, inflammatory bowel disease, and gastrointestinal disorders. Pro-inflammatory potential, prevention of surface eye disease, reduction in intestinal colonization of <i>Salmonella enterica</i> Typhimurium by competition.                                                                                                                                                     |

Source: Adapted from Fijan [47].

**Table 3.**  
 Probiotic microorganisms and their health benefits.

from children with autistic and gastrointestinal disorders revealed a strong increase in phylotypes belonging to the Alcaligenaceae family and the *Sutterella* genus, particularly the species *Sutterella wadsworthensis* and *Sutterella stercoricanis* [44]. This shows the influence of the absence of probiotic intestinal bacteria on the behavior of

these children. However, the administration of certain probiotics can prevent autism. Consumption of foods containing *Lactobacillus casei* for 2 months reduced anxiety in patients with chronic fatigue syndrome [45]. In 2013, a group of American researchers reported that the intestines of mice with autistic disorders contained lower levels of *Bacteroides fragilis* than those of control mice. The administration of *Bacteroides fragilis* thus enabled the elimination of psychomotor disorders linked to anxiety and communication between individuals [46].

### 3.4 Probiotic microorganisms and their health benefits

Several health benefits have been associated with probiotic consumption. These effects have been demonstrated either by in-vitro tests, in-vivo tests, or both. **Table 3** shows the diversity of microorganisms currently used as probiotics and their beneficial effects on health according to the scientific literature [47].

The metabolites secreted by probiotics provide physiological benefits to the host, preventing the proliferation of pathogens while improving intestinal well-being reducing the incidence of digestive disorders and strengthening the immune system [48]. In the animal domain, *Lactobacillus plantarum* CM49 has probiotic potential against the germs responsible for bovine mastitis [49]. Microorganisms with probiotic potential are safe and biotechnologically exploitable strains. LAB producing exopolysaccharides (EPS) plays a vital role in the industry for the development of functional foods. These are used as coadjuvants or starter cultures for yogurt development and other traditional fermented foods [50]. In strategies for the prevention and treatment of dental caries, certain probiotics such as *Lactobacillus* and *Bifidobacterium* are frequently exploited due to their beneficial effects on the oral cavity inhibiting cariogenic bacteria [51]. Despite the current use of certain probiotics, the search for new potentially probiotic and safe strains constitutes one of the most important subjects in medical microbiology, the food, and pharmaceutical industries [52].

## 4. Conclusion

Probiotics have therapeutic potential in digestive, neurological, and psychological disorders. This chapter will help many people to take an interest in probiotics and work to promote them in the future. However, clinical studies have demonstrated their beneficial effects and numerous interactions specific to the “probiotics-foods” concept on consumer health, such as the reduction of health risks, deficiency diseases, and intestinal disorders.

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## Conflict of interest

The authors declare no conflict of interest.

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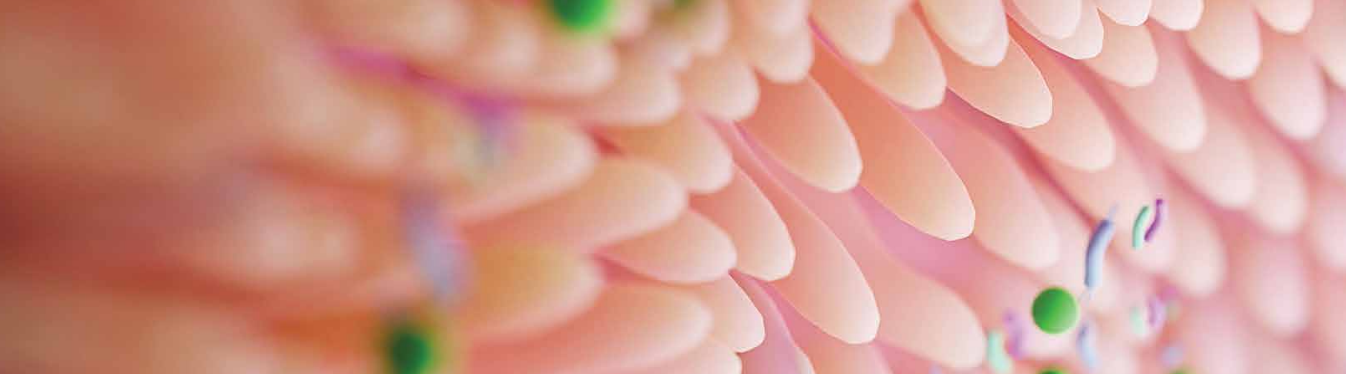
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Probiotics, prebiotics, and postbiotics are gaining attention for their health benefits and roles in sustainable food systems. These substances interact with gut microbiota to promote health and enhance the nutritional value of foods. Incorporating these elements into food systems can promote sustainability by utilizing locally sourced raw materials and functional foods. Fermented foods rich in probiotics are often made from seasonal ingredients, making them environmentally friendly. Additionally, foods enriched with prebiotics and postbiotics enhance diet quality and support sustainable agriculture. These biotics offer health benefits by improving gut microbiota and immune function, ultimately benefiting public health and environmental sustainability. This book provides a thorough overview of biotics' roles in the gut ecosystem, their global market presence, and the exploration of new probiotic species, all while emphasizing sustainability.

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