CHAPTER NINE

# Interactions of probiotics and prebiotics with the gut microbiota

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## Contents



## Abstract

The gut microbiota (GM) composition varies among individuals and is influenced by intrinsic (genetics, age) and extrinsic (environment, diet, lifestyle) factors. An imbalance or dysbiosis is directly associated with the development of several illnesses, due to the

*Progress in Molecular Biology and Translational Science*, Volume 171
ISSN 1877-1173
https://doi.org/10.1016/bs.pmbts.2020.03.008

potential increase in intestinal permeability leading to a systemic inflammation triggered by higher levels of circulating lipopolysaccharides and changes in the immune response caused by an overgrowth of a specific genus or of pathogens. These mechanisms may increase symptoms in gastrointestinal disorders or reduce glucose tolerance in metabolic diseases. Diet also has a significant impact on GM, and functional foods, namely prebiotics and probiotics, are a novel approach to reestablish the indigenous microbiota. Prebiotics, like inulin and polyphenols, are selectively utilized by GM, releasing short-chain fatty acids (SCFA) and other metabolites which may reduce the intestinal lumen pH, inhibit growth of pathogens, and enhance mineral and vitamin bioavailability. Probiotic microorganism may increase the microbial diversity of GM and improve the integrity of the intestinal barrier, leading to an improvement of baseline and pathologic inflammation. In this chapter, we will discuss the potential roles of prebiotics and probiotics in health and diseases throughout an individual's lifetime and proposed mechanisms of action.

## 1. Introduction

The term "microbiota" refers to the entire population of microorganisms in a given location. The largest population of microorganisms is the microbiota of the gut, which is comprised of more than 2000 bacterial species and is significantly influenced by intrinsic (genetics, age) and extrinsic (body mass index, smoking, physical activity, diet) factors, mainly by diet, which regulates microbial activity and gene expression (Fig. 1).[1–3] Studies have shown that several diseases are associated with an imbalance of the bacterial composition of the gut, which is referred to as a dysbiosis. Dietary interventions may be effective in restoring it to a healthier state.[4]

In this sense, functional foods, which in addition to their nutritional properties must confer a defined benefit to the consumer's health, seem to be interesting alternatives for dietary interventions targeting at improving the microbiota shape. The most known functional foods or ingredients are polyphenol, polyunsaturated fatty acids, prebiotics, and probiotics.[5,6]

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host."[7] While the definition of probiotics has been debated, an expert consensus from 2013 has agreed on this description.[8] The major mechanisms of action of probiotics on human health are multifold and include competition with pathogens for nutrients and adhesion sites, production of bacteriocin, vitamins, and short-chain fatty acids (SCFA), immunomodulation, improvement of the intestinal barrier, and production of neurotransmitters.[9]

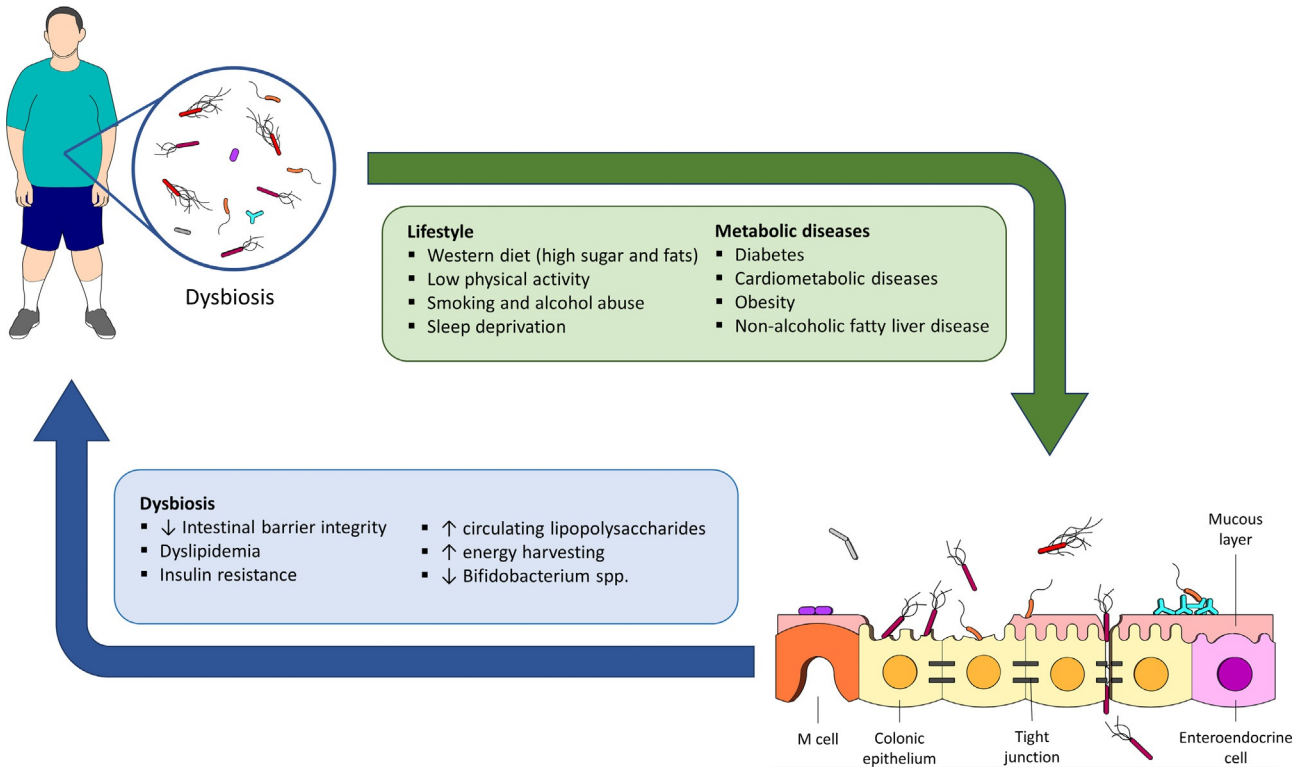


**Fig. 1** Factors that influence the gut microbiota.

In the last years, studies related to probiotics and their effects on human health have increased exponentially. The vehicles or food matrices used to deliver probiotics affect the viability of the microorganisms and, therefore, have different impacts on the host microbiota. In addition, prebiotics and bioactive food compounds may interact with the commensal microorganisms that inhabit the human gut.

Several clinical trials have been conducted in order to evaluate the effect of probiotics, primarily those containing bacteria from the *Lactobacillus* and *Bifidobacterium* genera, on different food matrices and their impacts on the human health, leading to heterogeneous results. In this chapter, we will delve into different bioactive and probiotic foods and their effects on the human health.

## 2. An overview into the human microbiota

The human microbiota consists of a complex ecosystem that holds approximately $10^{14}$ bacteria. Much has been discussed about the factors that may contribute to its shape in the prenatal period, since the mother's oral microbiota has recently been identified as being similar to that found in the placenta, comprising Firmicutes, Tenericutes, Proteobacteria, Bacteroides, and Fusobacteria phyla. One hypothesis is that these bacteria are transported from the oral cavity to the fetus through the bloodstream.[10]

Outside of any in utero exposures, the first contact of the newborn with various microorganisms occurs during birth, which is influenced by the mode of delivery.[11] In the vaginal delivery, the skin and mucous membranes of the baby are in contact with the local microbiota, which leads to colonization by *Lactobacillus* spp., reflecting the mother's vaginal microbiota. In contrast, when delivery occurs by cesarean section, the newborn's mouth, intestines, and skin are colonized by microorganisms present in the birth and skin environments, such as *Staphylococcus* and *Propionibacteria*.[12,13]

During the first months of life, the gut microbiota adapts to the conditions of the surrounding environment and are influenced by anaerobic conditions, nutrient availability, and microbial interactions during community succession.[13] Cesarean-born infants often have fewer important maternally-transmitted microorganisms, such as Bacteroides and Bifidobacteria. Infants delivered via c-section continue to exhibit a lower diversity and reduced Th1 response during the first two years of life, although this difference in colonization between delivery modes is gradually reduced over time.[12,13]

Breastfeeding is the other major contributor to early microbial colonization of infants. Breast milk may contain up to $10^7$ bacterial cells in 800 mL, mainly from the *Lactobacillus*, *Streptococcus*, and *Staphylococcus* genera.[14] In addition, it contains oligosaccharides that stimulate the selective growth of *Bifidobacterium* and *Lactobacillus* spp. and through fermentation in the gut, SCFA are released, reducing the colonic pH. Initially, this reduces the diversity of the infant's intestinal microbial community, which is likely due to acidification of the intestinal pH. Meanwhile, acidification acts as a defense against pathogens that cannot survive under these conditions.[15] Furthermore, the presence of immunoglobulin A, defensins, and lactoferrin in human milk provide other benefits to the infant's health,[16] and exclusive breastfeeding in the first months of life has been associated with a reduction in several childhood diseases, such as obesity, infections, and atopic diseases.[17–19]

After food introduction and, consequently, increased nutrient pool ingestion, the infant's intestinal microbiota resembles an adult's microbiota. It is generally reached in the third year of life and will be influenced together with the environment in which the child is inserted. A diverse microbiota enhances the vitamins and amino acids biosynthesis and the ability to metabolize carbohydrates.[13,20]

In adulthood, the microbiota is composed mainly of Firmicutes and Bacteroidetes, whereas members of other phyla, like Actinobacteria, Proteobacteria, and Verrucomicrobia are present in lower populations. The gut microbiota plays a role in various functions of the body, such as energy storage, intestinal barrier integrity, immune system development, including tolerogenicity, and neurotransmitter production. The host's microbiota is dynamic and, until now, it is difficult to establish how different factors, such as lifestyle, illnesses, geographical location, and age, may impact its stability.[21,22]

Presently, there is a collective effort to identify what would be a healthy microbiota, also known as a "core microbiota." However, due to the highly heterogenicity among individuals, it is easier to compare with the same individual along his lifetime. In fact, a core microbiome exists at the level of metabolic functions.[23] Throughout life, the gut microbiota is resilient, and transient or punctual situations do not seem to significantly affect its composition, such as adherence to a specific diet, use of antibiotics, and exposure to different environments.[24]

Several clinical trials have demonstrated that a number of diseases are related to changes in the relative abundance of a phylum or genus. For example, obesity or high caloric diet are associated with a greater relative

abundance of Firmicutes over Bacteroidetes. This also occurs in populations that eat a typical western diet, which is rich in processed foods, carbohydrates, and sugars.[25,26] Other metabolic diseases, such as diabetes, cardiometabolic diseases, and low-grade inflammation associated with obesity are inversely associated with *Akkermansia muciniphila* abundance.[27]

In conclusion, mechanisms involved in shifts of the gut microbiota are still not well understood. However, dietary interventions with functional foods, like probiotics, are being studied extensively and have been proposed as a way of ameliorating various diseases and as a potential adjunctive therapy or preventative strategy, in spite of the fact that they are not able to promote permanent changes in the host's microbiota.

## 3. Functional foods

It is well known that functional foods may be good alternatives in preventing or ameliorating the *status* of certain diseases. Moreover, they can be used in combination with traditional therapies as adjuvants and, unlike antibiotics, will not have a detrimental effect on beneficial populations of bacteria. The major functional foods or ingredients explored are prebiotics, probiotics, the combinations of both in synbiotic foods, in addition to dietary fibers and bioactive compounds.

### 3.1 Prebiotics

A prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit."[5] The potential health benefits related to prebiotics include gut microbiota modulation along with beneficial microbial metabolites release (e.g., SCFA and tryptophan). However, these effects should be verified in the target host (animal or human).[5,28,29] Several prebiotics are commercially available, like fructo-oligosaccharides (FOS), inulin, β-galactooligosaccharides (GOS), lactulose, isomalto-oligosaccharides (IMO), and resistant starches (i.e., the fraction of starch that resists to digestion in the small intestine).[30]

Innumerous studies explored the potential health benefits of fiber consumption, with or without prebiotic effect. The main pathway associated with prebiotics involves selective fermentation by beneficial microorganism present in the gut, including *Lactobacillus* and *Bifidobacterium*, which produce acetate and lactate and in turn may stimulate production of butyrate by other bacteria. Moreover, the production of SCFA bestows diverse health benefits like improvement of mineral absorption and barrier function.[5,28,29]

In addition, prebiotics may be used to enhance fermentation processes or as encapsulating material. According to Oliveira et al., inulin, one of the most studied prebiotic compounds, may increase the acidification rate of milk by co-cultures of probiotic and starter strains.[31] Meanwhile, Santos et al. also reported higher resistance to GIT stress simulated in vitro by the probiotic strain *Lactobacillus acidophilus* La-5 microencapsulated with inulin when compared to free cells. Moreover, when incorporated in a food matrix, the survival rate was further increased.[32] Similarly, Rosolen et al. stated that the combination of whey and inulin as a coating material for *Lactococcus lactis* R7 promoted resistance to heat treatment and GIT stress simulated in vitro.[33]

## 3.2 Polyphenols

Polyphenols are a large group of compounds that are well recognized for their anti-inflammatory and antioxidant properties and their potential health benefits on cardiometabolic diseases and brain function. The most common phenolic compounds are flavonoids (e.g., flavonols, isoflavones, and anthocyanins), phenolic acids (e.g., ellagic and caffeic acids), lignans, and stilbenes (e.g., resveratrol), which can be consumed in black and green tea, red wine, apples, blueberries, dark chocolate, onions, almonds, soy, pomegranates, and coffee, among others.[34–36]

After ingestion, some polyphenols are absorbed in the small intestines, while a significant amount can reach the large intestines intact where they can be metabolized by the gut microbiota and release active metabolites, like phenolic acids. Moreover, studies have shown that some compounds may stimulate beneficial bacteria, like *Lactobacillus* spp., *Bifidobacterium* spp., *Akkermansia muciniphila*, and *Faecalibacterium prausnitzii*. Conversely, other compounds may inhibit pathogens, like *Helicobacter pylori*, *Staphylococcus aureus*, and *Listeria monocytogenes*.[34–36]

Hence, there is evidence that polyphenols have potential as prebiotics and an innovative option for gut microbiota modulation. Furthermore, fermentation by lactic acid bacteria may be an alternative to improve bioavailability of these compounds, which are generally low in unprocessed foods.[36–38]

## 3.3 Omega-3

Consumption of omega-3 polyunsaturated fatty acids has been associated with a reduction in cardiovascular diseases and improvement of cognition,

depression, behavioral disorders, and brain function. Besides gut microbiota modulation, anti-inflammatory effects, and increased SCFA release, showing direct influence in the gut-brain axis.[39–42]

The major bioactive forms of omega-3 are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can be found in some fatty fishes or in nutraceutical supplements. Their precursors are α-linolenic acid (ALA), contained in nuts, flaxseed, and canola and soy oil.[39,40]

Studies have reported the potential of omega-3 fatty acids to modulate the gut microbiota and to act as prebiotic candidates. According to Prossomariti and colleagues, the consumption of EPA was able to improve the mucosal inflammation in ulcerative colitis (UC) patients, while decreasing *Clostridium* spp. in the feces in a short-term intervention.[41] In another study, a high omega-3 diet increased significantly *Bifidobacterium* and *Lactobacillus* population in mice throughout life. Meanwhile, it influenced positively brain function and behavior of the animals.[42]

## 3.4 Probiotics

Potential health benefits associated with probiotic consumption are widely known. Nonetheless, several factors should be taken into consideration when developing a food product or supplement with claims of health benefits. The microorganisms should be well recognized as GRAS (Generally Recognized as Safe), claims of health benefits may only be associated with select strains, not all genuses or species, or with the target population in that specific clinical trial (e.g., infants, chronic disease patients, elderly, etc.). In addition, a recommended daily dose of colony-forming unit (CFU) to achieve health benefits should be indicated and the microorganisms should be viable during the shelf-life of the products.[7,8,43]

The most common probiotics are species from the *Bifidobacterium* and *Lactobacillus* genera. However, other microorganisms may be classified as probiotics, such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Streptococcus thermophilus*, *Lactococcus lactis*, and *Saccharomyces bourlardii*.[8,44,45] Moreover, each strain has different multiplication and survival rates after passage through the gastrointestinal tract (GIT) depending on the food matrix (e.g., milk, soymilk, and fruit juice), pH, incorporated oxygen (e.g., stirred-yogurt), temperature of storage (e.g., room temperature, refrigerated or frozen), presence of prebiotics or any other food ingredient, and microencapsulation.[45–49]

In addition to health effects, probiotics can improve food matrices by reducing the level of undesirable compounds, like stachyose and raffinose in soymilk, or by producing compounds of interest, like vitamins.[46,47,50] Moreover, starter strains, such as *Streptococcus thermophilus*, are usually combined with probiotic cultures to reduce fermentation times, which when co-cultured with *Bifidobacterium* strains prevent off-flavors due to acetic acid release by species from this genus.[31,47,51]

Prebiotic compounds may be used in combination with probiotics, resulting in synbiotic foods, which have synergistic effects including reduction of fermentation time or enhancement of the survival of probiotics during passage through the GIT.[31,44] However, any changes in matrices may affect the behavior of strains differently. Bedani et al. reported that *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 in fermented soymilk had higher survival rates after GIT stress simulated in vitro when compared to their fresh cultures, without any food matrix.[52] On the other hand, the addition of tropical fruit pulps in probiotic fermented soymilk showed the opposite effect, despite an improvement in the consumers' acceptance of the product.[53]

Besides prebiotics or other ingredients, emerging food technologies, such as microencapsulation and ohmic heating, can be applied to improve fermentation processes or to increase the resistance to GIT stress.[31–33,47,51] Nevertheless, it has recently been suggested that microbial metabolites alone are enough to confer health benefits and viable cells in the gut are not necessarily essential. This concept might completely change the directions of probiotic food and supplements research.[47]

In conclusion, there are several factors that influence probiotic stability in different matrices and their survival through the GIT, and consequently, their health benefits. Nevertheless, there is a lot of heterogeneity in the intervention studies reported, like participants' age, sex, body mass index, metabolic diseases, daily dose, duration of the study, different prebiotic and/or probiotic cultures administered, and different food matrices. Therefore, it is difficult to compare outcomes and identify one unique answer to fit all cases and design of a tailored treatment is likely a more biologically plausible approach to probiotic use.

## 4. Effects on healthy individuals

A limited number of clinical trials have studied the impact of probiotics or bioactive food consumption on the gut microbiota, especially

in healthy individuals. Several studies evaluated the fecal recovery of probiotic strains in order to verify if the administered microorganisms survived through the GIT, in addition to checking the participants' adherence to the dietary intervention. Indeed, molecular biology techniques, like gene sequencing or real-time PCR quantification, are helpful in understanding the actual effects of probiotics on the gut microbiota. However, they are costly and are not always available to all researchers.

In a randomized double-blind placebo-controlled trial, Rampelli et al. administered a probiotic biscuit daily containing *Bifidobacterium longum* Bar33 and *Lactobacillus helveticus* Bar13, $10^9$ CFUs per serving versus placebo to 32 elderly participants living in Italy. Fecal samples were collected at baseline (T0) and after 30 days (T30). When compared to healthy adults, elderly subjects had higher levels of pathobionts, such as *Clostridium difficile*, *C. perfringens*, *Bacillus cereus*, *Campylobacter*, and Enterobacteriaceae, whose influence on the host occurs indirectly via stimulation of the immune system. After treatment, the placebo group microbiota remained the same while the intervention group had decreased opportunistic pathogens. Furthermore, increased content of health-promoting bacteria such as *Akkermansia muciniphila*, *B. longum*, and Lactobacillaceae was observed in the treatment group. These results suggest that dietary intervention with probiotic food may improve age-related dysbiosis, promoting a transitory healthier gut microbiota.[54]

Fermented milk containing *Lactobacillus casei* strain *Shirota* (LCS) is known to promote immune system function in healthy individuals. Mechanisms, such as an increase in natural Killer (NK) cell function, activation markers on circulating T cells, and increased concentrations of IgA1 and IgA2 in the oral mucosa, are well described.[55,56] Nevertheless, little is known about the effects of its consumption on the intestinal microbiota. In a study conducted in 25 healthy Chinese adults, subjects consumed daily 100 mL of a probiotic drink containing $10^8$ CFU/mL of viable LCS (Yakult). Fecal recovery showed that the strain survived the GIT and promoted the growth of beneficial bacteria, especially *Bifidobacterium* and *Anaerostipes*, an efficient lactate fermenter, suggesting a synergistic effect, once *Bifidobacterium* is a lactate producer. Furthermore, population of butyrate-producing bacteria, such as *Roseburia intestinalis* and *Clostridium*, declined. The strain demonstrated poor ability to persist in the GIT during follow-up (intervention, day 21: $10^7$ CFU/g of feces; endpoint, day 42: $10^4$ CFU/g of feces) and decreased ability to produce SCFA. This suggests that, despite modifying the microbiota composition of adults, regular consumption is necessary.[57,58]

Interestingly, Klein et al. conducted a cross-over study evaluating the impact of the consumption of a yogurt (300 g/day) containing probiotic strains *Lactobacillus acidophilus* 74-2 ($9.3 \times 10^8$ CFU/g) and *Bifidobacterium lactis* 420 ($3.0 \times 10^6$ CFU/g) on the host microbiota. After the run-in period (3 weeks), subjects consumed the probiotic or control yogurt for 5 weeks and then they have switched groups for another 5 weeks. Probiotics were significantly increased in fecal samples, mainly *B. lactis* 420 counts, but this was transient. The levels of cholesterol, LDL-c and HDL-c, and SCFA did not change, nor did other specific immune parameters.[59] It is noteworthy that adults were healthy, there was no washout period, and the probiotic doses administered were probably not sufficient to promote health benefits.[60]

The effects of the consumption of probiotic strains on healthy adults were recently reviewed by Khalesi et al. and found that current literature supports the use of probiotics to improve defection frequency, stool consistency, bowel movement, immune system responses, and vaginal lactobacilli concentration.[61] It seems that the use of probiotics in improving GI discomfort is most effective in healthy individuals.

The most well-known properties of prebiotics in healthy individuals is their ability to relieve intestinal discomfort or increase absorption of minerals. Improvement in stool consistency, defecation frequency, regulation of motility by SCFA-signaling hormones, flatulence and bloating, and increased calcium solubility by pH reduction are also described frequently.[62–64]

Supplementation of prebiotic GOS at 5 g/day increased calcium absorption, together with an increase in fecal bifidobacteria in young girls (9–13 years of age), suggesting that bifidobacteria may mediate this mechanism.[62] Similarly, the use of a mixture of inulin-type fructans (8 g/day) has been shown to be effective in increasing calcium absorption in adolescents and may be an interesting approach for improving or preventing bone mass loss.[65]

The effect of β-fructans, such as oligofructose and fructo-oligosaccharides, has been evaluated in healthy and ill subjects. A recent meta-analysis of human studies that tested β-fructans as a single supplementary ingredient and applied in a product or in the form of dietary supplementation demonstrated that short-chain and not long-chain β-fructans supplementation (degree of polymerization $<10$) contributed to an increased stool frequency (0.36 defecation $\pm 0.06$ per day; $P < 0.001$). In addition, β-fructans improved stool consistency and stool wet weight. The effects promoted by β-fructans appear to improve these features up to a dose of 18 g/day, with no substantial effects above that. Furthermore, bifidobacteria growth

stimulation appears to be dose-related, between 2.5 and 10 g/day, and doses above this only lead to marginal increases in stool frequency. Thus, different types of prebiotics might be useful for those with chronic constipation and may promote well-being by regulating bowel function.[66]

There are divergent opinions about studies designed to investigate the ability of probiotics to colonize the gut. Many studies fail to perform a follow-up of study participants' microbiota. However, since lifestyle is one of the factors shaping the autochthonous microbiota and probiotics have poor adherence to the intestinal mucosa, it is to be expected that the consumption of probiotic foods on a daily basis is recommended to produce the benefits observed in clinical trials.

## 5. Effects on various diseases

### 5.1 Application on early life conditions and diseases

Gastrointestinal disturbances are common in the early years of life. Therefore, many clinical trials are dedicated to preventing and treating diseases that affect this population, including acute and antibiotic-associated diarrhea, infantile colic, prevention of *Clostridium difficile*-associated diarrhea, necrotizing enterocolitis, *H. pylori* infection and neonatal sepsis.[67]

It is common for infants not to receive exclusive breastmilk and to minimally receive its benefits.[68] Thus, some infant formulas have been supplemented with prebiotics, such as GOS and FOS, aiming to prevent diseases. Enrichment of infant formulas with prebiotics has been effective in reducing respiratory tract infections to comparable levels observed breast fed children.[69,70]

#### 5.1.1 *Acute gastroenteritis, antibiotic associated diarrhea,* Clostridium difficile*-associated diarrhea*

The most well-established condition for probiotic supplementation in infants is acute diarrhea. The use of probiotics is strongly recommended by the European Society of Pediatric Infectious Diseases (ESPGHAN) guidelines as an adjunct with rehydration for reducing the length of hospitalization (1.12 days, 95% confidence interval [CI] −1.16 to 0.38) and disease symptoms. In addition, the best-established strains with high quality clinical trials conducted are *L. rhamnosus* GG and *S. boulardii* CNCM I-745.[71]

Antibiotic-associated diarrhea (AAD) is a major global health concern in children, especially in Asia-Pacific where antibiotics are overused.[72,73] The main causative agent is *C. difficile* and its complications are severe.

According to current guidelines for the Asia-Pacific region, *L. rhamnosus* GG and *S. boulardii* CNCM I-745 may be considered in pediatric populations, since they are effective in the prevention of AAD and *C. difficile* infections.[74] In contrast, the ESPGHAN group strongly recommends the use of *L. rhamnosus* GG or *S. boulardii* for AAD prevention. The recommendation to use *S. boulardii* to prevent *C. difficile* associated diarrhea is not as strong and includes the caveat that physicians should be cautious and include evaluation of risk factors for these conditions before prescribing use of these probiotic strains.[75]

### 5.1.2 Necrotizing enterocolitis (NEC)

The incidence of NEC in preterm infants is high, mainly in those with very low birth weight (VLBW) <1500 g, increasing the interest in evaluating probiotic effects that improve intestinal barrier function, prevent bacterial translocation, promote mucosal IgA production, and inhibit potential pathogens.[76,77]

Several meta-analyses show that the combination of *B. infantis* + *Str. thermophilus* + *B. bifidus* and *L. acidophilus* + *B. infantis*, were the most effective in reducing risk of NEC and mortality in VLBW infants, but not for nosocomial sepsis.[78] Thus, Latin-American (LATAM) experts' consensus grades 1a (Systematic Review with homogeneity of RCTs) recommend use of the following probiotic strains: *B. brevis*, *L. rhamnosus* GG, *L. acidophilus*, and *L. reuteri* DSM 17938 and mixtures of *Bifidobacterium* and *Streptococcus*.[67]

Although several studies have demonstrated the efficacy of probiotics from the *Lactobacillus* and *Bifidobacterium* species in reducing the incidence of NEC and NEC-associated mortality, recently conducted high quality RCTs have not been able to demonstrate that BBG-001 strain *B. brevis* is effective in reducing the risk of NEC. However, the latest expert consensus for the Asia-Pacific region states that probiotics may be considered to prevent NEC due to evidence of their effectiveness in reducing risk and mortality in high-risk populations.[74]

### 5.1.3 Infant colic

Colic is perceived as a condition that requires intervention in infants. There is usually an increase in gas production and changes in bowel motility, resulting in abdominal pain. The *L. reuteri* DSM 17938 strain was effective and safe in many clinical trials for the prevention of colic episodes, reducing infant fussiness, regurgitation, and constipation. Therefore, Latin-American experts classify the recommendation of this strain as grade 1a (prevention)

and 1b (treatment), while for Asia-Pacific population the evidence is considered weak, but the use of *L. reuteri* DSM 17938 may be considered.[67,74]

### 5.1.4 Inflammatory bowel disease

The role of probiotics in inflammatory bowel diseases is mainly targeted toward UC and Crohn's disease (CD). Probiotics are thought to modulate the host mucosal immune response, resulting in anti-inflammatory effects in IBD patients. Particularly for UC, guidelines suggest that commercial probiotic VSL#3, a multi-strain product including Bifidobacteria, Lactobacilli and Streptococcal species, is effective in inducing and maintaining remission of mild to moderate UC.[79] Another strain well studied is *E. coli Nissle*, for which the guidelines indicate that it may be as effective as standard mesalazine treatment in maintaining remission in UC.[74]

Crohn's disease management seems to be less effective, although several studies have shown changes in the intestinal microbiota to be relevant in this condition. One possible reason is that CD may be sub-classified, due to the genetic component present.[80] Current guidelines do not support effective probiotic therapy in maintaining remission of CD. The last expert panel conducted by Cameron et al. for the Asian-Pacific region concluded that the evidence is weak to indicate the use of probiotics in IBD in children, and did not recommend it overall.[74] In contrast, LATAM expert consensus recommended the use of VSL#3 only for UC (Evidence 1b—Individual RCT, with narrow Confidence Interval).[67,81]

### 5.1.5 Helicobacter pylori infection

With the increase in antibiotic resistance by pathogenic bacteria, interest for probiotics has grown with the objective of using them as a primary and adjuvant therapy. Some *Lactobacillus* species studied were unable to eradicate *H. pylori*, even in combination with drugs, such as antibiotics and proton pump inhibitors and probiotics only moderately improved eradication rates.[82,83] The recommendation for their use in the Asia-Pacific region is classified as "very low quality," but stronger evidence exists for the use of *S. boulardii*. There are currently no recommendations for probiotic use for *H. pylori* eradication in other geographic regions.[67,74,75]

It is noticeable that the evaluation of probiotic effects in the first years of life is directed toward health outcomes and not necessarily their effects on the intestinal microbiota. There are many RCTs conducted in infants, since they are a high-risk population with high mortality rates, garnering interest to improve the health status of this vulnerable population.

Additionally, specific guidelines have been developed for different regions, recognizing that each bacterial strain has unique properties that are affected by geography.

## 5.2 Adults

### 5.2.1 Metabolic diseases

With increasing prevalence of obesity and metabolic diseases such as diabetes mellitus type 2 (T2DM) in developed and emerging countries, the contributing factors for these diseases have been widely studied.[84] The GM plays a key role in energy homeostasis and low-grade inflammation, and its unbalance is associated with the development of the diseases mentioned above, a fact that has aroused the attention of researchers. Some bacterial species have been associated with obesity, such as *Lactobacillus* and *Bifidobacterium* species, Prevotellaceae and *Blautia coccoides*, as well as a high ratio between Firmicutes and Bacteroidetes.[85] Interventions that restore bacterial homeostasis in the gut may represent an important target for the use of probiotics and prebiotics and several studies have been conducted with the purpose of testing this approach. Such studies and respective results are discussed below.

In one study conducted by Stenman and colleagues, the strain *B. animalis* ssp. *lactis* 420 (B420) was administered to 225 healthy overweight or obese volunteers during a six-month period. The studied population was divided into four groups—probiotic only (B420, $10^{10}$ CFU/day), prebiotic Litesse® Ultra polydextrose alone (LU, 12 g/day), probiotic + prebiotic (B420: $10^{10}$ CFU/day + LU: 12 g/day), and placebo. In this study, B420 with or without LU was found to be effective in reducing body fat mass, waist circumference, energy intake, and body weight compared to placebo. This finding suggests a synergistic effect between the probiotic and the prebiotic and a major role for the chosen strain, as the prebiotic group did not show any difference when compared to the placebo. Results also demonstrated that expression of zonulin, a potential marker of intestinal permeability, was significantly correlated with changes in subjects' truncal fat mass ($r = 0.349$, $P < 0.0001$) in the B420 + LU group. Thus, the authors hypothesized that serum zonulin may be involved with gut microbiota modulation and intestinal integrity.[86]

Afterwards, Hibbert and colleagues aimed to explore possible changes in the microbiota and metabolites in the same population. The authors reported that *Lactobacillus* and *Akkermansia* were more abundant in the B420 group at the end of the intervention. *Methanobrevibacter*, Christensenellaceae, and *Akkermansia* were increased in B420 + LU, and LU alone groups, across

the intervention period and had a negative correlation with the waist-area body fat mass at the end of the intervention.[87]

*Akkermansia muciniphila*, a mucin degrading bacteria, has gained attention since its abundance is known to be inversely associated with obesity, diabetes, cardiometabolic diseases, and low-grade inflammation.[88,89] Animal studies have shown that treatment with *A. muciniphila* reduces obesity and associated comorbidities, such as insulin resistance and hepatic steatosis.[90,91] Due to difficulties cultivating this bacteria, its effects after heat treatment were evaluated in animal studies and demonstrated an even greater impact on adiposity, glucose tolerance, and insulin resistance after *A. muciniphila* was heat-treated (HT). Thus, Depommier et al. developed a synthetic medium and conducted a clinical trial on overweight/obese humans as a proof-of-concept exploratory study.[92]

The study was conducted with 40 volunteers, and 32 completed the 3-month protocol with daily administration of *A. muciniphila* at a concentration of $10^{10}$ CFUs/day. Subjects who received the HT *A. muciniphila* had improved insulin sensitivity ($+28.62 \pm 7.02\%$, $P = 0.002$), reduced insulinemia ($-34.08 \pm 7.12\%$, $P = 0.006$), and decreased total plasma cholesterol levels ($-8.68 \pm 2.38\%$, $P = 0.02$), when compared with the placebo group. Together, a small reduction in body weight ($-2.27 \pm 0.92$ kg, $P = 0.091$) was observed, compared with the placebo group. Fat mass ($-1.37 \pm 0.82$ kg, $P = 0.092$) and hip circumference ($-2.63 \pm 1.14$ cm, $P = 0.091$) were reduced compared to baseline. As this was the first human study with *A. muciniphila* administration, tolerability and safety were among the primary outcomes, while metabolic effects were the secondary. These interesting results will definitely arouse interest from other researchers to conduct other RCTs that may potentially replicate these results and validate beneficial effects on other metabolic disorders.[92] Despite the results obtained, supplementation with either HT or living cells of *A. muciniphila* did not affect the overall structure of the gut microbiome. In fact, it only increased the quantity of *A. muciniphila* recovered in the feces by 1.7 and 2.6 log, respectively, in the HT and living cells groups.[92]

In recent years, several clinical trials evaluating the effects of probiotics on obesity have been done, with positive results in weight reduction,[93–96] waist circumference,[93,97–100] and fat mass,[93,96,99,101] and mainly *Lactobacillus* and *Bifidobacterium* species were applied, besides mixtures of probiotic strains and synbiotics. Altogether, these studies demonstrated that the use of probiotics, especially if combined with a dietary intervention, were able to assist in weight loss and fat mass reduction in obesity, probably by modulating the intestinal microbiota.

The effects of probiotic and prebiotic food have also been investigated in other metabolic diseases such as T2DM and non-alcoholic fatty liver disease (NAFLD). Studies using the *Lactobacillus* La-5 and the *Bifidobacterium* Bb-12 strains as an intervention in individuals with T2DM showed positive results for fasting glucose[102] and hemoglobin fraction A1c (HbA1c),[103] as well as improved lipid profile.[102–104]

In a recent meta-analysis of RCTs, conducted by Zheng et al., the effects of different probiotics and synbiotics on inflammatory biomarkers and oxidative stress in diabetic individuals were evaluated. Despite the methodological limitations emphasized by the authors, the consumption of probiotics and synbiotics was able to reduce high-sensitivity C-reactive protein (standardized mean difference [SMD] = − 0.38; 95% confidence interval [CI] levels: −0.51, −0.24; $P=0.000$) and malondialdehyde (SMD = −0.61; 95% CI: −0.89, −0.32; $P=0.000$) levels, together with a total increase in the antioxidant capacity (SMD = 0.31; 95% CI: 0.09, 0.52; $P=0.006$), in nitric oxide (SMD, 0.62; 95% CI, 0.25–0.99; $P=0.001$), and glutathione (SMD = 0.41; 95% CI: 0.26, 0.55, $P=0.000$) levels, in diabetic patients compared to those in the placebo group. The authors highlighted the heterogeneity between strains and intervention time, as well as the low amount of available data. Nevertheless, the probiotic and synbiotic approach demonstrated efficacy in improving the evaluated biomarkers.[105]

Verifying the effects of probiotics on altered parameters in individuals with T2DM has been a challenge, due to the lack of literature, inappropriate experimental designs, and high heterogeneity in the administration of probiotics. In a meta-analysis that included only RCTs conducted with diabetic subjects or with associated risk factors, Sun et al. evaluated the following outcomes: fasting blood glucose, glycated hemoglobin (HbA1c), insulin, or HOMA-IR (Homeostatic Model Assessment-Insulin Resistance). The results of the 11 included studies showed that probiotics were able to reduce blood glucose (mean: −0.50 mmol/L; range: 0.09–1.29 mmol/L), HbA1c (mean: −0.48%; range: −0.3 to 1.21%), whereas they were not able to decrease insulin and HOMA-IR significantly.[106]

NAFLD is characterized by the accumulation of fat in the liver (over 5% of liver weight).[107] This classification involves simple steatosis to non-alcoholic steatohepatitis (NASH) and may lead to severe conditions such as fibrosis and cirrhosis.[108] Because obesity, inflammation, insulin resistance, and dyslipidemia are risk factors for the disease, researchers have been studying the potential effects of probiotics and prebiotics on the disease.[109]

Fatty liver improvement was reported by Ahn et al. in a study conducted with 34 obese and NAFLD individuals, using a mixture of *Lactobacillus* and

*Bifidobacterium*. Using 16S rRNA microbiome sequencing gene, an increase in *Ruminococcaceae-2*, *Lachnospiraceae-2*, *Coprococcus*, *Lachnospiraceae-1*, *Ruminococcus*, and *Dorea* was observed.[110] Oligofructose supplementation (8 g/day for 12 weeks followed by 16 g/day for 24 weeks) in adults with liver-biopsy-confirmed NASH was also able to modulate the intestinal microbiota, increasing *Bifidobacterium* and reducing *Clostridium* clusters XI and I, together with ameliorating liver steatosis histologically-confirmed in NASH patients, compared to placebo.[111]

There are studies supporting the use of probiotics and prebiotics in NAFLD. A recent review targeting the effect of synbiotics on NAFLD found evidence to suggest that synbiotics can reduce inflammation, insulin resistance, and anthropometric parameters in NAFLD patients. However, the effects on dyslipidemia and oxidative stress are inconsistent. Additional RCTs in larger study cohorts including more accurate methods for the evaluation of the severity of diseases, such as liver enzymes, are chief to improve our understanding of probiotic usage in NAFLD.[112]

### 5.2.2 Inflammatory bowel disease (IBD)

The two main forms of IBD are UC and CD. In these conditions, the intestinal immune system is imbalanced, leading to non-specific chronic inflammation of the intestinal tract.[113]

Studies assessing the intestinal microbiota of individuals with IBD found a dysbiosis that could contribute toward an intestinal barrier dysfunction. In CD it is common to observe a reduction in the complexity of the Firmicutes phyla, specifically a reduced abundance of *F. prausnitzii*, as well as an increase in enteropathogenic *E. coli*.[114,115] In UC, the intestinal microbiota is characterized by a high ratio of *B. fragilis*/*F. prausnitzii* and a low abundance of butyrate-producing bacteria.[116]

There is a lot of controversy in the literature about how effective and safe the use of probiotics and prebiotics in patients with IBD might be, due to the intestinal mucosal sensitivity and a potential worsening of the disease.

In a recent meta-analysis, Astó et al. proposed to evaluate the effects of prebiotics, probiotics, and synbiotics on UC. The meta-analysis included 12 placebo-controlled trials, involving a total of 886 individuals. At first, high heterogeneity between studies was found ($I^2 = 71\%$), but after a subanalysis using only the UCDAI (Ulcerative Colitis Disease Activity Index) and DAI (Disease Activity Index) indexes, FDA-recommended scales, the heterogeneity reduced dramatically ($I^2 = 29\%$). In this case, a statistically significant difference between the probiotic and the placebo groups was observed, for which relative risk (RR) was 1.55 (CI 95% 1.13–2.15,

*P*-value = 0.007), and the OR (odds ratio) was 2.12 (CI 95% 1.36–3.31, *P*-value = 0.000, $I^2 = 12\%$).[117,118] When a sub-analysis was performed for probiotics containing bifidobacteria, studies using this genus showed efficacy in inducing remission in patients with active UC (RR = 1.73 (CI 95% 1.23–2.43, *P*-value = 0.002, $I^2 = 35\%$)) and OR = 2.50 (CI 95% = 1.33–4.70, *P*-value = 0.005, $I^2 = 44\%$). For maintaining remission, no statistically significant differences were observed. The authors concluded that probiotics are a promising approach to achieve remission in patients with UC, especially bifidobacteria-containing products, but the results depend on the scale used in the studies (UCDAI and DAI).[117] The greater effect obtained with bifidobacteria-containing products seems to be related to a higher SCFA production in the intestinal lumen, which could reduce inflammation and maintain the gut barrier function.[119]

Although the administration of probiotics and prebiotics in CD appears to be a promising intervention, there is no current recommendation for how to use them in this disease. In fact, the European Crohn's and Colitis Organization recently published a review of complementary medicine in IBD. The organization maintained their position that evidence to support use of probiotics and prebiotics in CD for induction of remission, maintenance of remission, or even prevention of relapse in CD patients following surgically induced remission, is inconclusive. However, the organization recognizes that the use of *E. coli Nissle* 1917 or a multi-strain probiotic containing a combination of lactic acid bacteria, *Streptococcus* and *Bifidobacterium* may be effective in inducing and maintaining remission in UC.[120]

Here we discuss the main applications of probiotics and bioactive foods in various diseases explored more recently. With the new definition of prebiotics, it is likely that more RCTs, including phenolic compounds and omega-3 fatty acids, will be performed in the near future, as their beneficial health properties are recognized and well-studied. There are other applications for probiotics and bioactive foods which were not emphasized here but are worth mentioning including the use of galactooligosaccharides (GOS) and fructo-oligosaccharides (FOS) to reduce the incidence of atopic dermatitis[121] and prebiotic-supplemented formula to reduce the prevalence of allergies in the first years of life.[122]

## 6. Proposed mechanisms of action

There are several pathways associated with potential health benefits of prebiotic and probiotic consumption. Fig. 2 summarizes some of them that will be discussed hereafter.

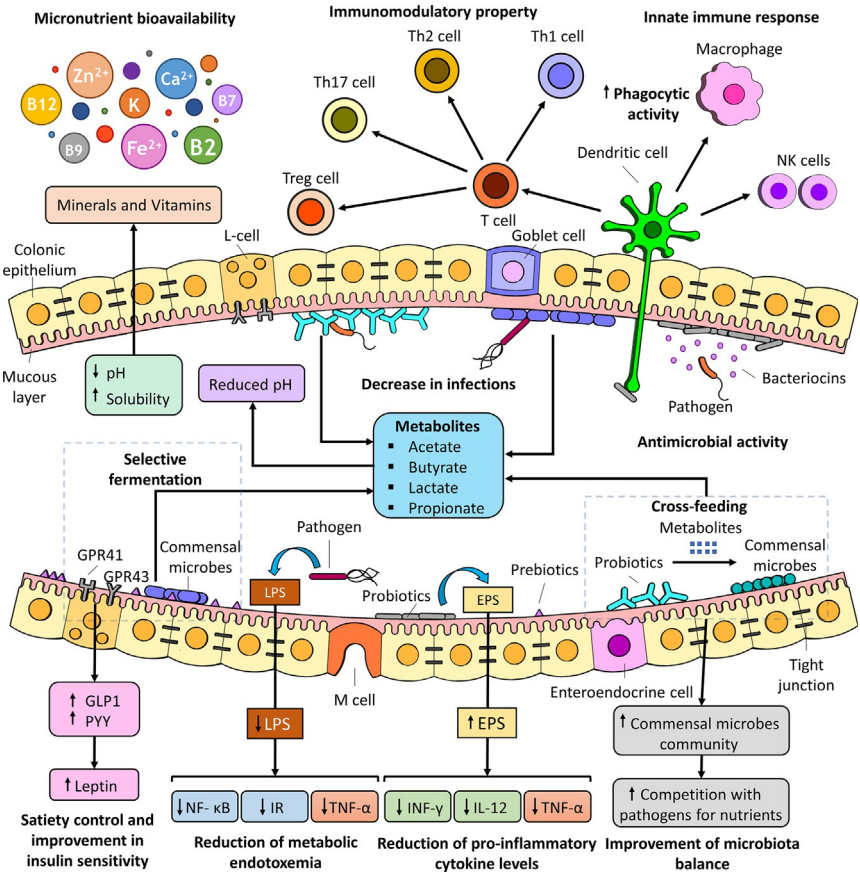


**Fig. 2** Main mechanisms of action of prebiotics and probiotics. Prebiotics are selectively utilized by the commensal microbiota, releasing metabolites like short chain fatty acids (SCFA) and organic acids, reducing the lumen pH and thus increasing the absorption of minerals and inhibiting the growth of pathogens. Metabolic products from probiotics may stimulate butyrate producer bacteria by cross-feeding mechanism. Probiotics may also increase the phagocytic activity and modulate the production of immunoglobulins, improving the immune response, promoting the microbiota modulation by competition for nutrients and adhesion sites, in addition to bacteriocin release, reducing the pro-inflammatory response, and enhancing the barrier functions. L-cell, enteroendocrine L-cell; Th1 cell, type 1T helper cell; Th2 cell, type 2T helper cell; Th17 cell, type 17T helper cell; Treg cell, regulatory T cell; NK cells, natural killer cell; GPR 41, G protein-coupled 41; GPR 43, G protein-coupled 43; GLP1, glucagon like peptide 1; PYY, peptide YY; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; IR, insulin resistance; TNF-α, tumor necrosis factor-α; EPS, exopolysaccharide; INF-γ, interferon-γ; IL-12, interleukin 12; M cell, microfold cell.

## 6.1 Immunomodulation of the host by humoral response and cell-mediated functions

The ability to increase phagocytosis, natural killer cell function, and dendritic cell activity has been attributed to some probiotic strains.[123–125] Probiotics interact with gut associated lymphoid tissue (GALT), composed mainly of Peyer's patches and lymphoid cells distributed along the gut mucosa, where antigen-presenting cells (APC), including macrophages and dendritic cells, first interact with antigens and initiate an immune response. Bacteria have a conserved structure known as microbial-associated molecular patterns (MAMPs), which are recognized by pattern-recognition-receptors (PRPs), such as Toll-like receptors, Nod-like receptors, and C-type lectin receptors. Recognition of MAMP by these receptors precipitates the maturation of APCs to define the type of immune response that will ensue—regulatory (Treg) or effector (Th1, Th2 or Th17). This immunomodulatory property of probiotic strains is the main target for allergies and inflammatory bowel disease (IBD).[126,127]

Together, probiotics demonstrate the ability to upregulate adaptive immune responses, improving vaccine response and defense against pathogens through modulation of IgA and IgM production.[128,129] There are many cell-surface components in probiotics regulating pro-inflammatory cytokine production, such as the exopolysaccharides from *Bifidobacterium breve*. These metabolites are able to reduce the expression of interferon-γ, TNF-α (tumor necrosis factor-α), and IL-12, and impair the persistence of the pathogen *Citrobacter rodentium*. *Lactobacillus rhamnosus* strain GG is capable of reducing cytokine-induced epithelial cell apoptosis and protecting against experimental colitis, due to activation of the epidermal growth factor receptor pathway and secretion of protein p40.[130,131] Bacterial strains in other probiotics express different cell-surface architecture, like fimbriae, flagella, secreted proteins, and cell wall associated polysaccharides.[123]

## 6.2 Reduced lumen pH by production of organic acids

The quantitatively and metabolically most important short chain fatty acids (SCFA) are acetate, butyrate, and propionate, occurring as end products of the human colon fermentation process.[132] The production of SCFA as primary end products of the carbohydrate metabolism is well known and described everywhere. Probiotic species from the *Bifidobacterium* and *Lactobacillus* genera produce lactic and acetic organic acids, which reduce the local pH, inhibiting the growth of pathogenic microorganisms and

provide a higher bioavailability of vitamins and minerals.[119,133] Although *Lactobacillus* and *Bifidobacterium* do not produce the main SCFA, butyrate, they promote the growth of commensal bacteria that do so, such as *Faecalibacterium prausnitzii* and *Roseburia intestinalis* by means of a cross-feeding mechanism.[134,135] It has been shown that SCFA can signal via cell-surface G protein-coupled receptors (GPCRs), such as GPR41 and GPR43, pathways related to the improvement in insulin sensitivity, regulation of energy intake through secretion of the hormones GLP-1 and PYY and satiety by increased levels of leptin. Increasing the levels of these organic acids in the intestinal lumen seems to be a promising approach against metabolic and intestinal diseases.[135,136]

## 6.3 Interactions with host gut microbiota

Probiotics may interact with the host microbiota in different ways: competition with pathogens for nutrients and epithelium adhesion, antagonism through the production of antimicrobial substances, cross-feeding other commensal bacteria, and inhibition of bacterial toxin production.[137,138] *Lactobacillus* spp. may produce antibacterial peptides like bacteriocins, including class II and III bacteriocins, which can be active in different mucosa inhibiting replication of pathogens.[139,140] The production of organic acids due to a saccharolytic property contributes to an acid environment that hinders the growth of pathogens.[141] As mentioned above, *F. prausnitzi* is able to use the *Bifidobacterium* fermentation end product acetate as substrate, characterizing the cross-feeding mechanism and consequently may improve the microbiota balance.[119] A probiotic *B. clausii* strain showed surprising results, inhibiting the cytotoxic effect of *Clostridium difficile* and *Bacillus cereus* through the secretion of an alkaline protease, elucidating a novel way to oppose enterotoxinogenic pathogens.[138]

## 6.4 Improvement in barrier function

The phenomenon known as "leaky gut" has been explored in the application of probiotics as a means of restoring intestinal barrier integrity in various diseases. Especially in metabolic diseases such as diabetes *mellitus* and obesity, probiotics have been associated with increased intestinal permeability, promoting the translocation of high amounts of LPS endotoxin from the cell wall of Gram-negative bacteria to the systemic circulation, causing what is called metabolic endotoxemia. When LPS is recognized by TLR-4 receptors in the intestinal mucosa, it promotes a pro-inflammatory response,

leading to the activation of NF-κB and production of cytokines such as TNF-α, which results in increased insulin resistance due to an IRS-1 phosphorylation in serine.[142,143] Interestingly, some scientists have attempted to clarify this mechanism and showed controversial results. *Bifidobacterium lactis* and the association of *Lactobacillus rhamnosus* 19070-2 and *L. reuteri* DSM 12246 have been shown to be effective in improving the integrity of the intestinal barrier. On the other hand, *L. rhamnosus* GG (LGG) and *L. plantarum* 299v did not affect or aggravate the intestinal integrity.[144–147] These results illustrate how the mechanism of each strain must be elucidated, as well as the pathophysiology of the disease in order to effectively use probiotics.

## 6.5 Production of enzymes and vitamins

Production of bile salt hydrolase (BSH) and β-galactosidase enzymes by some probiotic bacteria may improve cholesterol levels and lactose digestion. A large proportion of the world's population suffers from undesirable symptoms when consuming milk or dairy (lactose-containing products) in their diet. Several strains have β-galactosidase activity and in clinical trials have demonstrated symptom relief in individuals with lactose intolerance and malabsorption.[148,149] In one clinical trial, *Lactobacillus reuteri* NCIMB 30242 reduced cholesterol levels and increased BSH activity, showing that probiotic strains with high levels of BSH may be useful in the management of chronic diseases.[150] Besides, other microorganisms, such as yogurt starter cultures (i.e., *Streptococcus thermophilus* and *Lactobacillus bulgaricus*), may have a similar effect on lactose intolerance and malabsorption due to their capacity to produce β-galactosidase.[151–153]

It is well known and established that several food-related lactic acid bacteria (LAB) and commensal bacteria have the ability to produce B-group and K vitamins.[154,155] Some species of *Bifidobacterium* like *Bifidobacterium bifidum* and *Bifidobacterium longum* subsp. *infantis* can produce high levels of folate via de novo synthesis, and therefore, could help in the recovery of the nutritional status. Vitamins like riboflavin, cobalamin, niacin, and pyridoxine (the last two on a smaller scale) may also be produced by LAB, representing an interesting way to bio-enrich food products.[156] Since there are studies linking chronic fatigue syndrome with changes in the microbiota and the B-group vitamins are important cofactors for several enzymes that participate in the ATP generation in the citric acid cycle, these probiotics may provide an alternative to improve the fatigue status in these diseases.[157]

With the increased application of genome sequencing techniques allowing the selection and understanding of the mechanisms by which strains produce vitamins, it is likely that enrichment of fermented foods with B-producing vitamins will be stimulated, even within public health policies, in order to prevent nutritional deficiencies.[155]

## 6.6 Production of neurochemicals

Bidirectional interactions between the gut and the brain, referred to as the gut-brain-axis, have gained attention due to large amounts of neurochemicals produced directly or indirectly by the gut, which are now known to be modulated by the host microbiota.[158] Such neurochemicals include serotonin, dopamine, acetylcholine, and gamma-aminobutyric acid. Interactions between the gut microbiota and brain are complex and not yet well understood, but a dysbiosis is associated with neurological disorders such as anxiety, depression, and degenerative processes.[159] The use of probiotics has been shown to relieve part of the symptoms related to these diseases, such as, improving global scores and reducing cortisol levels.[160] In the next future, with studies advancing in this area, safer and more assertive approaches should emerge and benefit people under these conditions.

## 6.7 Microbiota modulation by prebiotics

Given that prebiotics stimulate selective growth of indigenous gut bacteria and that the gut is a complex ecosystem, the ingestion of different prebiotics may result in an increase of particular species depending on the individual metabotype (i.e., the metabolic responses of a group of individuals).[161,162] Just like probiotics, different prebiotics can modulate the gut microbiota through different ways, with effects primarily on the immune response, pathogen-defense mechanisms, bowel function and stool consistency, satiety-related hormone production, along with other secondary effects on metabolism. Although prebiotics are primarily known as carbohydrate-based, recent research has shown that bioactive compounds present in plants may also have the ability to shape the gut microbiota. Phenolic compounds and polyunsaturated fatty acids are two critical ones.

Reduction of Th2-type immune response seems to be the main mechanism related to the prebiotics' ability to modulate the immune function. Several clinical trials performed on infants have shown a reduction in both the incidence and the prevalence of allergic diseases such as atopic dermatitis.[163] In vitro studies have demonstrated that the inhibition of pathogens

occurs in the same manner as in the case of probiotics. So, the higher production of organic acids due to the increased proportion of beneficial bacteria leads to a reduction in the luminal pH, helping to maintain a stable microbiota in which commensal bacteria will reduce the availability nutrients to pathogens.[164]

Moreover, increasing SCFA concentrations due to prebiotic fermentation may result in changes in the intestinal motility and stool consistency, especially in infants.[63,165] However, it is important to emphasize that each SCFA may lead to different effects. As an example, higher propionate levels are related to delayed motility due to secretion of peptide YY (PYY), whereas higher butyrate levels increase motility, as shown in animal experiments.[64] Also, SCFA is capable of interacting with fatty acid receptors, GPR41 and GPR43, signaling the production of the anorexigenic hormones PYY and GLP-1, therefore suppressing appetite and improving insulin sensitivity. In addition, the administration of a synbiotic, containing L. *plantarum* ATCC 202195 + 150 mg of FOS and GOS alone, were able to prevent sepsis and decrease the intestinal permeability, showing that just as probiotics, prebiotics may have an influence on the expression of tight junction proteins, even though this mechanism has yet to be elucidated.[166,167] Together, these mechanisms suggest that the ingestion of prebiotics may be an adjuvant therapy in the prevention or management of infectious and inflammatory diseases.

Bioactive compounds have most recently been studied for their prebiotic-like effects on the gut microbiota. Among them, polyphenols and polyunsaturated fatty acids are the most frequently studied. Ingested polyphenols are metabolized by the microbiota, which may increase or decrease their bioavailability depending on the compound.[168] This biotransformation is highly dependent on the gut ecology and is heterogeneous inter-individual. Individuals can be stratified for their ability to produce specific polyphenol-derived gut microbiota metabolites (metabotypes), for example, is the low ability to produce equol from soy isoflavones in western populations (25–30%) compared to the eastern population (50%), who has the regular habit of consuming soy in their diet.[169–171] Thus, only the individuals producing higher amounts of equol benefit from the cardiometabolic effects of soy isoflavone consumption.[172]

The bioactive compounds of black and green tea, such as epigallocatechin, epicatechin, and catechin, are known potent inhibitors of pathogens including *Listeria monocytogenes*, *Salmonella typhimurium* DT104, and *Helicobacter pylori*. Other polyphenols, such as grape polyphenols and anthocyanidins, promote an increase in beneficial bacteria including

the genera *Bifidobacterium* and *Lactobacillus*, as well as *F. prausnitzii* and *Akkermansia muciniphila*.[173,174] These bioactive compounds might also be able to modify the relative abundances of specific phyla, reducing the ratio of Firmicutes to Bacteroidetes.[175] Although promising, some of these changes have yet to be tested in humans.

Omega-3 fatty acids also seem to impact the intestinal microbiota, although only a limited number of studies demonstrate effects on the microbial composition. An increase in the genus *Bifidobacterium* and a reduction in enterobacteria appear to promote a state of eubiosis, leading to increased SCFA production and suppression of metabolic endotoxemia, which results in the improvement of inflammation.[39]

## 7. Future perspectives

Throughout this chapter, we have discussed the various effects of probiotics and prebiotics and their implications on the human health (Fig. 3).

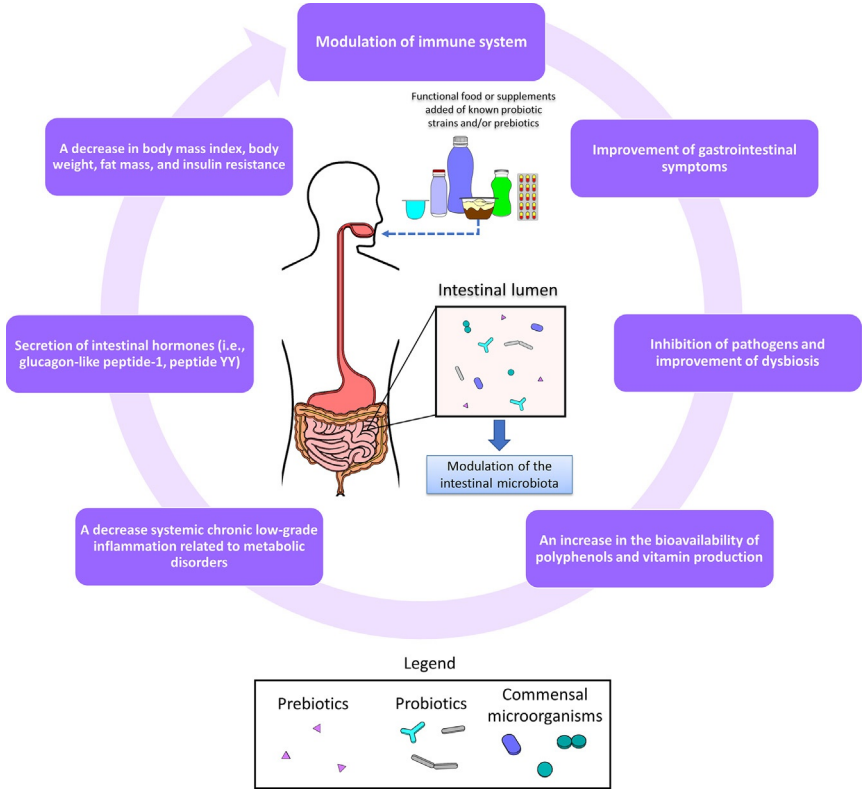


**Fig. 3** Major health effects resulting from the consumption of prebiotics and probiotics.

However, it is noteworthy to mention that results in human studies are still heterogeneous and may not be applied universally to diseases, bacterial strains, and individuals from different geographic regions.

The key understanding when we talk about probiotics is that their effects are strain specific and are usually studied in a particular pathophysiological condition. Herewith, we have the differences among microbiotas of individuals, which are influenced by the type of birth, geographical location, individual genetics, lifestyle, and diet, besides other factors.

The availability of advanced genome sequencing techniques, as well as the large number of data available in libraries such as KEGG (Kyoto Encyclopedia of Genes and Genomes) and GenBank, are providing a better understand of the human microbiota, together with bioinformatics, which facilitates the processing of these large datasets.

As appropriate strains for certain health conditions are established, it will be possible to assign health claims and to make safe and effective recommendations for health professionals who are qualified to prescribe them. Effects observed with the use of bioactive and probiotic foods tend to be more significant in individuals with impaired health statuses, usually with an intestinal dysbiosis, allowing the bioactive/probiotic food to restore a health microbiota or at least improve this imbalance in microbes.

A smart use of food matrices, food technology, and different pharmaceutical forms should contribute by enabling adequate viability of probiotic strains and regular consumption, ensuring that the end consumer enjoys the benefit provided by these foods/supplements.

Lastly, the mechanism of action of each strain must be established in in vitro, in vivo, and proof-of-concept assays. With a good understanding of the microbiota of each individual or population, predictive models and algorithms may help in establishing which groups of individuals may benefit from the use of probiotics and prebiotics. Conducting better designed, multi-center clinical trials in larger populations with relevant and objective clinical outcomes could make the use of these supplements and functional foods a routine therapy and preventive health strategy for gastrointestinal well-being.

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