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Gut microbiota

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Abstract.

The human gut is an intensively colonized organ containing microorganisms that can be health-promoting or pathogenic. This feature led to the development of functional foods aiming to fortify the former category at the expense of the latter. Since long, cultured products, including probiotics fortification, have been used for humans as live microbial feed additions. This review presents some of the microbes used as probiotics and discusses how supplementation with probiotics may help initiate and/or restore eubiotic composition of gut microbiota. Additionally, it considers safety and regulatory aspects of probiotics.

Keywords: microbiome; nutraceuticals; probiotics

1 Introduction

The number of cells belonging to the gut microbiota (including bacteria, archaea, fungi, and virus) exceeds by 10 times those of the human body (about 500 trillion versus 37 trillion), the number of their genes is 100-150 times the number of human genes, and their metabolic capacity is impressive.[1] The concept of probiotic bacteria is very old, and has been associated with the consumption of fermented foods by humans, for thousands of years. In fact, since ancient times, man has produced and eaten probiotic foods.[2] The gut microbiome plays a pivotal role in human health, competing with opportunistic bacteria for the same glycoconjugates (carbohydrates covalently linked with other chemical species such as proteins, peptides, lipids, and saccharides) present on the surface of epithelial cells.[3]

When changes in the enteral and external environments lead to a decline of intestinal-dominant microbiota, imbalance occurs, with pathogens or conditional pathogens increasing to the point of causing disease: this condition is called dysbiosis.[4–6] Dysbiosis is defined as the unfavorable alteration of an otherwise previously diverse and balanced microbiome, laying the basis for a significant disease list including diabetes, obesity, cardiovascular diseases, rheumatoid and intestinal dysimmune diseases, infective disorders (such as *Clostridioides difficile* infection), allergic diseases, chronic kidney diseases, neurologic diseases (such as multiple sclerosis, Parkinson's disease), mood disorders, colon and liver cancer.[7,8] The pathogenic implication of specific microbes in a disease, largely relies first on the identification of shifted bacterial populations based on high-throughput DNA sequencing of conserved 16S rRNA genes. There is evidence suggesting that antibiotics play a role in the pathogenesis of inflammatory bowel disease, in particular Crohn's disease, through induction of dysbiosis. In a meta-analysis, antibiotic therapy was identified as a significant risk factor for the development of Crohn's disease (odds ratio, OR, 1.74, 95% confidence interval, CI, 1.35-2.23), mainly in childhood (OR

2.75, 95% CI 1.72-4.38).[9] In irritable bowel syndrome (IBS), the microbiota is characterized by decrease in its diversity with reduction of Bacteroidetes; an increased Firmicutes/Bacteroidetes ratio, a reduction in Lactobacilli and Bifidobacteria, and an increase in Streptococci and Ruminococcus.[10,11] Change in the composition of the microbiota according to the predominance of symptoms (IBS-Diarrhea and IBS-Constipation) has also been observed.[12] Regarding atopic dermatitis (AD), it has been reported that in patients with AD, the proportion of Clostridia, *Clostridioides difficile*, *Escherichia coli*, and *Staphylococcus aureus* in the gut microbiota is higher than in healthy controls, whereas that of Bifidobacteria and Bacteroidetes is decreased. Dysbiosis might be one of the causes of AD.[13] It could act along two main pathways: a) Disruption of the epithelial lining cells; b) directly enhancing a noxious microbiota composition.[14] Regarding metabolic diseases, microbiota interacts with the host through multiple pathways, including the gut microbe-derived trimethylamine N-oxide (TMAO) pathway.[15] The important role of TMAO has been specified in the cardiovascular setting.[16] TMAO is oxidized in the liver and may affect plaque and thrombus formation. In patients with very high blood TMAO levels (like in red meat eaters), cardiovascular outcome after an acute coronary event is much worse compared to patients with low TMAO levels (like vegetarians).[17] These premises explain why the gut ecosystem is increasingly becoming more of a major therapeutic target in the management of the aforementioned diseases. Among the significant number of functions of the gut microbiome, the elaboration of food products occupies a leading position.

2 Functional foods

Diet and physical activity have been identified as two key factors that can promote health and reduce risk of disease onset. In fact, diversity, composition, and activity of gut microbiome are all influenced by diet. The spread of the so-called “Western diet”,

characterized by an excessive consumption of fats and sugars, seems to go hand in hand with the spread of incidence of metabolic diseases (i.e., obesity, hypertension, type 2 diabetes mellitus, non-alcoholic fatty liver disease [NAFLD])[18–20] and of dysimmune diseases (i.e., inflammatory bowel disease [IBD]).[21] In high-income countries, ultra-processed foods are now dominating the food supply, and are rapidly gaining ground in growing economies as well. A Western diet could lead to a permanent loss of bacteria important to microbiome function. Reversely, probiotic or fermented foods could contain beneficial bacterial metabolites enriching foods during fermentation. Studies have shown beneficial effects of fermented dairy products on metabolic markers in mice, also independently of the presence of live probiotic bacteria, either in the product or in the gut of the recipient. It has been demonstrated *in vitro* that metabolites from probiotic bacteria are capable of reducing the release of pro-inflammatory cytokines.[22]

A high-caloric and high-fat diet could cause dysbiosis too, decreasing the diversity of bowel microbiome, i.e., reducing the number of species in the bowel's ecosystem, and shifting homeostatic balance between the gut and the immune system.[23] A diet rich in saturated fats decreases the number of *Bifidobacterium* species, and intake of saturated fat increases the species from the *Bacteroides* genus.[24] A high intake of omega-6 fatty acids is associated with a decrease of *Bifidobacterium* species and consequently with an alteration of bowel-immune system homeostasis.[25] A high consumption of animal protein and saturated fats is associated with overgrowth of the *Bacteroides* enterotype.[26] The bacterial by-products that may be released by a diet rich in protein are reported to be connected with inflammation and increased colorectal cancer risk, as compared to a culture source of protein like yogurt or seitan.[27]

Classically, the "Mediterranean diet", characterized by high intake of virgin olive oil, nuts, fruits, vegetable, and cereal, a moderate intake of poultry, fish, and red wine, is

differentially stanced to the “Western diet” in terms of promoting health, with pivotaly beneficial bowel microbiome modulation reported.[28] The interactions between ingested fermented food and intestinal microbiota, and their correlations to metabolomic profiles and health, represent an important perspective, and independent research on health benefits is still emerging. The employment of fermentation for conserving foods and beverages while providing better taste, improving nutrient profile and food safety, organically preserving foodstuffs, and arguably promoting health properties, is a well-documented ancient practice. This process is not only beneficial for extending shelf-life, but also, enhancing nutritional properties in a safe and effective manner. Many types of food groups, including dairy, vegetables, legumes, cereals, starchy roots, and fruits, as well as meat and fish, can be fermented. When fermented foods and beverages are supplemented with probiotic bacteria, they present numerous extra nutritional and health characteristics. Not all fermented foods contain live organisms; beer and wine, for example, may undergo steps that remove the organisms, and other fermented foods like bread are heat-treated and the organisms are inactivated. Traditional and modern dietary practices utilize fermented foods and beverages, contributing significantly to the food chain value and contributing to a category of foods termed “functional foods” (e.g., probiotics, prebiotics, stanols, and sterols) by having an additional characteristic, i.e., health-promotion or disease prevention effect.[29] Fermentation converts carbohydrates, typically unaerobically, into organic acids, gases, alcohols, and carbon dioxide, thus providing a plethora of secondary compounds which in turn extend to the food item several benefits such as new and desirable tastes and textures, enhancement of nutrients (e.g., linoleic acid; bioactive peptides), removal of toxic or undesirable food constituents (e.g., phytic acid; bitter-tasting phenolic compounds), delivery of probiotic bacteria (e.g., *Lactobacillus delbrueckii subsp*. *bulgaricus*; *Streptococcus thermophilus*), and inhibition of foodborne pathogens.[30] In Mozambique and Zimbabwe, traditional fermented foods are used for

weaning from the age of four months. The most common fermented food in those locations is known as “mahewu”, a traditional, fermented, malted, sour, non-alcoholic maize or cassava thin porridge, sour milk, and sour porridge. The Tanzanian fermented gruel “togwa”, has been found to protect against foodborne illnesses in regions that exhibit sub-optimal sanitation and hygiene.[30]

Prebiotics are ingredients of functional foods. The term prebiotic refers to a compound that can be chemically characterized, that resists small bowel digestion and promotes homeostasis of gut microbiota and consequently the host’s health.[31] Alternatively, prebiotics can be viewed as compounds that promotes pro-biotics and/or beneficial bacteria in the gut environment. Prebiotics include polysaccharides (short chain carbohydrates) like inulin, oligofructose, fructooligosaccharides, galactose- and xylose-containing oligosaccharides, namely non-digestible foods ingredients that promote the growth of commensal bacteria.[32] Dietary fiber is heterogeneous, and thus different classifications are utilized to describe its characteristics, including, origin, chemical composition, and physicochemical properties with additional subcategorization based on the degree of polymerization (i.e. carbon chain length). Importantly, these properties could also impact microbial fermentation. With regard to origin, plant-based fiber can be separated into derived from cereal and grains, fruits, vegetables, nuts, and legumes. Such fiber types are naturally found in the diet in whole grains such as oats and barley (β-glucan) and fruits such as apples (pectin). Inulin-type fructans are naturally found in agave, artichoke, asparagus, banana, chicory root, garlic, onion, leek, and wheat.[33]

2.1 Bowel microbiota is able to metabolize food elements that escape digestion by human alimentary tract

Dietary fiber upon reaching the colon can be fermented by microbes and as such represent the main energy source of the microbiota.[7] Specifically metabolizing dietary fiber, *Bacteroidetes*, *Proteobacteria*, *Bifidobacteria*, *Clostridia*, *Eubacteria* do produce short chain fatty acids (SCFA) including acetate (directly produced from the aforementioned bacteria), propionate, and butyrate, with ensuing reduction of colonic pH value. *Eubacterium rectale* and *Roseburia* species convert acetate in butyrate.[34] The role of butyrate in promoting bowel homeostasis is solidly established in serving as a source of energy for intestinal epithelial cells, maintaining the normal barrier function of the epithelium and regulating immunity.[35] Furthermore, a small part of the overall produced butyrate in the colon, derives from the conversion of lysine derived from food proteins by *Intestinimonas* strain AF211.[36]

Habitual dietary patterns and changes in dietary elements influence the composition of bowel microbiota.[24,26] Functional foods such as whole grain maize and whole grain wheat cereal, contain a mixture of different fiber types that favor growth of beneficial bacteria, including *Bifidobacterium bifidum*, *Lactobacillus plantarum*, *Lactobacillus paracasei,* in the bowel.[37] A high intake of complex carbohydrate is associated with a *Prevotella* enterotype.[26] Prebiotics may disadvantage the growth of pathogenic bacteria such as *Clostridium perfringens*, *Escherichia coli*, *Campylobacter jejuni*, *Enterobacterium* species, *Salmonella enteritidis*, *Salmonella Typhimurium* by lowering colon pH value.

Prebiotics can be naturally found in fruits, vegetables, in particular in asparagus, garlic, leek, onion, banana, Jerusalem artichoke, chicory, and in whole grains. Average diets are often poor in prebiotics, making prebiotic addition desirable (Davis et al. 2020).

2.1.1 Fructooligosaccharides

Among the prebiotics, fructooligosaccharides, such as inulin and oligofructose, have demonstrated health promoting effects both *in vitro* and *in vivo*. Fructooligosaccharides are extracted from the blue agave plant as well as fruits and vegetables such as banana, onion, chicory root, garlic, asparagus, jícama, and leek.[38] Some grains and cereals, such as wheat and barley, also contain fructooligosaccharides. These are made of linear short chains of fructose molecules synthesized from sucrose in the vacuoles of plant leaves, stems and root cells or by hydrolysis of inulin. Fructooligosaccharides are present in garlic, onion, asparagus, artichoke, banana, wheat and yacon.[39] Similarly to other small soluble dietary fiber, fructooligosaccharides are not digested in the small intestine environment and consequently reach the colon intact, whereby are fermented by bacteria producing SCFA, favoring the growth of symbiotic *Bifidobacterium* species, and *Lactobacillus* species and counteracting the growth of pathogenic bacteria such as certain strains of *Clostridium* species and *Escherichia coli*, thus exhibiting prebiotic activity.[40–42]

2.1.2 Polyphenols

Polyphenols are plant-derived bioactive compounds. The characteristic phenolic structure consists of at least one aromatic ring and at least one hydroxyl group. Polyphenols are classically divided into flavonoids and nonflavonoids.[43]

The flavonoids of dietary interest include flavonols, isoflavones, flavan-3-ols, anthocyanidins, and others. Flavonols, like the well-known quercetin, are present in plants like onion.[44] Isoflavones are present in legumes and are known for their phytoestrogen activity.[45] Tannins belong to the group of flavan-3-ols, with green tea being the richest source of monomeric flavan-3-ols.[46] Anthocyanins, derived from anthocyanidins, give color to fruits with berries and red wine being rich sources of anthocyanins. Raspberries, strawberries, and pomegranate, nuts and coffee are all rich in nonflavonoids (phenolic

acids). Resveratrol also belongs to the group of nonflavonoids and is present in red grapes and red wine.

Gut microbiota is involved in the metabolism (dietary polyphenols are not completely absorbed from the gastrointestinal tract and are metabolized by the gut microflora) and bioavailability of polyphenols and dysbiosis could lead to an altered absorption of polyphenols.[47] In turn, polyphenols (in particular tea and red wine polyphenols) modify the gut microbiome profile due to their action against pathogenic microbes thus increasing commensal bacteria, counterbalancing, at least partly, any negative effects of a high-fat sucrose diet: phenolics exert significant effects on the intestinal environment by modulation of the intestinal bacterial population, probably by acting as metabolic prebiotics.[48]

2.1.3 Omega-3 fatty acid

A diet rich in omega-3 fatty acids is associated with higher amount of beneficial *Lactobacillus* species and with a more physiologic bowel barrier function.[49] In response to this type of diet, gut microbiota shifts to butyrate-producing bacterial species.[50]

2.1.4 Infant formula enriched with functional compounds

It has been reported that certain types of infant formula (necessary when human milk is unavailable) enriched with functional elements were associated with an increase in *Bifidobacteria* and a decrease in *Clostridia* concentration, making gut microbiota similar to that of breast-fed infants, and demonstrating that oligosaccharides, acidified milk, and β-palmitate can lead to a favorable bowel environment, increasing *Bifidobacterium* species.[51]

2.2 Probiotics

The concept of “probiotics” was crafted in 1907, when Metchnikov hypothesized that lactic acid bacteria in fermented foods could be beneficial from a health standpoint. Probiotics are live microorganisms that are used as functional foods or supplements thus extending health benefits to humans when consumed at adequate amounts. Functional foods include digestible forms of probiotics used in bio-yogurt starter, table olives, fermented soymilk, and other fermented products.[52] Most probiotics, consumed for centuries as yogurt and fermented milks, are sold as foods or dietary supplements.[53]

Some of the metabolites produced during food fermentation (e.g., organic acids, bacteriocins) also serve to preserve foods and inhibit the growth of pathogenic organism. Depending on the microorganism, the final format could be a fresh preparation (e.g., yeast cake), frozen liquid, or dry. The popularity of fermented foods and beverages is due to their enhanced shelf-life, safety, functionality, sensory, and nutritional properties. Regarding enhanced nutritional properties, the most important include the presence of bioactive molecules, vitamins, and other constituents with increased availability due to the process of fermentation. Many fermented foods also contain live microorganisms that might improve gastrointestinal health and provide other health benefits, including lowering the risk of type 2 diabetes mellitus and cardiovascular diseases. The number and type of organisms in fermented foods can vary significantly, depending on how products were manufactured and processed, as well as conditions and duration of storage.[54]

The fact that a particular food or beverage is produced by fermentation does not necessarily indicate that it contains live microorganisms. Although bread, beer, wine, and distilled alcoholic beverages require yeast for fermentation, the organisms produced are either inactivated by heat (in the case of bread and some beers) or physically removed by filtration or other means (in the case of wine and beer). According to both tradition and various national and international standards of identity, yogurt is made with a culture

containing strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus*. However, many commercial products are supplemented with probiotic bacteria, particularly strains of Bifidobacterium and Lactobacillus to obtain added benefits. There are a large number of recent human clinical studies in which the so-called probiotic yogurts and other probiotic-containing cultured milk products have been examined, with specific clinical end points measured.[55]

Probiotic consumption has been shown to increase microbiome diversity in animal model and in humans.[56,57] With reference to the ability of a given bacterial species to initiate/restore microbiome diversity, a great number of studies have identified *Lactobacillus* and *Bifidobacterium* over some 1,000 microbiota genera. Also strains of bacterial groups once considered pathogenic, like *Bacteroidetes* and *Clostridia*, could be associated with beneficial effects.[58]

The choice of appropriate probiotic(s) can be challenging, as a variety of factors are relevant: the strain-specific and disease-specific efficacy of probiotic products, differences in the mechanisms of action for different probiotic strains, differences in manufacturing processes and quality control of the products and differences in international regulatory requirements.

2.2.1 Probiotic used in inflammatory bowel disease (IBD)[59]

Faecalibacterium

Faecalibacterium, an anaerobe of *Clostridium* cluster IV, is reported to contrast inflammation by producing butyrate: consistently, it seems to ameliorate colitis in mouse models, and is depleted in IBD patient cohorts.[60,61]

Faecalibacterium prausnitzii levels are reportedly reduced in ileal Crohn's disease patients, although increases were noted in pediatric Crohn's disease. There are differing functional capabilities between strains, with the tantalizing potential that differing phylotypes may exert opposite effects.[62]

Oral administration of *Faecalibacterium prausnitzii* was found to favor induction of remission in ulcerative colitis patients.[63]

Escherichia coli Nissle 1917

A large clinical trial (in total, 327 patients were recruited and assigned to a double blind, double dummy trial) was conducted to investigate the efficacy of *Escherichia coli* Nissle 1917, a nonpathogenic strain, on maintaining remission of ulcerative colitis. *Escherichia coli Nissle* 1917 achieved comparable efficacy and safety outcomes to salicylate in the maintenance of remission in ulcerative colitis patients.[64]

VSL#3

VSL#3 is a probiotic mix with 4 *Lactobacilli* (*Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii species*., *Lactobacillus Bulgaricus*), 3 *Bifidobacteria* (*Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*) and a *Streptococcus* (*Streptococcus salivarius* subsp. *thermophilus*).

A clinical study found that VSL#3 was effective in inducing remission in patients with mild-to-moderately active ulcerative colitis.[65] In addition, VSL#3 was found to be effective in maintenance of remission. A meta-analysis has shown that VSL#3 added to standard treatment achieved better outcomes when compared to standard treatment alone in the induction of remission and response: five studies with 441 patients were identified. The pooled remission rate was 49.4% (95% CI, 42.7-56.1). A >50% decrease in the Ulcerative

Colitis Disease Activity Index was achieved in 44.6% of the VSL#3-treated patients versus 25.1% of the patients given placebo (P = 0008; OR, 2.793; 95% CI, 1.375-5.676; number needed to treat = 4-5).[66]

Lactobacillus GG

A randomized study investigated the efficacy of *Lactobacillus GG* in quiescent ulcerative colitis. One hundred eighty-seven ulcerative colitis patients with quiescent disease were randomized to receive Lactobacillus GG 18 x 10(9) viable bacteria/day (65 patients), mesalazine 2400 mg/day (60 patients) or Lactobacillus GG + mesalazine (62 patients). Disease activity index, endoscopic and histological scores were determined at 0, 6 and 12 months and in case of relapse. The primary end point was to evaluate sustained remission. The treatment with *Lactobacillus GG* seemed to be more effective than standard treatment with mesalazine in prolonging the relapse-free time (P < 0.05).[67]

2.2.2 Prevention of antibiotic-associated diarrhea

Antibiotic-associated diarrhea may occur in 5–50% of patients after use of almost any type of antibiotic, owing to microbiome disruption.[68]

The consequences of antibiotic-associated diarrhea may include prolonged hospitalization, increased healthcare costs, higher risk of acquiring other nosocomial infections and poor compliance (especially for outpatients), with inadequate cure rates.[69]

On Table 1 controlled studies that favor probiotic use in prevention of antibiotic diarrhea are listed.[70]

Table 1.

It has been reported that certain foods and food components (such as soy), can improve gut microbiota: in general, both animal and human studies have shown that consumption of soy foods can increase the levels of bifidobacteria and lactobacilli and alter the ratio between Firmicutes and Bacteroidetes. These changes in microbiota are consistent with reported reductions in pathogenic bacteria populations in the gut, thereby lowering the risk of diseases and leading to beneficial effects on human health.[71] The fiber-derived fermentation products including SCFAs butyrate, propionate and acetate contribute to a variety of positive health effects both locally in the intestine and systemically.[72] For example, benefits have been reported in animal models of inflammatory bowel disease, whereby reduced levels of SCFAs in the feces were associated with inflammatory bowel disease severity,[73] and in humans, in whom the absence of bacteria with potential for SCFA production has been seen as a signature of Crohn's disease patients.[74]

2.2.3 Prevention and treatment of Clostridioides difficile infections

Clostridioides difficile may result in often fatal disease (pseudomembranous colitis toxic megacolon) and is the leading cause of nosocomial infections.[75]

The incidence of *Clostridioides difficile* infection continues to increase in hospitals and long-term care facilities.[76]

Randomized controlled trials supporting probiotics use in prevention of *Clostridioides difficile* infections are reported in Table 2. In a Canadian study, all in-patients on antibiotics and symptomatic *Clostridioides difficile* infection, participants received a three-strain Lactobacilli mixture.[77] Intervention achieved a significant reduction of *Clostridioides difficile* infection rates, which was sustained over 10 years of the follow-up.[78] The reduction of *Clostridioides difficile* infection rates was also observed in other Canadian

hospitals which added the three-strain Lactobacilli formulation during their infection control programs.[79,80]

Table 2.

About 20–30% of patients with *Clostridioides difficile* infection may experience disease recurrence. Studies supporting the use of probiotics to avoid or reduce the rate of bacterial re-infection are listed on Table 3 below.[70]

Table 3.

2.2.4 Eradication of Helicobacter pylori (H. pylori) infection and prevention of adverse reactions to H. pylori therapy

The treatment of *H. pylori* infection (2-3 types of antibiotics, along with a proton-pump inhibitor) may be poorly accepted due to the common occurrence of adverse reactions to the antibiotic treatment.[81] Most of the adverse reactions associated to antibiotic treatments (antibiotic-associated diarrhea, nausea, vomiting or abdominal pain), may result in poor compliance to the eradication therapy. Probiotics use may effectively prevent side effects of therapy, whilst not affecting *H. pylori* eradication rates.[82]

Table 4 and Table 5, respectively, report the figures achieved in prevention of adverse reactions, and eradication rates.[70]

Table 4

Table 5

Several studies have documented the beneficial consequences of fermented dairy food products in preventing *H. pylori*-associated gastric carcinogenesis.[83] Oh et al., reported the inhibitory effect on *H. pylori* extended by two strains of yeast and several strains of Lactobacilli isolated from probiotic yogurt.[84] Sachdeva and Nagpal conducted a meta-analysis of randomized controlled trials and concluded that there was a significant improvement in *H. pylori* eradication rates while using fermented milk-based probiotics, compared to capsule/sachet-based bacteria-only preparations. An interview-based study conducted on the Mexican population documented the protective effect of yogurt against *H. pylori* infection when eaten one serving or more per week.[85] A similar study conducted on *H. pylori*-infected children demonstrated the protective role of probiotic-containing yogurt by restoring Bifidobacterium in their gut along with the reduction of *H. pylori* load.[86] The effectiveness of fermented dairy product was also investigated by Lin et al. and the results demonstrated an anti-*H. pylori* effect of three strains of lactic acid bacteria, namely LY1, LY5, and IF22, present in the supernatants of fermented milk.[87] Similarly, in Turkey, a traditional fermented milk product named Kefir also showed an increase in the eradication rates of *H. pylori* when combined with triple therapy along with reducing the side effects of therapy.[88] Finally, a popular fermented dairy product in Japan containing the probiotic bacteria *Lactobacillus gasseri* OLL 2716 (LG21), showed improvement in the efficacy of triple therapy when administered in *H. pylori*-infected patients.[89] All the aforementioned studies postulated the beneficial role of fermented food particularly made from dairy products and the probiotic action of microorganisms by *in vitro* or clinical evidence.

2.2.5 Prevention of traveler's diarrhea

Traveler's diarrhea affects as many as 24–40 million travelers worldwide, with symptom duration ranging from 12 hours to 3.5 days. One incapacitation day in Western populations results in a loss of $290–490 million dollars of lost revenue, warranting efforts to avoid this diarrheal disorder.[90] However, prevention options are limited and behavioral practices are often overlooked by travelers.

Randomized controlled trials of prevention of traveler's diarrhea, by using probiotics, are reported in Table 6.[70]

Table 6

2.2.6. Treatment of acute diarrhea

Randomized controlled trials of probiotics as an adjunctive treatment of acute diarrhea, (otherwise treated with oral rehydration only), are reported in Table 7.[70]

Table 7

2.3 Safety of prebiotics and probiotics

Excessive consumption of prebiotics might cause abdominal discomfort, bloating, and diarrhea due to osmotic phenomena caused in the gut environment.[91]

Any microbe can cause illness in a compromised host (opportunistic infections), with safety depending on several variables: the intrinsic characteristics of the organism, its use, exposure levels and features of the target population (e.g., healthy adult, elderly, immunocompromised patients, patients who are hospitalized, post-surgery, critically ill).

Once the specific probiotic strain or mixture of strains is selected for the targeted disease, the next challenge is finding a product containing those specific strains that meet the requirements of quality and stability. Microbes to be included in probiotic products must be raised with aseptic technique from pure cultures, with isolation, characterization, and maintenance of the microorganisms used as “starter cultures”. The International Dairy Federation (IDF) and the European food safety authority have listed the microorganisms that are considered safe.

Development of pure and characterized seed stocks is crucial. The number of transfers of the seed stocks and isolation of new strains from the seed stocks is restricted to limit the risk of progressive functional loss via genetic drift (an excessive number of bacterial replications from the original strain leads to daughter colonies genetically different from the original strain).[92]

The seed scale-up fermentations are routinely done in a sterile fermentation medium, with the number of scale-up fermentation limited again to reduce the risk for changes in the culture genotype and/or phenotype.[92]

All microbial food culture contact surfaces are sanitized and heat-treated to minimize risk of introduction of contaminating microorganisms. At the appropriate temperature, seed cultures are added to the fermentation vessels via sterile transfer.[92]

Lyophilized capsules maintain high concentrations for longer than probiotics in dairy products. Probiotic capsules requiring refrigeration are heat-dried (not lyophilized): limited stability at room temperature thus limits their portability. Enteric-coated capsules show higher survival rates compared to non-enteric coated capsules. So, given two probiotic products with similar strains, reviewing how well the product complies with required label information might be useful. Severe adverse events due to probiotic use are rare but might occur.

2.3.1 Lactobacillus species

The literature reports five neonates with underlying diseases, receiving *Lactobacillus rhamnosus* GG to prevent small-intestinal bacterial overgrowth or antibiotic-associated diarrhea. Use of this probiotic resulted in bacteremia in all cases and endocarditis in one case with valvulopathy.[52]

2.3.2 Bifidobacterium species

A newborn with omphalocele showed sepsis after 10 days administration of *Bifidobacterium breve* BBG01.[93]

2.3.3 Saccharomyces boulardii

Thirty cases of preterm newborns and adults with underlying diseases who underwent surgery showed fungemia due to *Saccharomyces boulardii* administration to prevent or to treat diarrhea.[52]

3 Regulatory aspects

The regulatory aspects concerning the release of probiotics into the market make a serious technical issue with differences between countries in a continuous change. Because probiotics may be available in many countries as dietary supplements, national drug regulatory agencies cannot provide the same level of clinical guidance that they do for prescription medications and the responsibility involves health providers.

Under the current regulatory framework, in the United States the producer can choose between 4 categories: food, medical food, dietary supplement, drug or biological

product.[94] Probiotics are available to consumers mainly in the form of dietary supplements and conventional foods (i.e., yogurt). The most common claims raised in the marketing of probiotics are structure and function claims.[95]

3.1 Food

Probiotics are “generally recognized as safe” (GRAS) (we do not have evidence to specifically believe there is a safety issue with a food, but it does not necessarily mean that it is indeed absolutely safe especially when considering long term exposure/consumption) and subjected only to the Food and Drug Administration (FDA)’s post-market controls.[94]

Although there is a genuine dispute among experts, the product must be considered as a food additive and must go through a rigorous pre-market clearance process.[94]

3.2 Medicinal food

The regulatory process is the same as that of food and claims must be limited to a focus on the dietary management of disease, conditioned by appropriate medical supervision, and the product must address a distinctive nutritional need.[94]

3.3 Dietary supplement

No pre-market approval is required; however, notification requirements apply for “new dietary ingredients”. Claims must regard the effect that the product has on the structure or function of the body.[94]

3.4 Drug or biological product

If the intent of a study is to substantiate a drug claim, researchers must submit an Investigational New Drug (IND) application to FDA.[53] As a part of the process, the FDA must have required investigator to conduct safety studies prior to efficacy studies.[96] An IND application, generally, includes three phases of human studies for development of the new product.

4 Concluding remarks

One of the achievements of modern science applied to gastroenterology is the demonstration that the gut is not simply the site where energy is extracted from diet constituents to make fuel for life. The gut plays rather the role of a barrier organ where the outer and the inner worlds come into an array of complex contacts swinging from reaction (inflammation) to tolerance.[97] There are at least two rulers in this interplay: the gut immune system, and the microbiota. The immune system patrols the borders and acts in a tolerance-biased fashion to encourage acceptance of the outer world coming as diet constituents. By contrast, in their multifaceted action, the billions of microbiota can preciously metabolize diet fibers, for example, but can also respond with excessive inflammation to the introduction of a protein-rich diet, or any other misconduct. The appreciation of inflammation as a leading cause of cell wear-and-tear and precocious demise is fundamental but goes beyond the scope of this paper, which is intended to emphasize the central role of the diet in health and disease. The divining and maintenance of a safe diet (i.e., poorly inflammatory and boosted by pro- and prebiotics) is in the hands of anyone willing to preserve man's life on earth.

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Table 1. Probiotics supported by several evidence for the prevention of antibiotic-associated diarrhea

Probiotics	**Evidence**	**Prescription**
Saccharomyces *boulardii* I-745	RCTs in favour: 18 RCTs against: 9	started 1–2 days of antibiotic admission, and continued for 1–4 weeks after antibiotics were discontinued
Mixture of Lactobacillus *acidophilus* CL1285 + Lactobacillus *casei* LBC80R + Lactobacillus *rhamnosus* CLR2	RCTs in favour: 3 RCTs against: 1	started 1–2 days of antibiotic admission, and continued for 1–4 weeks after antibiotics were discontinued
Lactobacillus *casei* DN114001	RCTs in favour: 2 RCTs against: 0	started 1–2 days of antibiotic admission, and continued for 1–4 weeks after antibiotics were discontinued

Table 2. Probiotics supported by several evidence for the prevention of *Clostridioides difficile* infection

Probiotics	**Evidence**	**Prescription**
Mixture of Lactobacillus *acidophilus* CL1285 + Lactobacillus *casei* LBC80R + Lactobacillus *rhamnosus* CLR2(Gao et al. 2010)	A significant reduction of *Clostridioides difficile* infection for both the low-dose (9.4%, p = 0.03) and the high dose (1.2%, p = 0.002) compared with the control group (23.8%)	Probiotic prophylaxis began within 36 h of initial antibiotic administration, continued for 5 days after the last antibiotic dose

Table 3. Probiotics supported by several evidence for the reduction of *Clostridioides difficile* recurrence after antibiotic therapy

Probiotics	**Evidence**	**Prescription**
Saccharomyces boulardii I-745	RCTs in favour: 2 RCTs against: 0 Reduced *Clostridioides difficile* infection recurrence rates in those with recurrent *Clostridioides difficile* infection	started from the 7th day of antibiotic therapy and continued for 28 days

Table 4. Probiotics supported by several evidence for the prevention of antibiotic-associated side effects during the treatment *H. pylori* infection

Probiotics	**Evidence**	**Prescription**
Saccharomyces boulardii I-745	RCTs in favour: 7 RCTs against: 2	During all the days of antibiotic therapy
Mixture of *Lactobacillus helveticus* R52 + *Lactobacillus rhamnosus* R11	RCTs in favour: 2 RCTs against: 0	For 10 days

Table 5. Probiotics supported by solid evidence for increasing the eradication rate of *H. pylori* infection

Probiotics	**Evidence**	**Prescription**
Mixture of *Lactobacillus helveticus* R52 + *Lactobacillus rhamnosus* R11	RCTs in favour: 4 RCTs against: 1	For 20 days Plus Amoxicillin, Clarithromycin and proton-pump inhibitor triple therapy for 7-14 days

Table 6. Probiotics supported by solid evidence for the prevention of traveler's diarrhea

Probiotics	**Evidence**	**Prescription**
Saccharomyces *boulardii* I-745	RCTs in favour: 4 RCTs against: 1	begun a few days before travel, continued during travel and then for 2–5 days afterwards to allow time for intestinal microflora restoration

Table 7. Probiotics supported by several evidence for the treatment of acute diarrhea

Probiotics	**Evidence**	**Prescription**
Saccharomyces boulardii I-745	RCTs in favour: 25 RCTs against: 4	for about 5 days
Lactobacillus rhamnosus GG	RCTs in favour: 10 RCTs against: 3	for about 5 days
Lactobacillus reuteri 17938	RCTs in favour: 3 RCTs against: 0	for about 5 days
Lactobacillus acidophilus LB	RCTs in favour: 3 RCTs against: 1	for about 5 days
Lactobacillus casei DN114001	RCTs in favour: 3 RCTs against: 0	not stated in paper
mix of 4 strains of *Bacillus clausii* (O/C, N/R84, T84, Sin8)	RCTs in favour: 3 RCTs against: 1	for 5 days
Mix of *Lactobacillus paracasei* BP07, *Lactobacillus plantarum* BP06, *Lactobacillus acidophilus* BA05*, Lactobacillus delbrueckii subsp. Bulgaricus BD08*, *Bifidobacterium longum* BL03, *Bifidobacterium breve* BB02 and *Bifidobacterium infantis* BI04, *Streptococcus thermophilus* BT0	RCTs in favour: 2 RCTs against: 0	At least 6 weeks