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The potential for probiotic manipulation of the gastrointestinal microbiome

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Multiple internal and external sites of the healthy human body are colonized by a diversity of symbiotic microbes. The microbial assemblages found in the intestine represent some of the most dense and diverse of these human-associated ecosystems. Unsurprisingly, the enteric microbiome, that is the totality of microbes, their combined genomes, and their interactions with the human body, has a profound impact on physiological aspects of mammalian function, not least, host immune response. Lack of early-life exposure to certain microbes, or shifts in the composition of the gastrointestinal microbiome have been linked to the development and progression of several intestinal and extra-intestinal diseases, including childhood asthma development and inflammatory bowel disease. Modulating microbial exposure through probiotic supplementation represents a long-held strategy towards ameliorating disease via intestinal microbial community restructuring. This field has experienced somewhat of a resurgence over the past few years, primarily due to the exponential increase in human microbiome studies and a growing appreciation of our dependence on resident microbiota to modulate human health. This review aims to review recent regulatory aspects related to probiotics in food. It also summarizes what is known to date with respect to human gastrointestinal microbiota – the niche which has been most extensively studied in the human system – and the evidence for probiotic supplementation as a viable therapeutic strategy for modulating this consortium.

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Introduction

The concept of purposeful microbial ingestion to manipulate the microbial ensemble in our intestine for the benefit of human health is not novel. The Russian

biologist and Nobel laureate Ilya Ilyich Mechnikov ('Eli Metchnikoff') suggested in 1907 '*to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes.*' He hypothesized that consumption of lactic-acid-producing bacteria (LAB), such as lactobacilli found in yogurt, enhanced longevity [1]. Based on Metchnikoff's work, Cheplin and Rettger [2,3], a decade later, fed *Bacillus acidophilus* (now *Lactobacillus acidophilus* [4]) to rats and human volunteers and upon examination of stool samples found changes in the composition of the fecal microbiota which they described as '*the transformation of the intestinal flora*'. The word 'probiotic' (meaning 'for life') was probably first used in 1953 by Werner Kollath to describe organic and inorganic food supplements applied to restore health to patients suffering from malnutrition and to distinguish these 'pro-biotics' from *anti*-biotics [5]. Since that time the term has undergone several transformations, but it was not until 1974 that the expression 'probiotic' was used in relation to the interactions of microorganisms with the whole animal or human host [5]. Even so, for many years there was a lack of international and scientific accord on the definition of probiotics. Based on the consensus of a multi-national expert group of scientists convened in 2001 on behalf of the Food and Agriculture Organization of the United Nations (FAO), probiotics are today defined as '*live microorganisms which when administered in adequate amounts confer a beneficial health effect on the host*' [6]. In addition to probiotics, additional means of dietary modulation of the intestinal microbial community include prebiotics and synbiotics. Briefly, prebiotics are defined as food ingredients that cannot be digested by the human digestive system but are metabolized by discrete enteric microbes, thus stimulating proliferation of selected gastrointestinal (GI) bacteria species thought to be beneficial for human health [7]. A synbiotic, on the contrary, represents a defined supplement comprised of a mixture of probiotics and prebiotics aimed at enhancing survival and colonization of the supplemented species in the GI tract [8]. The beneficial effects of food containing probiotics (or prebiotics or synbiotics) on human health – and in particular of dairy products such as yogurt and milk – are increasingly being promoted by food manufacturers, but also by health professionals. Probiotics (and foods containing them) are advertised to contribute to overall well-being and are sought to prevent and alleviate many diseases, especially digestive, immunological and respiratory disorders. However, regulatory bodies in both the US and Europe have recently restricted label claims that were made without rigorous scientific support. This has prompted expanded research efforts in the field to better

Table 1

Microbes commonly used as probiotics, examples of their potential disease targets, and references of recent (2006–2011) human trials.

Species	Target	Study type ^a	Type of probiotic ^{b,c}	Outcome ^e	Reference
<i>Lactobacillus acidophilus</i>	Type 2 diabetes	R, DB, PC	Capsules with freeze-dried <i>L. acidophilus</i> NCFM; about 10 ¹⁰ CFU per capsule	Ingestion of <i>L. acidophilus</i> preserved insulin sensitivity versus placebo, but did not affect systemic inflammatory response	[76]
	Incidence of cold and influenza-like symptoms in healthy children	R, DB, PC	Dried <i>L. acidophilus</i> alone (10 ¹⁰ CFU/day) or in combination with <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> (10 ¹⁰ CFU/day combined) mixed into milk	<i>L. acidophilus</i> alone or in combination with <i>B. animalis</i> subsp. <i>lactis</i> reduced incidence and duration of fever, coughing, and rhinorrhea in healthy children	[77]
<i>Lactobacillus fermentum</i>	Mucosal immunity in endurance athletes	DB, PC, CO	Daily dose of 1.26 × 10 ¹⁰ CFU of <i>L. fermentum</i> VRI-003 as a freeze-dried powder in gelatin capsules	Substantial reduction in the number of days and severity of respiratory illness as reported by participants	[50]
	Hypercholesterolemia	R, DB, PC	Two capsules containing <i>L. fermentum</i> twice daily (each capsule containing 2 × 10 ⁹ CFU)	No change in serum lipids	[78]
<i>Lactobacillus delbrueckii</i>	Immune senescence and resistant to the common cold	NA	Yogurt fermented with <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	Augmented natural killer cell activity and reduced the risk of catching the common cold in elderly individuals	[11]
<i>Lactobacillus johnsonii</i>	Crohn's disease	R, DB, PC	<i>L. johnsonii</i> LA1 (Nestec) in freeze-dried form and blended with maltodextrin at 10 ¹⁰ CFU/day	No effect on endoscopic recurrence after ileo-caecal resection	[79]
	Skin immune status following UV exposure	R, DB, PC	1 × 10 ¹⁰ CFU of <i>L. johnsonii</i> LA1 (NCC533) per day	Accelerated recovery of skin immune homeostasis after UV-induced immunosuppression	[80]
<i>Lactobacillus rhamnosus</i>	Allergic sensitization and asthma in infants at risk	R, DB, PC	6–24 months old infants at risk received 10 ¹⁰ CFU of <i>L. rhamnosus</i> strain GG (LGG; ATCC 53103) twice daily for 6 months	No clinical effect on atopic dermatitis or asthma-related events, and only mild effects on allergic sensitization	[51]
	Prevention of (atopic) eczema in children	R, DB, PC	Daily dose of 1 × 10 ¹⁰ CFU of <i>L. rhamnosus</i> strain GG (LGG) postnatally for 6 months	Frequency of eczema reduced during the first 7 years of life	[81]
<i>Lactobacillus reuteri</i>	Prevention of antibiotic-associated diarrhea	R, DB, PC	Patients receiving antibiotics were given 1 × 10 ¹⁰ CFU of <i>L. reuteri</i> twice daily	Significant decrease of antibiotic-associated diarrhea	[82]
	Infantile colic	R, DB, PC	Suspension of freeze-dried <i>L. reuteri</i> DSM 17 938 in a mixture of sunflower oil and medium-chain triglyceride oil; 10 ⁸ CFU/day	Improved symptoms of infantile colic	[83]
<i>Lactobacillus casei</i>	Allergic asthma and/or rhinitis in children	R, DB, PC	Long-term consumption of fermented milk containing 10 ⁸ CFU/mL of <i>L. casei</i>	Improved allergic rhinitis but no effect in asthmatic children	[84]
	Small bowel injury in chronic low-dose aspirin users	R, PC	<i>L. casei</i> for 3 months	Effective for the treatment of aspirin-associated small bowel injury	[85]
<i>Lactobacillus paracasei</i>	Incidence of eczema in infants	R, DB, PC	Infants were fed cereals with <i>L. paracasei</i> strain F19 from 4 to 13 months of age	Decreased incidence of eczema and increased Th1/Th2 ratio	[86]
<i>Bifidobacterium adolescentis</i>	Allergic rhinitis	R, DB, PC	Combination of <i>B. adolescentis</i> and <i>L. rhamnosus</i> mixed into yogurt	Potentially effects on cytokine profile but few clinical benefits and no significant effect on quality of life score	[87]

<i>Bifidobacterium breve</i>	Necrotizing enterocolitis (NEC) in preterm infants	R, DB, PC	Human milk supplemented with <i>B. breve</i> and <i>L. casei</i>	Reduced occurrence of NEC	[88]
	Prevalence of asthma-like symptoms in infants with atopic dermatitis	R, DB, PC	Hydrolyzed formula with <i>B. breve</i> alone or in combination with a galacto/fructo-oligo-saccharide mixture	Combination prevented asthma-like symptoms	[89]
<i>Bifidobacterium bifidum</i>	Irritable bowel syndrome (IBS)	R, DB, PC	One capsule containing 1×10^9 CFU of freeze-dried <i>B. bifidum</i> MIMBb75 daily for 4 weeks	Significantly alleviated IBS symptoms and improved quality of life	[90]
	Incidence of eczema in high-risk children	R, DB, PC	Mixture of <i>B. bifidum</i> , <i>B. animalis</i> subsp. <i>lactis</i> , and <i>Lactococcus lactis</i>	Reduction in parental-reported eczema during the first 3 months	[91]
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	Risk of infections in infancy	R, DB, PC	Tablets containing <i>B. animalis</i> subsp. <i>lactis</i> BB-12 (10^{10} CFU/day)	Decreased incidence of respiratory infections but no significant differences between the groups in reported gastrointestinal symptoms, otitis media or use of antibiotics	[92]
	Maintaining remission in patients with ulcerative colitis	R, DB, PC	<i>L. acidophilus</i> La-5 and <i>B. animalis</i> subsp. <i>lactis</i> BB-12	No significant clinical benefit observed	[93]
<i>Bifidobacterium infantis</i>	Irritable bowel syndrome (IBS)	R, DB, PC	Freeze-dried, encapsulated <i>B. infantis</i> at a daily dose of 10^6 , 10^8 , or 10^{10} CFU for 4 weeks	Significant improvement of IBS symptoms with 10^8 CFU but not with other doses	[94]
<i>Bifidobacterium longum</i>	Allergic rhinitis	R, DB, PC	<i>B. longum</i> BB536	Significant decrease in subjective symptom scores; marked improvements in medical scores	[95]
	Bowel movements in the elderly	R, DB, PC	Fermented oat drink with 10^9 CFU/day of <i>B. longum</i> strains BL46 and BL2C or 10^9 CFU/day <i>B. animalis</i> subsp. <i>lactis</i> strain Bb12 or without viable bacteria	Probiotic supplementation normalized bowel movements	[96]
<i>Escherichia coli</i>	Impact on carriage of multidrug-resistant <i>E. coli</i> (MDREC)	R, DB, PC	Encapsulated <i>E. coli</i> strain Nissle 1917 (5×10^9 to 5×10^{10} CFU/day)	No effect on MDREC carriage	[97]
	Persisting diarrhea in infants and toddlers	R, DB, PC	<i>E. coli</i> Nissle 1917	Significant reduction of daily stool frequency after 14 days	[98]
<i>Streptococcus thermophilus</i>	Irritable bowel syndrome (IBS) in children	R, DB, PC, CO	Mix of 8 lyophilized strains (<i>S. thermophilus</i> , <i>B. breve</i> , <i>B. longum</i> , <i>B. infantis</i> , <i>L. acidophilus</i> , <i>L. plantarum</i> , <i>L. paracasei</i> , <i>L. bulgaricus</i> ; commercial product VSL#3 [®]); daily dose of 4.5×10^{11} total CFU	Significantly ameliorated IBS symptoms and improved quality of life	[99]
<i>Bacillus coagulans</i>	Intestinal gas symptoms in adults with no gastrointestinal diagnosis	R, DB, PC	<i>B. coagulans</i> GBI-30, 6086 (commercial product GanedenBC ³⁰ [®]); 2×10^9 CFU/day	Reduced GI symptoms; strong placebo effect was evident	[100]
<i>Saccharomyces cerevisiae</i> subsp. <i>boulardii</i> ^d	Intestinal permeability in patients with Crohn's disease in remission	R, PC	Encapsulated, lyophilized <i>S. cerevisiae</i> subsp. <i>boulardii</i> (' <i>S. boulardii</i> -17'; commercial product Floratil [®]); one capsule (4×10^8 CFU) every 8 h for 3 months	Improved intestinal permeability; complete normalization was however not achieved	[101]

^a R, randomized; DB, double-blind; PC, placebo controlled; CO, cross-over; NA, data not available.

^b CFU, colony forming unit.

^c As reported in the referenced studies.

^d Commonly referred to as '*S. boulardii*' in probiotic products. Currently classified as a subspecies of *S. cerevisiae* due to its highly similar genotype, *S. cerevisiae* subsp. *boulardii* differs from other strains of *S. cerevisiae* by several key metabolic and genetic characteristics and may represent a unique species [102].

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understand the efficacy of these food products in promoting human health.

Selection of probiotics and health claim substantiation

Lactobacillus and *Bifidobacteria* species represent the microbes most commonly researched for conferring health benefits. Other LAB (*Streptococcus* spp. and *Enterococcus* spp.) as well as a strain of *Escherichia coli* Nissle 1917, some Bacilli, and even the yeast *Saccharomyces cerevisiae* subsp. *boulardii* (often referred to as '*Saccharomyces boulardii*') have been studied for their use as probiotics [9]. Table 1 represents an overview of key microbes used as probiotics as well as their application in human trials as reported between 2006 and the Spring of 2011. Many of those probiotics are found in traditional, fermented foods that for generations were believed to be beneficial for human health. This includes dairy products such as yogurt (milk fermented with *L. bulgaricus* and *S. thermophilus*) and kefir (milk fermented by a mix of several LAB), as well as non-dairy fermented foods such as sauerkraut (cabbage fermented with LAB) and pickles (cucumbers fermented with LAB). Few recent studies of fermented foods not supplemented with probiotics exist, but reports from human trials suggest that yogurt consumption may improve lactose digestion and may reduce the risk for infections in the elderly [10,11], while the ingestion of kefir has been associated with improved efficacy and tolerability of antibiotic therapy in eradicating *Helicobacter pylori* [12]. However, modern production processes such as pasteurization may reduce the viable number of beneficial microbes in traditionally fermented products. Thus, food manufacturers have more recently supplemented foods with probiotics, both to maintain a high viable cell count and to provide a probiotic organism (or several) not ordinarily present in the product. Commonly used probiotic additives include *Lactobacillus* spp. such as *L. acidophilus* or *L. casei*, and bifidobacteria like *B. lactis* or *B. longum*. While dairy products may be suitable vehicles for the delivery of probiotics the efficacy of such products on consumers' health and well-being is still under investigation.

Several factors impact the suitability of a bacterial strain as a probiotic and the traits necessary may differ depending on the target site of probiotic use (e.g. oral, vaginal, or intestinal application), as well as the mode of application. Figure 1 summarizes important characteristics for probiotic strains supplemented into food, but some attributes may also apply to other probiotic products (based on guidelines published by the group of experts convened in 2001 by FAO [6]). Besides the selection of novel probiotic strains, the demonstration of their health benefits represents a tremendous challenge for science as well as industry. This obstacle is magnified by the lack of standardization for the assessment of probiotic efficacy (e.g. selection of the target population, study design, and

definition of end points), making it difficult to effectively communicate results and to validate and compare outcomes with other studies. Two expert groups of academic scientists and industry representatives independently published reports in 2010 with guidelines for the evaluation and validation of probiotic health claims [13^{••},14^{••}]. Some of the key recommendations arising from these reports included:

Selection of an appropriate target population and comparison group for the proposed study as well as an appropriately powered study size. Selection of a reasonable duration of the trial, and effective monitoring of participant adherence to the study regimen (allowing the efficient detection of impending benefits, assessment of potential confounding factors, and extrapolation to the general consumer and broader population).

Precise identification (genotypic and phenotypic characterization) of the tested microorganism as well as detailed description of strain viability and probiotic administration, such as food matrix or probiotic carrier (to help resolve differences in outcome between studies that superficially use the same probiotic species; e.g. strain-specific discrepancies).

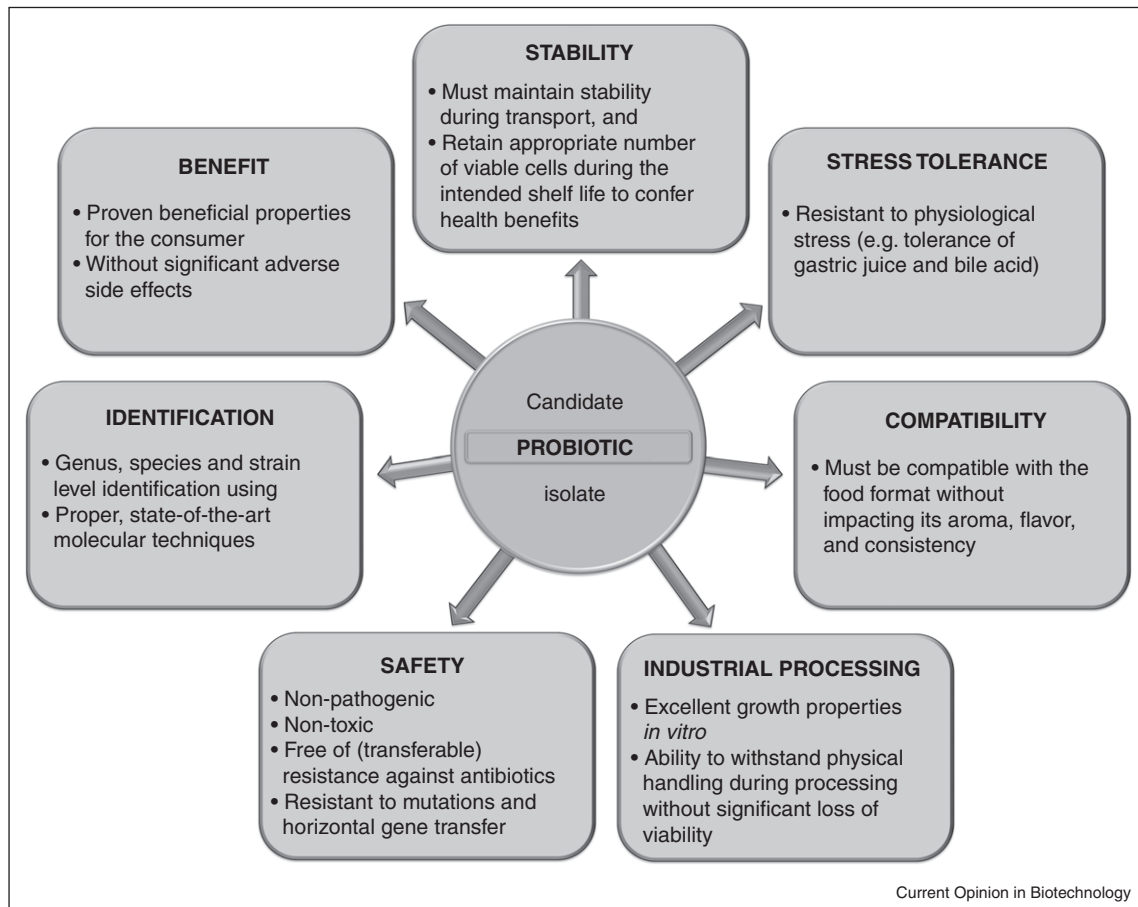
Determination of easily identifiable endpoints that are significant for the proposed health claim such as biomarkers that are indicative of disease abatement and health. Improvement of participant reports using standardized and validated questionnaires particular to the disease of interest, as well as use of a harmonized way to express results (allowing objective measurement of the effects of the probiotic intervention and comparison with other studies).

Appropriate safety assessment (consideration of genome plasticity of the selected probiotic strain, possible adverse effects of live bacteria on the trial population but also the potential for over doses by future consumers).

Regulatory aspects of probiotics and of foods containing them

In addition to the challenges of demonstrating the beneficial effects of their probiotic products on human health, that will withstand rigorous scientific scrutiny, manufacturers of probiotics also have to ensure their products are in compliance with the regulations set forth by various government agencies when applying for approval for market release. Probiotics, and foods containing them, are regulated very differently in different countries and currently there is little global consensus among the regulatory authorities. For example, the Food and Drug Administration (FDA), controlling safety, efficacy, and security of food and drugs in the USA, and the European Food Safety Authority (EFSA), the European agency responsible for the regulation of probiotics, have

Figure 1



Important considerations for the selection of novel food probiotics.

Clearly demonstrated health benefits, risk assessments (including unambiguous strain identification and safety evaluation), physiological characteristics (e.g. stress tolerance and viability), and product considerations (food production and format) are all factors critical for the successful selection of new probiotic microbes.

very different standards as to when a health claim is sufficiently substantiated by the manufacturer [15]. Even within the United States, different federal policies apply to particular probiotics and are regulated by specific centers within the FDA that have little overlap. Foods that contain probiotics, but for which no health benefits are claimed, fall under the jurisdiction of the Center for Food Safety & Applied Nutrition (CFSAN), while the Center for Biologics Evaluation and Research (CBER) oversees probiotic products (including foods) that do make a health claim. The latter, referred to by FDA and CBER as 'live biotherapeutic products' (LBPs), are regulated under Section 351 of the Public Health Service Act, 41 U.S.C. 262 and require application as an Investigational New Drug (IND) through CBER. During the IND application and review process the FDA will assess product safety and drug effectiveness. At present, the FDA is in the process of modifying its current non-binding recommendations regarding IND submissions for early clinical trials with LBPs. In September 2010,

the agency released a Draft Guidance for Industry titled 'Early Clinical Trials with Live Biotherapeutic Products: Chemistry, Manufacturing, and Control Information'. Once finalized, it will represent the FDA's current opinion on LBPs. The guidance, however, does not establish legally enforceable responsibilities and does not change current regulations unless specific regulatory or statutory requirements are cited [16].

Regulatory issues and the substantiation of health benefit claims of probiotic and prebiotic products were discussed by an expert group of US and European participants, who convened during the 2010 annual meeting of the International Scientific Association for Probiotics and Prebiotics (ISAPP). Key points from this discussion were recently published [15]. The authors point out a lack of a unified perspective in regards to health claim substantiation among consumers, healthcare providers, regulators, legislators, and scientists and that a harmonized approach and regulation (nationally and globally) would simplify

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requirements for industry and help to decrease consumer confusion. Moreover, the authors criticize the compartmentalization of the current regulatory framework as too rigid in that it only distinguishes between ‘food and drugs’, ‘health and disease’, or ‘emerging evidence and supported with significant scientific agreement’, but does not take into consideration the continuum and at times subtle variations between these entities. More discussions are clearly necessary to identify common ground among all interest groups involved and to define cogent compromises that will satisfy the needs of consumer advocates, industry representatives, and researchers.

Structure of the human-associated gastrointestinal ecosystem of microbes

The healthy human body is host to several diverse microbial communities [17–21]. The human gut is the most complex of these assemblages, and multiple human diseases, particularly chronic inflammatory diseases, have been shown to be associated with aberrations in the GI microbiota (reviewed in [22,23]). Therefore, the intestinal microbiota represents an appropriate target for probiotic intervention in the form of dietary supplement or food ingredient, with the specific aim of influencing and restoring community composition and functional capacity.

Presumed sterile at birth, the first microbial colonizers of the infant bowel are thought to be acquired during delivery and immediately after birth [24,25]. Eventually, the number of prokaryotes residing in the intestine far exceeds that of all other microbial communities associated with the body and outnumbers an individual’s human cells by approximately 10-fold [26]. Conservative calculations estimate the presence of at least 800 bacterial species [27], but recent data suggest the true number could be as high as 40,000 [28,29]. Defining a core GI microbiota, shared by all individuals, has so far been elusive, because the composition of the GI microbiota varies significantly between individuals [30[•]]. Recently, however, the existence of three robust clusters of GI microbiomes (referred to as ‘enterotypes’) that vary in species and functional composition and that span several nations and continents was demonstrated [31^{••}]. Interestingly, the authors found that rare, low abundance members of these defined enterotypes contributed the greatest functional gene repertoire and metabolic capacity to these communities; this may have important implications for probiotic supplementation strategies. Promoting diversity among these low-abundance species may prove most effective in combating disorders, particularly those in which a loss of community diversity typifies the condition (e.g. inflammatory bowel disease).

Impact of diet on enteric microbiota composition

Clearly, more work is required to understand the importance of the GI microbiota composition and its contribution

to human health and disease. Not surprisingly, diet has a profound impact on the structural and functional composition of the intestinal microbiota of mammals and humans [32–34]. A recent extensive study reviewing and summarizing reports on how the mode of feeding influences GI microbiota composition found that Clostridia, *Bacteroides*, Enterococci, and *Enterobacteriaceae*, especially *Klebsiella* and *Enterobacter*, tend to occur more frequently and in higher abundance in formula-fed babies, while *Lactobacillus rhamnosus* was more common in breast-fed infants [25]. However, the reviewers also pointed out that the overwhelming majority of studies conducted in the last 30 years found no difference regarding the occurrence of *Lactobacillus* and *Bifidobacteria* species, both long thought to be hallmarks of breast-fed infants. The authors suggested that the narrowing gap in GI microbiota composition between breast- and formula-fed infants may be due to modern formulas that are more faithful replicas of breast milk than those used previously [25] and infant formulas are now commonly supplemented with probiotics. However, it should be noted that many of the previous studies relied on the ability of investigators to grow these organisms in culture. Given the fastidious nature of many gastrointestinal bacterial species, it is possible that these studies underestimated both the burden and diversity of the target genera. Furthermore, a recent report demonstrated that the fecal microbiota of Florentine children raised on a typical high-fat, low-fiber ‘Western’ diet was significantly less diverse than that of children from a rural village in Burkina Faso, Africa, where the diet is rich in plant polysaccharides and fiber [35^{••}]. In fact, the relative ratio of the two dominant phyla detected in the GI microbiota (*Bacteroides* and Firmicutes) was reversed in these distinct populations. This indicates that a Western diet and lifestyle, which has been associated with soaring prevalence of autoimmune and allergic diseases [36,37[•]], could conceivably be attributed – at least partly – to a downward selective pressure on GI microbial diversity, thus resulting in functionally inept assemblages that impact both metabolic and immune homeostasis [30[•],38[•],39]. Supplementation with probiotics may be one approach to overcome this phenomenon by increasing microbial diversity and thus functionality in these communities; however, studies suggest that without long-term dietary intervention the effect of supplementation may only be transient.

Function of the intestinal microbiota

The intestinal microbiome confers a multitude of important functions to the mammalian host. Enteric bacteria increase caloric uptake both by degrading indigestible dietary polysaccharides into absorbable monosaccharides and by fermenting complex carbohydrates to short chain fatty acids (SCFA), which are subsequently assimilated by the host [40]. SCFAs, such as acetate, butyrate, and propionate also stimulate epithelial cell proliferation and differentiation, and through their anti-inflammatory

properties modulate immune responses [41]. The GI microbiome also regulates energy storage and provides essential vitamins for the host [39,42]. Furthermore, a phenomenon known as ‘colonization resistance’ exists in which intestinal bacteria inhibit pathogen invasion by competing for the same nutrients and by occupying available niches in the gut [43]. The GI microbial consortium also impacts the development and morphology of the intestine, and contributes to angiogenesis and to the fortification of the intestinal epithelial barrier [44,45]. Apart from the digestive tract, a role for enteric bacteria has been demonstrated for extra-intestinal sites including mammalian brain development and hepatic lipid metabolism [46]. Moreover, and with substantial consequences for human health, the intestinal microbiota plays a key role in locally and systemically shaping and modulating the host’s immune system.

Enteric microbes influence the development of appropriate immune function

Enteric microbes constantly prime the innate immune system, thus facilitating a rapid response to pathogens [47]. A growing body of literature is focused on the complex interplay that exists between initial events in the assembly of the GI microbiota and the development and maturation of the host’s immune system and whether manipulation of microbiota during this key developmental stage can impact inflammatory disease outcomes [48–51]. Appropriate microbial colonization plays a key role in the development of the gut-associated lymphoid tissue (GALT), a primary mechanism of defense against enteric pathogens [52,53^{*}]. Furthermore, intestinal colonization stimulates the production of effector molecules such as secretory IgA [54], the differentiation of TH17 cells [38^{*}], and the development and activation of regulatory T (T_{Reg}) cells [55,56]. Significantly, it has also been demonstrated that early stimulation of the immature immune system by a diversity of appropriate commensal microbes and the presence of a GI microbiota is fundamental to establishing and maintaining the essential balance between Th1, Th2, or Th17 cytokine expressing T-cells [57–60].

Diseases and disorders associated with aberrations in the GI microbiota

Considering the intimate relationship between the host and its microbial inhabitants and the multi-faceted effects intestinal microbes have on host physiology, immune system development and maturation, it is not surprising that the development and progression of many intestinal as well as systemic disorders and diseases have been linked to GI microbiota. These include the more obvious conditions such as colon cancer [61], irritable bowel syndrome (IBS) [62], inflammatory bowel disease (IBD) [63] and obesity [64], but also less obvious disorders such as Type 1 diabetes [65], rheumatoid arthritis [66], progression and severity of HIV infections [67],

development of nonalcoholic fatty liver disease [68], cardiovascular disease [69], autistic spectrum disorders (ASDs) [70], and allergy and asthma [71]. (For detailed reviews see Fujimura *et al.* [22] and Sekirov *et al.* [23].) Some of these diseases, such as obesity, IBD, or allergies have been linked to dysbiosis, that is aberrations in the composition of the gut microbial community in conjunction with significant loss of microbial diversity and, presumably, key functional groups. This knowledge has led to the proposal that thwarting the onset of microbiota perturbation and restoring the composition of the GI microbiota to a more diverse and functionally redundant consortium represents a feasible therapeutic strategy. In fact, many of the disorders associated with dysbiosis have been the target for probiotic intervention (Table 1). The majority of studies to date focus on GI microbiota restoration in patients with IBD (Table 1, [72]) as well as on influencing the assemblage of the developing GI microbiota of infants in order to prevent allergic diseases such as allergic rhinoconjunctivitis, atopic eczema, and asthma; all of which are some of the most promising areas for probiotic therapy (Table 1, [73–75]).

Conclusions

The consumption of probiotics, either as food additives or dietary supplements, for both health maintenance and disease alleviation is gaining popularity with consumers and is also increasingly advocated by health care professionals. Advances in culture-independent molecular technologies have significantly increased our understanding of structure and function of the resident microbial communities associated with the human body in health and disease. In particular, our knowledge of the complex gastrointestinal microbiota, and how supplementation with probiotics may help initiate or restore appropriate microbiota composition has been greatly advanced. Several recent human trials have demonstrated the potential for live biotherapeutic products in disease management and prevention, but larger, better controlled, and universally standardized studies are needed for the rigorous scientific evaluation of probiotic therapies and the comparison of diametric outcomes. Furthermore, taking into consideration globalization of the probiotic market and the access consumers have to information and products across national borders, the field would benefit tremendously from internationally harmonized regulations.

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