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REVIEW ARTICLE

Gut microbiome, gut function, and probiotics: Implications for health

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Abstract New insights from a rapidly developing field of research have ushered in a new era of understanding of the complexity of host-microbe interactions within the human body. The paradigm shift from culturing to metagenomics has provided an insight into the complex diversity of the microbial species that we harbor, revealing the fact that we are in fact more microbes than human cells. The largest consortium of these microbes resides in the gut and is called the gut microbiota. This new science has expanded the ability to document shifts in microbial populations to an unparalleled degree. It is now understood that signals from the microbiota provide trophic, nutritional, metabolic, and protective effects for the development and maintenance of the host digestive, immune, and neuroendocrine system. Evidence linking changes in the gut microbiota to gastrointestinal and extraintestinal disorders like irritable bowel syndrome, inflammatory bowel disease, obesity, diabetes, and celiac disease have begun to emerge recently. Probiotics act through diverse mechanisms positively affecting the composition and/or function of the commensal microbiota and alter host immunological responses. Well-controlled intervention trials, systematic reviews, and meta-analysis provide convincing evidence for the benefit of probiotics in prevention and treatment of gastrointestinal as well as extraintestinal disorders.

Keywords Clinical evidence · Gut microbiota · Probiotic Mechanisms · Metagenomics · Probiotics

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Introduction

It has been estimated that humans host approximately 10^{14} microorganisms, which is ten times more than the total number of somatic and germ cells in the body. The gene pool of the microbial habitants is diverse and considerably larger than the gene pool of the host and determines a number of metabolic capacities that are necessary for the survival of these microbes in the host. Of the vast array of microorganisms that reside within the human body, the majority resides in the gut and is called the gut microbiota [1]. The physiology of the human gut, which works as a chemostat bioreactor and a continuous culture system, is inextricably linked to the microbial population it hosts. The expanse of understanding of the gut microbiota and its role in human nutrition and metabolism has become clear in light of the advancements in genomic tools including functional genomics, transcriptomics, and proteomics that have accelerated research for deciphering the interactions between the gut microbiota and its host [2].

It is now realized that the role of the gut is no longer limited to energy harvest, nutrient acquisition, and intestinal homeostasis; it also plays a significant role in postnatal terminal differentiation of mucosal structure and function, stimulating

both the innate and adaptive immune system. The gut microbiota therefore acts as one of the first lines of protection against incoming pathogens, hosting an arsenal of defense mechanisms to counter potential pathogenic invasion [3].

Gut bacteria use mostly fermentation to generate energy, converting sugars in part to short-chain fatty acids (SCFA) that are used by the host as an energy source [4]. The main end-products are acetate, propionate, and butyrate. SCFA help increase gut motility, decrease gut pH, and provide energy for commensal bacteria. Besides SCFA, a number of amino acids that are indispensable to humans are provided by commensal bacteria. It has been well established that some microbial species may be responsible for the synthesis of vitamins like biotin, phylloquinone, and vitamin K, and deficiencies may directly or indirectly be associated with reduction in abundance of specific components of the gut microbiome. The gut microbiota also plays an important role in preventing comorbidities and infection in addition to influencing mood regulation, obesity, diabetes, insulin resistance, and cognition [5].

Paradoxically, there appears to be no absolute requirement for a functional resident microbiota; however, gnotobiosis also does not occur in nature. Animal models have been used to provide crucial information regarding host-microbiota interactions. These studies suggest that the presence of the colonic microflora is essential for the development of mucosal integrity, in the absence of which the mucosal and systemic limbs of the immune system are structurally and functionally defective. This can be restored on colonization with commensal bacteria, clearly indicating that the luminal microbiota must be a source of immunomodulatory signals [3]. Remarkably, colonization with a single bacterial commensal strain has been sufficient to reveal the impact of microbial signaling on the expression of host genes controlling gastrointestinal (GI) structure and function.

Gut microbiota during life

While the adult microbiota is extremely complex and has a significant contribution to health and disease, the gut of the newborn is essentially sterile. The colonization process commences immediately after birth and successive development in the infant is influenced by a number of factors including early environmental exposure (especially route of delivery—vaginal or cesarean section), gestational age, and use of antibiotics especially in the perinatal period in neonatal intensive-care units [6].

The strong influence of the maternal microbiota on neonatal colonization was observed in infants born vaginally who have greater numbers of *Bifidobacteria* as compared to those born by cesarean section that have increased colonization by *Klebsiella*, *Enterobacter*, *Clostridia* [7], and organisms prevalent in-hospital settings. Delivery route also influences immunological function during the first year of life, with babies delivered by cesarean section having lower bacteria cell counts in fecal samples and a higher number of antibody-secreting cells. Infant-feeding patterns greatly affect microbial colonization—breast milk contains antimicrobials, antibodies, and lactobacilli [8] whereas formula-fed infants have an increased prevalence of *Clostridia* and *Bacteroides* in the gut [7].

The diversity of the bacterial count in an infant gut is initially very low both in complexity and species richness and then climbs through early development and converges to a more stable, phylogenetically diverse, adult-like profile by 2 years of age. There is a critical window of sensitivity with the introduction of solid foods when the infant is exposed to environmental and dietary challenges and triggers, which has an impact on the makeup of the gut microbiota. The development of the biodiversity and functionality of the gut microbiota is therefore far from gradual during the growth of the child and evolves during different stages of life from infancy to adulthood to old age [6].

The microflora hypothesis suggests that reduced microbial exposure at an early age inhibits normal maturation of the intestinal microbiota and delays maturation of the mucosal immune system, increasing the risk of aberrant immune response and allergic disease. The developing microbiota of the newborn therefore plays a significant role in protecting the host from infection and influences the development of oral tolerance and the infant's susceptibility to allergies and inflammation, now being recognized as an important cause in the pathogenesis of non-communicable diseases [9].

It has been hypothesized that healthy postnatal development of the gut microbiota is perturbed in malnourished children and decreased microbial diversity in infancy appears to be associated with an increased risk of malnutrition and may be responsible for the pathogenesis of malnutrition. This was reflected in a recent study in Bangladesh that identified a total of 220 bacterial taxa that were significantly lower in their proportional representation in the fecal microbiota of children with severe acute malnutrition compared to healthy children [10]. Valuable insights have also been provided by a recent Indian study that investigated the gut microbiome of 20 children with varying nutritional status from a rural setting in West Bengal and revealed that impaired nutritional status was not only due to an abundance of pathogenic microbial groups but also as a result of depletion of several commensal genera that have a positive influence on the nutritional status of children [11]. A study by Smith et al. implicated the gut microbiome as a causal factor in kwashiorkor, an enigmatic form of severe acute malnutrition [12]. The distinct signature patterns of gut microbiota in healthy and malnourished children may therefore have profound implication in determining the health of children in developing countries.

Studies have revealed that the composition of the gut microbiota also changes markedly with age, and elderly

individuals (>65 years) tend to have greater interindividual variation than younger adults. Additionally, there is a decrease in species diversity. The altered microbial community may be pro-inflammatory, and the presence of inflammatory markers correlates with frailty [13]. Such changes in the gut microbiota of elderly individuals have been linked to poorer health and nutritional status, increasing their susceptibility to disease and infection. The microbiota therefore undergoes substantial changes in the extremes of life, the ramifications of which are still being explored.

Alterations of gut microbiota in disease

The human gut microbiota is currently the focus of advanced techniques, and 16S ribosomal RNA gene sequence-based methods have revealed that two bacterial phyla—Bacteroidetes and Firmicutes—constitute over 90 % of the known phylogenetic categories [14]. Other phyla present to a lesser extent include Actinobacteria, Proteobacteria, Fusobacteria, Spirochetes, and Verrucomicrobiota [15]. The bacterial diversity in the human body is striking in its richness of distinct species and strains along different parts of the human body. Time-series data have shown that the composition of the otherwise stable gut microbiota fluctuates over time and can be negatively altered by external perturbations such as intercurrent infections, poor diet, lifestyle habits, stress, aging, and treatment with oral antibiotics and other medication.

Metagenomic analyses have successfully captured the breadth of microbial functional and metabolic potential, revealing significant metabolic discrepancies between diseased and healthy individuals. A decreased diversity of the gut microbiome has been observed in patients with Crohn's disease and ulcerative colitis, with reduced levels of both *Faecalibacterium prausnitzii* [16] and *Akkermansia mucinphilia* [17]. The colonic microbiota has been suspected for a long time to be involved in the pathogenesis of bowel cancers. A reduction of *Faecalibacterium prausnitzii* and *Eubacterium rectale* and an increase in *Enterococcus fecalis* was observed in Indian patients suffering from bowel cancer [18]. Attempts have been made to understand the perturbations in the predominating and subdominating gut microbiota during the active stage and during the process of reassembly (remission stage) in ulcerative colitis. In a study involving 26 patients and 14 controls at the All India Institute of Medical Sciences, New Delhi, major fluctuations were seen in six main genera: *Bacteroides*, *Bifidobacteria*, *Clostridia*, sulfate-reducing bacteria, *Campylobacter*, and *Lactobacilli* as determined by qPCR using genus-specific primers and probes. Significant reduction of SCFA concentration was observed by gas chromatography in these patients. It was concluded that reduction in all these genera may have functional consequences on the ability of the host to repair the epithelium and to regulate inflammation [19].

Irritable bowel syndrome (IBS) is a widely prevalent but poorly understood GI disorder. Although a causal role has not been established, attempts to characterize the gut microbiota in IBS confirm alterations in both community stability and diversity of microbes. The discovery that differential microbial composition is associated with alterations in behavior and cognition has significantly contributed to establishing the microbiota-gut-brain axis. Bercik et al. showed that proliferation of *Lactobacillus* and *Bifidobacteria* in the mouse gut increased non-anxious behavior, clearly indicating a link [20].

Recent insights have also suggested that the gut microbiota plays a crucial role in regulating energy homeostasis and the development and progression of obesity and its associated metabolic disorders [21, 22]. Backhed and colleagues found that alteration of the gut microbiome in germ-free mice with microbiota harvested from conventionally raised, genetically obese mice resulted in a 60 % increase in body fat when compared to germ-free mice whose microbiome was unaltered [23]. Subsequently, Turnbaugh et al. confirmed these results and found that transfer of microbiota from conventionally raised mice into germ-free mice resulted in phenotypically obese mice. Phylum level changes in the microbiota in the two main phyla, a decrease in Bacteroidetes and an increase in Firmicutes, coupled with reduced bacterial diversity, were observed in obesity. They calculated that a 20 % increase in Firmicutes and corresponding decrease in Bacteroidetes was associated with an increase in energy absorption equivalent to 150 Kcal/day [24]. Recently, Ridaura et al. found those communities with microbiota from obese twins were correlated with differences in fermentation of SCFA, metabolism of branched-chain amino acids, and microbial transformation of bile acid species, with a net result of an increase in body mass and adiposity in the obese subset as compared to its lean counterpart [25]. Animal studies have been challenged by more recent studies in humans demonstrating that the relationship may be more complicated than simply the ratio of Bacteroidetes to Firmicutes [26].

A recent study involving 91 pregnant women suggests that gut microbiota is profoundly altered during pregnancy. The microbial composition changed markedly between the first and third trimesters, with an increase in the abundance of Proteobacteria and Actinobacteria. This was followed by a decrease in the bacterial diversity when the women progressed from the first to the third trimester [27].

A critical link has also been recently established for the residential microbiota in the promotion of atherosclerosis. Wang et al. delineated a two-step metabolic pathway involving the microbially mediated metabolism of dietary phosphotidylcholine resulting in the production of the metabolite trimethylamine-N-oxide (TMAO), a predictor of cardiovascular diseases [28].

Dietary modulation of gut microbiota

Defining a core microbiome among individuals is a challenging task although the pivotal work by Arumugam and colleagues suggested that the microbiota of most individuals can be classified into three "enterotypes" dominated by *Bacteroides*, *Prevotella*, and *Ruminococcus* [29]. The basis for the enterotype clustering appears to be independent of nationality, sex, age, or body mass index, and it appears that these broad patterns are driven primarily by dietary effects, with the *Bacteroides* enterotype being associated with animal protein, a variety of amino acids and saturated fats, which suggests that meat consumption as in a Western diet characterized this enterotype. The *Prevotella* enterotype, in contrast, was associated with high intake of carbohydrates and simple sugars [1]. Comparison of long-term and short-term dietary data showed that only the long-term diet could be correlated with enterotype clustering whereas short-term dietary changes triggered a significant and rapid response in the gut microbiome but the magnitude of change was modest and not sufficient to switch individuals between the enterotype clusters associated with protein/fat or carbohydrate consumption.

The dietary association seen here parallels comparative studies between rural communities from Africa and South America and industrialized Western communities of Europe and North America, providing interesting insights into specific gut microbiota adaptions based on their diet and lifestyles. The adaptations include higher levels of microbial richness and biodiversity and an enrichment of Bacteroidetes and Actinobacteria in rural communities and overall reduction and stability in the Western population. European children who consume a typical Western diet high in animal protein and fat were dominated by taxa typical of the *Bacteroides* enterotype, and children in Burkino Faso who consumed a high carbohydrate diet low in animal protein were dominated by the *Prevotella* enterotype, the same pattern as described above [30].

Such diet-induced changes to gut-associated microbial communities are now suspected to contribute to the growing epidemics of chronic illness in the developed world, including obesity and inflammatory bowel diseases [10].

The science of nutrition reiterates using a diet-based approach as an integral part of management of health and disease. As the gut microbiota plays an important role in nutrient extraction from food, the nutritional value of food is influenced particularly by a person's gut microbial community. The gut microbiota therefore represents an attractive target for improving the nutritional status of a population. In the last decade, there have been several human studies evaluating the potential of probiotics and prebiotics to improve health generally by modulating the composition of the gut microbiota. Increasing the diversity of the general diet and including probiotic and prebiotic products could be a simple affordable way of modulating the intestinal microbiota to promote beneficial microbial metabolism.

Probiotics

Probiotics have been investigated for many years for their potential to improve digestive function and to mitigate the effects of infectious and inflammatory diseases. Defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [31], they are derived from traditional fermented foods, from beneficial commensals, or from human origin. They are the subject of increasing basic and clinical research while also being incorporated into an expanding array of foods, nutritional supplements, and pharmaceutical products (Table 1). In addition to safety, the selection of a probiotic strain is driven primarily by its potential to confer a health benefit on the host.

There is growing evidence that probiotics can be used to improve the absorption of micronutrients (such as calcium and iron) from ingested foods. They do so by increasing the bioavailability of micronutrients through several mechanisms and therefore represent an avenue for potentially alleviating micronutrient deficiencies. The increased SCFA production due to probiotic fermentation decreases pH, increases mineral solubility, and enlarges enterocyte absorption surface. One placebo-controlled study showed that the iron status in young children could be improved significantly by intake of milk fortified with synbiotics (*Bifidobacterium lactis* HNO19, oligosaccharide) for 1 year [32]. Furthermore, the absorption of iron was improved significantly in healthy women of childbearing age after intake of probiotics in a placebo-controlled crossover study [33]. However, the effects appear to be highly dependent on the probiotic strain. In some cases, probiotics have very specific beneficial effects, such as in the case of vitamin production. Genome sequencing has shown that some strains of *Lactobacillus reuteri* have biosynthetic pathway for vitamin B_{12}, folate, and thiamine synthesis [34].

The matrix for the delivery of the probiotic strain is an important factor that plays an important role in influencing probiotic viability and efficacy. Dairy products that constitute an integral part of diet represent an excellent food matrix, ensuring stability, viability, and optimal expression of probiotic functionality.

Mechanism of action

Most of the studies of probiotic mechanisms have been conducted by immunologists, who have tried to understand the "probiotic effect" by studying the response of innumerable cell lines and biochemical readouts to various microbes. A

Table 1 Common probiotic strains and products found in India

Probiotic strains	Probiotic food
Bifidobacterium BB-12	B-Activ
Lactobacillus acidophilus	Actiplus
LA5 and *BB-12*	Nutrifit
Lactobacillus casei Shirota	Yakult
	Probiotic drugs
Mixture of one strain of *Streptococcus thermophilus*, four *Lactobacillus* spp., and three *Bifidobacterium* spp. strains	VSL#3
Bacillus subtilis	Enterogermina
Saccharomyces boulardii	Econorm
Lactobacillus acidophilus, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Saccharomyces boulardii*	Darolac

reductionist approach can be very useful in determining the mechanism of action of probiotics. As exemplified in one case, it was possible to definitively identify a mechanism of action of a probiotic *Lactobacillus salivarius* UCC118, where the health benefit imparted was in protecting mice against an otherwise lethal infection with *Listeria monocytogenes* [35].

Other probiotics tested in the same model had no such protective effect. It had previously been demonstrated that *Lb. salivarius* UCC118 produces a bacteriocin which was effective against *L. monocytogenes* in vitro [36]. Bacteriocins are antimicrobial peptides produced by some bacteria that are effective against specific groups of organisms. It was speculated that the probiotic effect was due to bacteriocin production in vivo, which eliminates the pathogen in the small intestine. The bacteriocin-negative mutant was unable to protect mice against infection with *Listeria*, confirming the role of the bacteriocin as the "probiotic" mechanism (Fig. 1).

It is worth noting that strain UCC118 also protects against murine salmonellosis, but the protection can be imparted by a bacteriocin-negative mutant as well, confirming that a single strain can have multiple mechanisms of action directed against different targets. One of the benefits of determining the probiotic mechanism of UCC118 was that it allowed for screening of other bacteriocin-producing commensal strains, which resulted in the identification of thuricin CD, a narrow-spectrum bacteriocin that can control *Clostridium difficile* infections in an artificial colon model of the disease [37, 38].

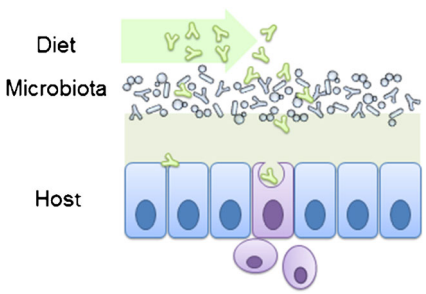

Fig. 1 Probiotic mechanisms. Probiotics can act through multiple mechanisms to affect the health of the host. They can transform dietary components into bioactive molecules, modulate the microbiota, or interact directly with the host immune or nervous system. Reproduced with permission from the Yakult India Microbiota and Probiotic Science Foundation, New Delhi

Another example of a probiotic strain with a defined mechanistic basis is the case of *Bifidobacterium breve* expressing linoleic acid isomerase activity that is capable of converting linoleic acid into conjugated linoleic acid (CLA) in the GI tract [39]. This activity leads to detectable changes in CLA levels in the liver and adipose tissues in murine and porcine models. Interestingly, when the gene encoding this enzyme was cloned in a strain of *Lactobacillus*, the resulting clone could also modify fatty acid composition in host tissues [39]. This illustrates how determining the mechanistic basis of a probiotic effect can lead to precise engineering of other commensal strains to introduce a beneficial trait.

Evidence of specificity

There are several examples in the literature of the specific effects of probiotics. An elegant study by Van Baarlen and colleagues showed that the human immune system responds very differently to three commercially available probiotics [41]. The example presented in an earlier section in which only one of many probiotic strains tested was capable of protecting mice against infection with *L. monocytogenes* is another example of specificity [35].

Evidence of lack of specificity

There are also many examples in which one can see generic or non-specific effects of probiotics. For example, the probiotic *Lactobacillus rhamnosus* GG was isolated many decades ago on the basis of its technological attributes (biological robustness, adherence to epithelial cells, and inhibition of other bacteria). Since then, this strain has been tested in numerous preclinical and clinical settings, with an extraordinary range of benefits. The benefits range from ameliorating viral infections

in children, to fussing and crying in preterm infants, to protecting against nonalcoholic fatty acid liver disease in mice, and many others [42–51]. Since GG was not isolated based on any of these health targets, one can conclude that there are many conditions that respond favorably to the ingestion of large numbers of a safe bacterium, and it may well be that almost any safe bacterium would function equally well.

A lack of specificity is seen in the outcomes of many meta-analyses and systematic reviews conducted on probiotic trials in humans, which often conclude that probiotics are generally beneficial across a wide range of strains, sample sizes, and clinical endpoints measured [52–60]. It seems obvious that there are some probiotics that work across multiple targets, and there are some health conditions which are amenable to probiotic intervention from a wide array of strains (Fig. 2). This should not undermine the usefulness of probiotics in any sense, but simply indicates that some mechanisms are likely to be rare and strain-specific, while others are more widespread.

Clinical evidence

An insight into the mechanism of action of probiotics has provided a stepping-stone for discovering new ways in which they can improve human health. Given that the intestinal tract is the largest reservoir of microbes in the human body, it is not surprising that the use of probiotic microorganisms has been investigated extensively in intestinal disorders; however, the past decade has also witnessed tremendous progress in the possible role of probiotics beyond the gut.

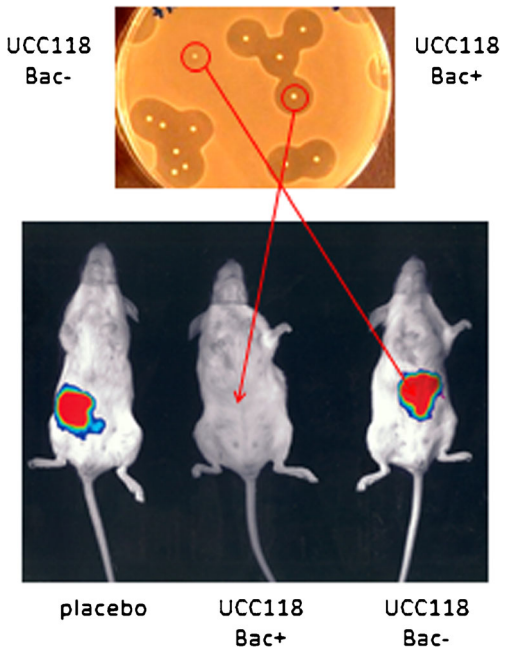

Fig. 2 Bacteriocin-mediated probiotic effect. A bacteriocin-producing *Lactobacillus salivarius* UCC118 is able to protect mice against normally lethal infection with lux tagged *Listeria monocytogenes*, whereas the bacteriocin-negative mutant offers no protection. Reproduced with permission from the Yakult India Microbiota and Probiotic Science Foundation, New Delhi

Gastrointestinal diseases

Acute diarrhea

Acute diarrhea is an important cause of childhood mortality and morbidity in developing countries, and the development of preventive and therapeutic measures remains an important goal. The rationale for using probiotics in acute infectious diarrhea is based on the assumption that they act against enteric pathogens, synthesize antimicrobial substances that competitively inhibit the adhesion of pathogens, modify toxin and non-toxin receptors, and stimulate both specific and non-specific immune responses to pathogens. The evidence from studies on viral diarrhea is however more convincing than from bacterial or parasitic infections.

A Cochrane review on probiotics for acute infectious diarrhea from 63 randomized and quasi-randomized placebo-controlled trials (56 of these studies recruited infants and young children) that comprised 8,014 participants from various geographical areas, in a wide range of settings, and tested different organism and doses, found that there was a diarrhea reduction following probiotic treatment compared with controls, although effect sizes were highly variable between trials. Probiotics appear to be safe and have clear beneficial effects in shortening the duration and reducing stool frequency in acute infectious diarrhea in trials that used rehydration therapy alongside [61]. A meta-analysis of studies on acute pediatric diarrhea concluded that there was significant data for probiotic-based reduction of diarrhea duration, treatment failure, and prevention [62]. Evidence from several meta-analyses of randomized controlled trails has consistently shown the effect and clinical benefits of probiotics in acute infectious diarrhea, often rotaviral, primarily in young infants and children [63].

Studies involving Indian children have shown that specific probiotics can significantly reduce the duration and occurrence of diarrhea and significantly increase the weight and height of these children relative to those fed a supplement with similar caloric value but lacking probiotics [64]. An Indian study conducted at the National Institute of Cholera and Enteric Diseases in Kolkata showed that the diarrhea frequency was reduced by 14 % among children in India who received daily doses of *Lactobacillus casei* strain Shirota for 12 weeks with a 12-weeks follow up period [65]. This raises the possibility of using probiotics to improve the outcomes of nutritional interventions in the treatment of undernourished children.

The European Society for Pediatric Gastroenterology, Hepatology and Nutrition and the European Society of

Pediatric Infectious Diseases Expert Working Group have stated that selected probiotics with proven clinical efficacy and in appropriate dosage, according to the strain and population, may be used as adjunct for the management of children with acute gastroenteritis [66].

Antibiotic-associated diarrhea and Clostridium difficile infection

Antibiotic-associated diarrhea (AAD) and *Clostridium difficile* infection (CDI) are associated with high morbidity, mortality, and health-care costs. Probiotics have been used prophylactically to reduce both these conditions. A recent meta-analysis that included 82 RCTs comprising 11,811 participants indicated a statistically significant association of probiotic administration with reduction in AAD [58]. Majority of the trials used *Lactobacillus*-based interventions alone or in combination with other genera and *Saccharomyces boulardii*. A 2009 review has shown that the effectiveness of a probiotic is mainly related to the strain used [67]. Therefore, additional research is needed to determine which probiotic is associated with the greatest efficacy and the antibiotic against which the probiotic would be efficacious.

Clostridium difficile is the pathogen most often associated with opportunistic proliferation during or after antibiotic administration. The severity of CDI ranges from mild, usually self-limiting diarrhea to toxic megacolon and death. A recent Cochrane review that included 31 randomized controlled trials with a total of 4,492 participants concluded that probiotics when given with antibiotics reduce the risk of developing *Clostridium difficile* diarrhea by 64 % [68].

A systematic review and meta-analysis that evaluated the incidence of both antibiotic and *C. difficile* diarrhea reviewed 16 trials; pooled analyses revealed significant reductions in the risk of AAD (RR 0.61, 95 % CI 0.47–0.49) and CDI (RR 0.37, 95 % CI 0.22–0.61) among patients randomly assigned to co-administration [69].

A Cochrane review on pediatric AAD suggested a protective association of probiotic use in preventing AAD in children [70]. Low cost and low incidence of adverse effects may make probiotics an attractive intervention for the prevention of AAD and CDI in adults and children. The role of probiotics in preventing nosocomial infectious diarrhea has contradicting evidence.

Norovirus gastroenteritis

Norovirus is one of the most common causes of acute gastroenteritis in Japan. The illness can last longer and is more severe in young children and elderly who are more prone to infection. It has therefore become a major challenge to control infections among elderly who suffer from norovirus gastroenteritis.

A recent open case-controlled study that included 77 elderly people who were infected with norovirus gastroenteritis concluded that regular consumption of *Lactobacillus casei* strain Shirota for 1 month reduced the duration of fever by approximately 1.5 days [71].

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a chronic debilitating functional GI disorder that may result from visceral hypersensitivity, intestinal dysmotility, dysregulated brain-gut axis, bacterial infection, and chronic low-grade mucosal inflammation. Preliminary evidence suggests alterations in the gut microbiota in IBS although it remains to be determined whether these alterations are a cause or a consequence of the disease. Diet and lifestyle changes are important management strategies in the treatment of IBS.

Meta-analyses vary in their conclusions on the effectiveness of probiotics against IBS. A systematic review of 19 randomized controlled clinical trials that included 1,650 patients with IBS concluded that various *Lactobacillus* species when taken either alone or co-administered with *Bifidobacterium* resulted in improvement of symptoms in patients [72]. The result of this analysis has been underpinned by another study of 42 RCTs: 34 reported benefit in at least one of the endpoints studied [73]. A recent meta-analysis and systematic review that examined the efficacy of probiotics, prebiotics, and synbiotics in IBS and chronic idiopathic constipation that included 43 RCT's concluded that probiotics are effective treatment for IBS although which individual species and strains are most beneficial remains unclear [74]. Studies have suggested that maintenance of epithelial barrier function, changes in gut motility, and modulation of visceral pain sensitivity are potential mechanisms of action of probiotics in the setting of IBS.

However, given the heterogeneity of symptoms of IBS, studies that focus on specific strains for specific IBS-related symptoms would provide a greater insight for strain-specific benefits of probiotics in this condition [75].

Inflammatory bowel disease

There is growing evidence that altered gut microbiota may play an important role in inflammatory bowel disease (IBD), especially in Crohn's disease. This comes from the finding that *Faecalibacterium prausnitzii*, an anti-inflammatory commensal, is decreased in Crohn's disease patients as compared to healthy individuals [16], and adherent invasive *E. coli* are observed in greater numbers in these patients [76]. The intestinal microbiota of patients with IBD also seems to drive an overactive immune response leading to disease expression and concurrent morbidity. The potential for probiotics to modulate the microbiota, provide beneficial immunomodulatory

effectors, and restore epithelial barrier defects suggests that a probiotic strategy might prove a viable future treatment option for patients with IBD.

Benefits have been observed with a combination of *Lactobacillus*, *Bifidobacterium*, or *Streptococcus* species or with *E. coli* Nissile 1917 in inducing and maintaining remission of disease activity in mild to moderately severe ulcerative colitis [77]. A recent Indian study also underpinned the findings and showed that probiotics help in inducing remission in patients with ulcerative colitis as measured by the disease activity index [78]. A recent systematic review that included 14 studies in patients with Crohn's disease, 21 studies in patients with ulcerative colitis (UC), and 5 studies in patients with pouchitis concluded that there is insufficient data to recommend the use of probiotics in Crohn's disease; however, there is sufficient evidence to recommend the role of probiotics for induction and maintenance of remission in UC and pouchitis [79]. Future quality studies are required to make definite recommendations. Twenty-three randomized controlled trials that included 1,763 participants concluded that VSL#3 was particularly useful in increasing remission rates compared with controls in patients with active UC. Interestingly, VSL#3 also significantly reduced the clinical relapse rates for maintaining remission in patients with pouchitis [80]. In fact, the role of probiotics in primary prevention of pouchitis and reducing the likelihood of relapse after successful antibiotic treatment has received an "A" recommendation.

Disparity between the therapeutic potential of probiotics in different forms of IBD can be explained on the complex interactions between genetic, microbial, and environmental influences, leading to heterogeneous phenotypes in patient subsets that are uniquely responsive to specific microbial manipulations.

Constipation

Constipation is a very common condition in children and adults. Although traditional treatment is safe and well established, for many patients it does not provide satisfying improvement, prompting interest in other therapeutic strategies. The success of nutritional treatment for constipation, such as the ingestion of larger amounts of fiber, may be due to elevated metabolic activity of colonic flora resulting in a lowering of pH in the colon. These results emphasize the importance of intestinal flora in the treatment and prevention of constipation. In constipated children, the number of *Bifidobacteria* was decreased while nonpathogenic *E. coli*, *Bacteroides* and the total number of microorganisms increased [81]. Data suggest that adults with constipation might benefit from ingestion of *Lactobacillus casei* strain Shirota [82, 83] and *B. lactis* DN-173010 and *E. coli* Nissle 1917, which are known to increase defecation frequency and improve stool consistency. In children, *L. casei* Lcr 35 but not *L. rhamnosus* GG has shown a beneficial effect [84].

Helicobacter pylori

H. pylori, an ancient member of the human microbiota, generally dominates in the human gastric niche. The standard treatment for the eradication of *H. pylori* consists of triple therapy, which includes a proton pump inhibitor along with two antibiotics. In recent years, the success of eradication therapies has declined, in part due to the development of antibiotic-resistant *H. pylori* strains, and alternative anti-*H. pylori* treatments are currently becoming popular. Probiotics represent a promising strategy with a recent meta-analysis of 14 randomized trials suggesting that supplementation of anti-*H. pylori* antibiotic regimens with certain probiotics may also be effective in increasing eradication rates and may be considered helpful for patients with eradication failure. However, there is currently insufficient evidence to support the concept that a probiotic alone without concomitant antibiotic therapy would be effective [85]. Probiotics may be helpful as adjunct therapy with antibiotics for the eradication of *H. pylori*. The antibiotics (amoxicillin and clarithromycin) used for the treatment of *H. pylori* often induce diarrhea and therefore probiotics when used as an adjunct may also be useful in overcoming that side effect. In children, probiotics were generally ineffective in eradicating *H. pylori* infection but can reduce side effects of the recommended antimicrobial therapy [86].

Neonatal necrotizing enterocolitis

Neonatal necrotizing enterocolitis (NEC) is one of the major causes of mortality and morbidity in preterm infants [9]. It affects 10 % of all infants less than 1500 g in weight and is associated with 30 % mortality rates [87]. Deep sequencing studies before the development of NEC suggest that individual operational taxonomic units differ between patients with NEC and controls. A recent meta-analysis that included 11 trials on preterms less than 34 weeks and less than 1500 g in weight showed that enteral administration of a probiotic supplement initiated within the first 10 days of life and continued for up to 7 days resulted in 30 % reduction in incidence of NEC and decreased mortality risk [88]. Yet, another meta-analysis concluded that "enteral probiotic supplementation decreases the incidence of severe NEC in low-birth-weight (less than 2500 g) preterm neonates" [89]. The American Academy of Pediatrics recognizes that there is evidence for the role of probiotics in preventing NEC and has called for more studies to clarify the effective dose and strain of probiotic before issuing clinical recommendations.

Liver cirrhosis and hepatic encephalopathy

Little is known about whether probiotics can affect the outcomes of patients with cirrhosis and hepatic encephalopathy (HE). HE is a serious but potentially reversible disorder with a wide spectrum of neuropsychiatric abnormalities. Small intestinal bowel overgrowth is common in cirrhosis and associated with systemic endotoxemia and delayed oro-cecal transit time. Abnormal intestinal motility may play an important role in increasing the growth of pathogenic bacteria and increased absorption of gut toxins. It has been hypothesized that probiotics may replace the harmful urease-producing bacteria, decrease the production of neurotoxic ammonia, and thereby prevent the development of HE.

A recent Indian study revealed that over a 6-month period, daily intake of VSL#3 significantly reduced the risk of hospitalization for HE, as well as the Child-Turcotte-Pugh and model for end-stage liver diseases scores in patients with cirrhosis [90]. Yet, another study indicated that probiotics and lactulose are effective for secondary prophylaxis of HE in patients with cirrhosis [91]. Minimal hepatic encephalopathy was reversed in 50 % of patients treated with a synbiotic preparation [92]. However, a Cochrane review that encompassed seven trials and 550 patients concluded that probiotics were not useful in the treatment of HE but did help in reduction of plasma ammonia levels.

Pancreatitis

A study of intraduodenal administration of a probiotic mixture of six bacteria in patients with severe pancreatitis showed no reduction in acute complications of acute pancreatitis. Moreover, the risk of mortality in the probiotic group was significantly higher. This study, which was initiated because of benefit indicated in animal studies, is the only study that has shown an increased risk of death in a probiotic group. Nevertheless, this study indicates that probiotics should be used with caution in immune-compromised patients or patients at high risk of disease [93].

Table 2 provides a listing of clinical efficacy of probiotics in various GI disorders.

Extraintestinal diseases

Allergy and atopic diseases

In recent years, industrialized countries have witnessed a significant increase in autoimmune diseases and allergies. The progression of infant allergy to atopic diseases such as atopic eczema, allergic rhino conjunctivitis, and asthma is becoming increasingly common and is now referred to as the allergic march. Responsible factors are an impaired immune system that involves a Th1/Th2 switch and an altered microbiota [9]. The intestinal microbiota also appears to be different in allergic individuals with lower numbers of *Lactobacilli* and *Bifidobacteria* and more numbers of aerobes, coliforms, and *Staphylococcus aureus*. Decreased microbial diversity in infancy seems to be associated with an increased risk of atopic diseases later in childhood.

The rationale for the use of probiotics in allergic disorders is primarily their ability to modulate the composition of intestinal microbiota, improve barrier function of the intestinal mucosa, and reduce leakage of antigens [95]. Probiotic interaction with gut-associated lymphoid tissue (GALT), such as in the Peyer's patches, elicits indirect enhancement of respiratory immunity by activation of pro-inflammatory NK cells and/or macrophages within the airway mucosa. Direct modulation of the immune system through induction of anti-inflammatory cytokines increased production of secretory IgA, activation of T_{reg} cells, and skewing of Th1, Th2, and Th17 cell activation, or alterations in macrophage function are other benefits of probiotic therapy [96].

Various meta-analyses and systematic reviews have shown positive effects of probiotics with regard to prevention of atopic dermatitis particularly in infants who were administered the probiotic during the perinatal period [97]. However, the optimal dose, effective probiotic strains, time, and duration of supplementation need to be more thoroughly investigated.

Randomized controlled trials have yet not yielded sufficient evidence for the role of probiotics in the primary prevention of asthma, and results with rhinitis have yielded mixed results. Efficacy depends on several factors: the nature and severity of allergy, subject's age, time frame of the probiotic intervention, and the probiotic strain used.

Probiotics and respiratory infections

Recent studies have shown positive effects of probiotics in preventing and reducing the severity of respiratory infections due to an increase in IgA-secreting cells in the bronchial mucosa [98]. A meta-analysis conducted on the effectiveness of probiotics in preventing acute respiratory infections analyzed 10 trials involving 3,451 participants and found that probiotics reduced the number of participants experiencing acute upper respiratory tract infection. The role of probiotics in the prevention of childhood respiratory infections was evaluated in a recent systematic review and meta-analysis of four randomized controlled studies that involved 1,805 participants. *Lactobacillus rhamnosus* GG administration was associated with reduced incidence of acute otitis media, reduced risk of upper respiratory infections, and reduced need for antibiotic treatment. However, there was no difference in the risk of overall respiratory infections and the incidence of lower respiratory infections, except in children >1 year old [99].

A recent Cochrane Database systematic review concluded that probiotic use prevented upper respiratory tract infections with an odds ratio (OR) of 0.53 (95 % CI 0.36, 0.80) [56].

Table 2 Clinical efficacy of probiotics in various disease conditions

Clinical condition	Results/conclusions	References
Gastrointestinal diseases	This meta-analysis reviewed 84 trials including 10,351 patients; probiotics are generally beneficial in treatment and prevention of gastrointestinal diseases.	[52]
Treatment of acute infectious diarrhea	This Cochrane review analyzed 63 studies which includes 8,014 subjects; shortened duration of diarrhea and reduced stool frequency.	[61]
Prevention and treatment of antibiotic-associated diarrhea (AAD)	This meta-analysis reviewed 82 RCTs involving 11,811 subjects which suggests that probiotics are associated with reduction in AAD with an RR of 0.58. The treatment effect equates to an NNT of 13.	[58]
Prevention of *Clostridium difficile*-associated diarrhea (CDAD)	This review includes 31 RCTs with 4,492 subjects that reduce the risk of developing CDAD by 64 % in adults and children.	[68]
Treatment of irritable bowel syndrome (IBS)	The study includes 19 RCTs involving 1,650 patients that concludes probiotics appear to be efficacious in IBS but the magnitude of benefit and the most effective species and strain are uncertain.	[72]
Constipation	This meta-analysis reviewed 5 RCTs involving 377 subjects and found that in adults, effect of treatment with *Bifidobacterium lactis* DN-173010, *Lactobacillus casei* Shirota, and *E. coli* Nissle 1917 was observed in defecation frequency and stool consistency. In children, *L. casei rhamnosus* Lcr35 showed beneficial effect.	[84]
Necrotizing enterocolitis (NEC)	This meta-analysis reviewed 11 RCTs with total of 2,176 subjects; The probiotic studies using strains of *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, and/or *S. thermophilus* to prevent NEC shows reduction in the frequency and reduction in overall mortality.	[88]
Common infectious diseases	A meta-analysis conducted on the effectiveness of probiotics in preventing acute upper respiratory tract infections (URTIs) analyzed 10 trials involving 3,451 participants and found that probiotics reduced the number of participants experiencing acute URTI.	[56]
Nonalcoholic fatty liver disease (NAFLD)	This review includes 4 RCTs with a total of 134 patients that shows probiotic therapies can reduce liver aminotransferases, total-cholesterol, TNF-α, and improve insulin resistance in NAFLD patients.	[94]

NNT number needed to treat, *RR* relative risk, *RCTs* randomized controlled trials

A meta-analysis of five randomized controlled trials concluded that the administration of probiotics, compared with control, was beneficial in terms of the incidence of ventilator-associated pneumonia, length of ICU stay, and colonization of the respiratory tract with *Pseudomonas aeruginosa* [100]. There was no difference in ICU mortality, in-hospital mortality, duration of mechanical ventilation, and diarrhea.

Another meta-analysis on the role of probiotics in the prevention of the common cold showed a marginally favorable outcome with probiotics use (risk ratio 0.92; 95 % CI 0.84, 1.00) [101]. There are also reports of the beneficial use of probiotics in viral respiratory infections, with or without asthma (e.g. *L. casei* strain Shirota in influenza), respiratory infections in hospitalized children, and ventilator-associated pneumonia [102–105].

Probiotics and metabolic syndrome

More intriguing is the role of probiotics in metabolic syndrome (MS), a constellation of obesity, hypertension, diabetes, and disturbed lipid and carbohydrate metabolism. Recently, investigators related imbalances in gut microbiota with obesity and insulin resistance. It has been hypothesized that lipopolysaccharide (LPS) derived from gram-negative bacteria residing in the gut acts as a triggering factor linking inflammation to a high-fat diet-induced MS. This results in endotoxemia which induces obesity, insulin resistance, and diabetes [106]. Manipulating the gut flora with pre- and probiotics has shown to increase glucagon-like peptide-1, glucagon-like peptide-2, and peptide YY responses [107]. This is associated with higher expression of the zona occludens that improves mucosal barrier function and acts favorably on the intestinal barrier and thereby decreases the influx of LPS, thus lowering LPS-induced endotoxemia. This has resulted in increased insulin sensitivity. These studies provide strong evidence for using probiotics in formulation of dietary strategies in the management of metabolic syndromes.

Evidence suggests that probiotic bacteria could contribute to the prevention of coronary heart disease as well as to the control of blood pressure. Proposed mechanisms include interference with cholesterol absorption from the gut, direct cholesterol assimilation, and production of end-fermentation products that affect the systemic levels of blood lipids and mediate an antihypertensive effect [108]. These effects are still a matter of debate and further research is needed to draw conclusions.

Probiotics and cancer

It is interesting to note that tumor incidence and mass is greater in conventional than germ-free mice. In addition, germ-free mice and animals in whom the gut microbiota has been modified by antibiotics are more resistant to radiation toxicity, providing a basis for suspecting that interventions targeting microbiota may be effective in cancer [109]. Several mechanisms have been proposed such as augmentation of immune surveillance (including NK cell activity), downregulation of severe inflammation, and excretion of carcinogens by adsorbing them [110]. Studies have demonstrated that certain members of the *Lactobacillus* and *Bifidobacterium* sp. decrease the levels of carcinogenic enzymes produced by colonic flora through normalization of microbiota balance as well as production of antimutagenic organic acids and enhancement of the host immune system [111]. *L. casei* strain Shirota has been shown to stimulate immune response and to inhibit tumor development [110]. A similar preventive role in relapse prevention in colorectal and bladder cancer has been documented [112, 113]. *Lactobacillus casei* strain Shirota is probably the only probiotic strain for which favorable effects have been documented in other cancers. One population-based case-control study retrospectively evaluated 306 cases with breast cancer and 662 matched controls. The odds ratio of *Lactobacillus casei* strain Shirota consumption (≥4 times a week against <4 times a week) was 0.65. Moreover, consumption of soy isoflavone was associated with a lower OR of breast cancer: adjusted OR in the second, third and fourth quartiles against the first quartile was 0.76, 0.53, and 0.48, respectively (trend p=0.0002). The authors concluded that regular consumption of *Lactobacillus casei* strain Shirota and isoflavones since adolescence was inversely associated with breast cancer incidence in Japanese women [114].

The SYNCAN (which examined effect of synbiotics in cancer) project confirmed the beneficial effect of probiotic or synbiotic supplementation on primary prevention or prevention of recurrence of colorectal cancers [115].

Probiotics and urogenital infections

Beneficial effect in urinary tract infection was noted mainly with *Lactobacilli*, the predominant urogenital flora in healthy premenopausal women. A recent meta-analysis of data from 294 patients across five studies showed no significant difference in the risk of recurrent urinary infection in patients receiving lactobacillus vs. controls. However, on excluding studies using ineffective strains and studies testing for safety, a significant decrease was found in patients given lactobacillus (pooled risk ratio 0.51; 95 % CI 0.26–0.99) [116]. *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 seemed most effective among lactobacilli for prevention of urinary infections [117].

Bacterial vaginosis is a syndrome characterized by a change in vaginal ecology, where normal flora that is predominant in Lactobacilli is replaced by a mixed flora consisting of facultative and obligate anaerobic bacteria resulting in an increase in vaginal pH over 4.5. Lactobacilli strains, administered orally or preferably intravaginally, could be effective in prevention and treatment of bacterial vaginosis [118].

Probiotics in oral disease

In a recent systematic analysis, in two thirds of the selected papers, probiotics demonstrated a capacity to reduce *Streptococcus mutans* counts in saliva and/or plaque in the short-term. The authors concluded that the effect of probiotics on the development of caries seems encouraging, but there is insufficient data to provide conclusive clinical evidence. There is also some evidence that probiotics may be useful in periodontal infections, halitosis, oral candidosis, and in prolonging the life of voice prostheses [119].

Conclusion

The progress and challenges for elucidating the interactions between the human gut microbiota and host through metabolic modeling is an area of increasing interest. Manipulating and mining the microbiota promises much, but this will be realized with greater understanding of the diversity and complexity of the normal microbiota in different populations with different lifestyles. One of the most compelling and persuasive examples to highlight the importance of the gut microbiota comes from the ground breaking research and study in cases of antibiotic resistance *Clostridium difficile* infection where fecal microbiota transplantation has shown to be highly effective in clearing infection and associated symptoms. In the coming years, it is envisioned that the plasticity of the gut microbiota will be exploited to provide new categories of therapeutics, providing modification of the gut microbiota, on the basis of specific microbe-microbe modulation and microbe-host interaction aiming to correct and improve lifestyle conditions. The role of probiotics in the maintenance of gut health and controlling disease has been fairly well established and it is only in recent years that their benefit outside the gut is being evaluated gainfully. However, unlike antibiotics and the other weapons of offence, such defensive modulation cannot be expected to reverse disease but only to prevent it.

Therefore, it is too optimistic to expect a single probiotic organism to have benefit across an array of conditions, especially since the gut microbiota is diverse between individuals and communities. Lessons learnt with one organism obviously cannot be extrapolated to others; benefit seen in one condition again cannot be extrapolated to others. We are far away from a panacea but there is reason to look at

probiotics as a feasible intervention for the improvement of health and prevention of diseases.

Further insights will come from interdisciplinary approaches progressively provided by enlarged consortia, including researchers and clinicians able to exploit high-throughput technological platforms to apply translational work flows to diagnostic pipelines and finally to patient care and treatment programs.

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