Function

Probiotic actions on diseases: implications for therapeutic treatments

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The ecology of gut microflora, which colonizes all body surfaces, has long coevolved with its hosts in a complicated fashion. Health benefits conferred by gut microflora include defense against invading pathogens, improvement of nutritional bioavailability, and development of the regional and systemic immune systems. The past decade has witnessed growing interest in the fact that the gut microflora affects the host's energy homeostasis by means of various mechanisms, including supplying nourishment from indigestible compounds, producing small biomolecules responsible for lipid profiles, and participating in the absorption, distribution, metabolism and excretion of nutrition. Much *in vitro* and *in vivo* research has indicated that aberrant gut microflora plays an important role in the pathogenesis of a wide spectrum of diseases. This is accomplished by a shift in focus, from laying an emphasis on pharmacotherapy to placing more effort on gut microflora normalization. The objectives of this review include illustrating trends in the clinical application of probiotics on diseases, as well as discussing current methodology limitations on probiotic selection. Furthermore, it is expected to shed light on the nature of probiotics, with the aim of giving greater insight into the implications for clinical use of probiotics in the treatment of diseases.

Introduction

The human gastrointestinal tract is an ideal residence, which is full of nutrition, supporting the growth of complicated and dynamic microflora. Our body surfaces that are exposed to the

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outer environment are colonized almost everywhere by microflora, especially the skin, respiratory tracts, genitourinary tracts and gastrointestinal tracts. It is estimated that the gut microflora in humans contains approximately 10^{14} microbes (including prokaryotes, protists, parasites, and viruses), ten times more than the total number of human body cells.[1]

The composition and function of gut microflora have been demonstrated to be important factors in human health and diseases.[2] Although the definitions of probiotics remain ambiguous and diverse, the Food and Agriculture Organization (FAO) and World Health Organization (WHO) research teams suggest that probiotics are live microorganisms which, when administered in adequate quantities, confer a health benefit on the host. Taking into account that some literature proposed that administration of probiotics had no pharmacological effect on humans, compared with a placebo group,[3] the hypothesis of a host–microbe symbiotic relationship was addressed elaborately by means of a germ-free animal experiment, which proved that germ-free mice showed reduced organ weight, reduced cardiac output and reduced immune functions compared to normal mice.[4]

To date, there has been a lack of concrete characterization of the "healthy gut microflora" conditions, whether in human beings or other organisms. However, accumulating research has revealed that dysbiosis plays an important role in the pathogenesis of a wide spectrum of diseases.[5] Even though the probiotic effects on diseases are controversial, probiotics have in fact been reported to be quite relevant to metabolic diseases,

infectious diseases, gastrointestinal diseases and gastrointestinal oncological diseases.[6]

The ecology of human gastrointestinal microflora

Obligate anaerobes dominate the main population of microflora, a number that is roughly two to three times greater than that of facultative anaerobes and aerobes. Until now, over fifty prokaryote phyla have been published. However, the majority of GI microflora can be grouped into merely two phyla: the *Bacteroidetes* and the *Firmicutes*.[7] The phylum *Bacteroidetes* comprises the main classes of bacteria, which have Gram-negative, non-sporeforming, anaerobic, and rod-shaped characterization, for example the most famous bacterial species *Escherichia coli*. The phylum *Firmicutes* comprises two main classes of *Bacilli* and *Clostridia*. A large amount of bacteria under the two classes is Gram-positive, sporeforming, with low-GC content of genomic DNA. The *Bacilli* class includes two orders: *Lactobacillales* and *Bacillales*. Almost all probiotic products on the market belong to the *Lactobacillales* order, for example, lactic acid bacteria. By the way, the *Bifidobacteriales* order is also a popular target of probiotic products. Nevertheless, the order belongs to the *Actinobacteria* phylum, which is only present in small proportions of GI microflora, but is enriched in the small intestine and colon.[8]

The number of microbes in our body varies from area to area. It is assessed that the number of microbes in human bodies ranges from 10^1–10^3 in the stomach and duodenum, 10^4–10^7 in the jejunum and ileum, 10^{11}–10^{12} in the colon and 10^9 CFU per gram in feces. Furthermore, GI microflora composition also differs between sites. In the small intestine, the *Actinobacteria* phylum and the *Bacilli* class of *Firmicutes* phylum account for the majority of the microflora, whereas the *Bacteroidetes* phylum and the *Clostridia* class of *Firmicutes* phylum dominate the majority in the colon.[9]

Factors known to be involved in GI microflora composition alteration include acquired habits (*e.g.* smoking and diet), maternal colonization (*e.g.* breast milk and the birth canal), pathogens, genetic profiles, immune system development, and pharmaceutical therapy.[10–12] Even though the GI microflora composition fluctuates with so many factors, Eckburg *et al.* proposed that alteration in GI microflora appears to occur mainly within classes, orders, genera and species. At the phylum level, it is relatively stable and conserved between human individuals.[13] This research revealed that even under a variety of intrinsic and extrinsic pressures, the GI microflora might possess redundant functionalities. As a result, it is difficult to shape what "healthy GI microflora" is. On the other hand, thanks to the functionality redundancy property of GI microflora, our physiological state and metabolic capacity are not easily influenced by a single factor.

To date, due to well-developed next-generation sequencing technology, comparative metagenomic and metaproteomic investigation of gut microbial communities has gained substantial understanding. The typical changes in composition

of the gut community in patients with certain disorders and the functional roles of gut microflora are increasingly clear. Ferrer *et al.* reported that in the gut of obese subjects, the phylum *Firmicutes* was more plentiful out of the total microbiota (94.6%) in comparison with *Bacteroidetes*, while the gut of the lean subjects exhibited a significant shift toward increasing amounts of the phylum *Bacteroidetes*.[14] Similar results were also revealed by Larsen and his colleagues.[15] They showed that the proportions of phylum *Firmicutes* and class *Clostridia* were drastically decreased in the diabetic group. Moreover, the ratios of *Bacteroidetes* to *Firmicutes* and the *Bacteroides–Prevotella* group to the *C. coccoides–E. rectale* group correlated positively with blood glucose level but not with body-mass index (BMI). The increased ratios of phylum *Bacteroidetes* to *Firmicutes* not only occur in obese/diabetic individuals, but also in those suffering from GI inflammation diseases. In addition to variation in the ratio of phylum *Bacteroidetes* to *Firmicutes*, Wouters *et al.* discovered that the intestinal microbial composition of IBD patients showed a reduced ratio of phylum *Firmicutes* to *Proteobacteria*.[16]

In summary, recent research has proposed that "healthy GI microflora" is characterized by a high amount of obligate anaerobes and a low concentration of oxygen, as well as a low redox potential environment. Dysbiosis of the gut microbiota has been observed in patients with IBD and metabolic diseases, but the underlying mechanisms resulting in this imbalance remain vague. Even though growing evidence has proposed that "healthy GI microflora" might be linked to increased ratio of phylum *Firmicutes* to *Bacteroidetes*, do dietary habits or physiological state contribute more to compositional changes of microflora? In other words, is composition alteration of gut microflora perhaps a consequence of disease-caused changes of the physiological state rather than a primary event? If it is correct that the change in microbiota is simply a consequence of the change in physiological state, why have so many clinical trials yielded positive results? Still, more complementary studies are needed to support this point of view.

Several lines of study have reported that human GI microflora coevolves with its host. Although Gill and Guarner performed metagenomics analysis, finding that the GI microflora composition is conserved and remains stable within the phylum level,[17] a prospective therapy, intestinal microbiota transplantation (IMT), is suggested as one alternative treatment for *Clostridium difficile* infection; in this approach indigenous intestinal microflora is transplanted from a healthy donor to patients.[18–20] Controversy hence arises as to whether administration of probiotics is able to alter the original host's GI microflora or not. We reviewed four categories of human diseases proposed to be associated with dysbiosis that have been clinically treated by probiotics.

Probiotics in treating metabolic diseases

Obesity is threatening all over the developing and developed world. Unfortunately, obesity rarely comes by itself. High

development of adipose tissues gives rise to abnormally increased secretions of pro-inflammatory cytokine tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), as well as protein hormone leptin, adiponectin and resistin. All these increased risk factors are directly proportional to both body fat deposition and serum lipid concentration. Moreover, they also play roles in diabetes mellitus, non-alcoholic steatohepatitis and cardiovascular diseases, such as atherosclerosis.[21–23]

The last two decades have seen growing importance placed on research into two metabolic diseases: hyperlipidemia/hypocholesterolemia (Table 1) and diabetes (Table 2). Although there is an abundance of clinical evidence showing the excellent cholesterol-lowering capacity as well as the anti-diabetic properties of probiotics, challenges such as bacterial strain-dependent properties and adequate administration dosages need more well-established research to overcome.

Hyperlipidemia and hypocholesterolemia

Hepner et al. recruited 54 subjects for studying the cholesterol-lowering activity of yoghurt fermented by Lactobacillus bulgaricus and Streptococcus thermophilus.[24] The result showed that serum cholesterol was dramatically reduced by 5 to 10% after 1 week of fermented milk consumption. Kiessling et al. assessed the hypocholesterolemic effect of yoghurt fermented with a mixed culture of Lactobacillus acidophilus 145 and Bifidobacterium longum 913 in 29 females aged 19–56 years old.[25] The findings indicated long-term supplementation with 300 grams fermented milk over a period of 21 weeks improved the serum level of HDL cholesterol and gave rise to a decreased LDL/HDL cholesterol ratio. Another study done by Hlivak et al. assessed the effects of long-term orally administered probiotic strain E. faecium M-74 on lipid profiles in humans.[26] Their results revealed that after 56 weeks of probiotic administration, a decrease in total cholesterol of 12% was observed, but there was no significant impact on HDL and triglycerides. For comparison of probiotics and conventional yogurts with regard to their efficacy on lipid profiles, a clinical trial was performed among 70 pregnant women, and found both probiotics and conventional yogurts showed similar results in the reduction of serum total cholesterol, LDL, HDL as well as serum triglyceride (TC) concentrations.[27]

In discussions of probiotic clinical use in hyperlipidemia and hypocholesterolemia diseases, Lactobacillus reuteri NCIMB 30242 has been emphasized in recent years.[84,85] Jones et al. have pointed out that consumption of yoghurt containing L. reuteri NCIMB 30242 is both effective and safe for lowering LDL-C, TC, apoB-100 and non-HDL-C in hypercholesterolemic subjects. The efficacy of L. reuteri NCIMB 30242 seems to be superior to traditional probiotic therapy and akin to that of other cholesterol-lowering ingredients. In addition, L. reuteri NCIMB 30242 was reported to be capable of increasing the circulation of 25-hydroxyvitamin D (lower-than-normal levels of serum 25-hydroxyvitamin D may be a sign of osteoporosis, cardiovascular disease, diabetes, and cancer) in a trial of a total of 127 healthy hypercholesterolemic adults.[86]

Differences of gastrointestinal microbiota between obese and lean phenotypes were primarily observed in leptin-deficient (ob/ob) mice by Ley et al. (2005), showing that compared to lean mice, ob/ob animals have a 50% decrease in the abundance of the Bacteroidetes phylum and a proportional increase in the abundance of Firmicutes.[87] Similar results reported by Turnbaugh et al. suggested that an increase in the proportion of the Firmicutes phylum was associated with more microbial genes responsible for encoding enzymes relevant to carbohydrate metabolism being detected, which might increase the capability for digesting foods and supplying more energy to the host.[88] However, due to a lack of models of germ-free human beings, it is difficult to reproduce the obese phenotype in ethically compromised human studies. Although the hypothesis that obesity alters gut microbial ecology has been reproduced in clinical studies, a consensus has not been reached on whether or not gut microbial ecology alters the obese phenotype in humans.

Even though dysbiosis and obesity are not confirmable as having a cause–effect relationship, there is an increasing trend in developing approaches (such as probiotics, prebiotics and synbiotics) to restructure human gut microflora towards an increased ratio of Firmicutes to Bacteroidetes, the latter of which has been found overpresent in the intestinal tracts of those obese individuals. As Kalliomaki et al. revealed in their prospective follow-up study, obese children had diverse gut microbiota in comparison with lean ones.[89] This difference may imply that early differences in fecal microbiota composition in children may predict overweight, and thus offer new opportunities for preventive measures in early life weight management before obesity occurs.

Table 1 Hyperlipidemia/hypocholesterolemia clinical trials evaluating the effect of probiotic therapy

Subjects	Probiotics	Dose (CFU per day)	Duration	Ref.
54	Yogurt (L. bulgaricus, S. thermophilus)	N/A	12 weeks	24
29	Yogurt (L. acidophilus 145, B. longum 913)	10^8 to 10^9	7 weeks	25
43	E. faecium M-74	2×10^9	6–56 weeks	26
60	L. plantarum CECT 7527/7528/7529	1.2×10^9	12 weeks	28
70	L. acidophilus LA5, B. animalis BB12	1×10^7	9 weeks	27
114	L. reuteri NCIMB 30242	5×10^9 (twice)	6 weeks	84

Table 2 Diabetes clinical trials evaluating the effect of probiotic therapy

Subjects	Probiotics	Dose (CFU per day)	Duration	Ref.
64	Yogurt (*L. acidophilus* La5, *B. lactis* Bb12)	10^8 to 10^9	6 weeks	32
120	Probiotic mixture Ecological®Barrier	2.5×10^9	4–26 weeks	35
70	Yogurt (*L. bulgaricus*, *S. thermophilus*)	10^7	9 weeks	33
45	*L. acidophilus* NCFM	1×10^{10}	4 weeks	36
200	*L. rhamnosus* GG/LC705, *B. breve* Bbi99, *P. freudenreichii* ssp. Shermani JS	5×10^9, 2×10^8, 2×10^9	6–24 weeks	34

Diabetes mellitus

Much evidence in both animal and clinical research has proposed that oxidative stress is of importance in the progression of type 1 and type 2 diabetes. Maillard reactions of high-concentration reducing glucose and protein lead to radical chain polymerization, and in turn irregular oxidative stress increases lipid peroxidation, membrane disruption and gene mutation. All these radical-induced types of damage are hypothesized in relation to the dysfunction of pancreas β-cells and the development of insulin resistance.[29–31]

Ejtahed *et al.* performed a clinical trial to evaluate the effects of probiotic supplementation on blood glucose and antioxidant status in type 2 diabetic patients.[32] Each patient consumed 300 grams of probiotic yoghurt fermented by *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12. The final result showed that probiotic administration improved patients' fasting blood glucose levels and antioxidant capacity, which are assessed by the status of erythrocyte superoxide dismutase, glutathione peroxidase, catalase and serum malondialdehyde.

Because of overgrowth of adipose tissue and excess body weight, increased inflammatory cytokine secretions (especially IL-6) occur for the most part during the third trimester, thus leading to elevated insulin resistance in pregnancy. Asemi *et al.* investigated whether probiotic yoghurt (*L. acidophilus* LA5 and *B. animalis* BB12) or conventional yoghurt (*S. thermophilus* and *L. bulgaricus*) had a more beneficial effect on pregnant women in terms of serum insulin levels. They found that, contrary to conventional yogurt, daily intake of probiotic yogurt for 9 weeks had a more significant effect on maintaining insulin levels and might improve insulin resistance in pregnant women.[33]

Unlike type 2 diabetes, which is caused mostly by obesity, type 1 diabetes results from the failure of the pancreas insulin-producing β cells to produce insulin. This type can be further classified as an immune-mediated disease, and was referred to as "juvenile diabetes". A 200-subject PRODIA study was conducted with the aim of investigating whether or not probiotic intervention could prevent beta cell autoimmunity in children at genetic risk of type 1 diabetes.[34] In this research, three beta cell autoantibodies (GAD, IA-2, and IAA) were selected as markers to detect the occurrence of type 1 diabetes. The results showed that only one case was analyzed positive for IAA at 6 months of age. There was no case that showed positive at 12 months of age. Yet at 24 months of age, one case was exhibited positive for GADA and another one for IA-2A. However, no sample presented as positive for more than one autoantibody.

Probiotics in treating infectious diseases

Research on probiotics applied to infectious diseases has been mounting steadily for a number of decades. Several lines of evidence have suggested that probiotic agents, as an alternative form of treatment or prevention of infectious diseases, pose much fewer side effects than typical medicinal therapies. Among the clinical studies, oral health, gastrointestinal infections, respiratory and genitourinary tract infections have attracted the most attention.[37–39] A hypothesis, which proposed that the enhancement of systemic immune responses by probiotics is an important characteristic of successful defense against invading pathogens, still remains controversial due to a lack of direct evidence.

For the application of probiotics to the treatment or prevention of urogenital and gastrointestinal tract infections, in addition to outstanding antibacterial capacity, the most essential characteristic probiotics must have in this respect is dominant colonization ability, and to maintain the acidity of the environment at a pH lower than 4.5.[40] Since the mechanisms underlying probiotic action on infectious diseases are supposed to be intensification of epithelial and mucosal barriers, competition and inhibition of pathogen adhesion and colonization, criteria for probiotic selection are usually established according to these distinctive properties. Table 3 summarizes some probiotic clinical use on infectious diseases.

Hemmerling *et al.* conducted phase 1 (ref. 41) and phase 2a (ref. 42) of a study evaluating the colonization efficiency, safety, tolerability, and acceptability of *Lactobacillus crispatus* CTV-05 in women with bacterial vaginosis. Abad and Safdar published a systemic review, revealing the feasibility of the application of lactobacilli bacteria, like *L. rhamnosus* GR-1 and *L. reuteri* for prevention and treatment of recurrent bacterial vaginosis.[43] Jeppsson *et al.* reported numerous clinical trials, which have suggested that probiotics may decrease the number of opportunistic pathogenic bacteria and restore an impaired barrier function, suggesting potential for the prevention of post-operative infections.[44] In terms of gastrointestinal infections, the effectiveness of the alternative probiotic treatment in decreasing the duration of acute infectious diarrhea in the pediatric emergency department was evaluated by Nixon and his colleagues.[45] They revealed that among children, administration of *L. rhamnosus* GG decreased the duration of acute diarrheal illness presenting with more than 2 days of symptoms. Probiotic treatment for infectious diseases not only elicits potent effects on adults and children, but also on infants, as in

Table 3 Infectious disease clinical trials evaluating the effect of probiotic therapy

Subjects	Probiotics	Dose (CFU per day)	Duration	Ref.
12	*L. crispatus* CTV-05	10^8 to 10^9	5 days	41
24	*L. crispatus* CTV-05	2×10^9	2 weeks	42
159	Probiotic mixture BIO-THREE	10^9	7 days	44
129	*L. rhamnosus* GG	10^9 to 10^{10}	5 days	45
109	*B. animalis* subsp. *lactis* BB-12	5×10^{10}	8 months	46

the clinical trial reported by Taipale *et al.*[46] A randomized, double-blind, placebo-controlled study done by their research team suggested that the infants (1 month-old) receiving *B. animalis* subsp. *lactis* BB-12 were experienced no significant impact on the occurrence of gastrointestinal symptoms, but had a reduced risk of respiratory infections compared to those of the negative control group.

In summary, much research has supported the idea that probiotics, especially *Lactobacillus* spp., is highly promising and safe as a prophylaxis for infectious diseases, while the effectiveness of *Bifidobacterium* spp. seems to be limited to GI disorders.

Probiotics in treating gastrointestinal diseases

The integrity of gastrointestinal microflora is demonstrated to be highly correlated with human health, and their disintegration gives rise to not only gastrointestinal diseases but also to a wide range of immune and metabolic diseases.[47,48] Until now, various studies have boosted our knowledge of the mechanisms of probiotic action and the evidence for support of their use in practice. Probiotic alternative therapy has been suggested as being beneficial for the treatment of GI diseases in recent years because of the role of microbiomes, which suggests that IBDs result from an overaggressive immune response to a subset of commensal enteric microbes in genetically predisposed people. It has been shown that anti-inflammatory responses are mediated by TGF and IL-10 production by epithelial cells and mononuclear cells, thus suppressing Th1 polarization. Until now, there has been much literature indicating that some strains of probiotics are capable of up-regulating anti-inflammatory cytokine secretions, both *in vitro* and *in vivo*.[49-51]

Clinical applications of probiotics to GI diseases include diarrhea caused by antibiotic use, *Clostridium difficile* infections, inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS).

Inflammatory bowel diseases

IBD is a collection of inflammatory situations occurring on the colon and small intestine. Two major types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). The two diseases share numerous symptoms. However, the main difference between CD and UC is that in CD, the inflamed area might occur throughout the digestive tract, while in UC the inflammation is characteristically located in the colon. Since the exact etiology of a wide spectrum of pathogenic factors in CD and UC has not yet been well elucidated, in fact many patients still have a reduced quality of life even under pharmacological treatment. Table 4 and Table 5 show the recent clinical trials of probiotics therapy on UC and CD, separately.

Anderson *et al.* undertook a systematic review of fecal microbiota transplantation in patients with inflammatory bowel diseases and infectious diarrhea.[52] The results showed that the majority of patients under probiotics treatment experienced a reduction of symptoms (76%), and disease remission (63%). Surprisingly, all of the cases of *C. difficile* infections were resolved. Jonkers *et al.* conducted a systematic review, and concluded that the application of *Escherichia coli* Nissle 1917 in inactive ulcerative colitis (UC) and VSL#3 probiotics in active UC is promising and warranted. However limited evidence is available to support the use of probiotics in Crohn's disease so far.[53]

More specifically for UC treatment, the probiotic mixture VSL#3 was suggested to be safe and able to decrease UCDAI (ulcerative colitis disease activity index) scores in patients. A total of 144 patients were treated with probiotic mixture VSL#3 at a dose of 3.6×10^{12} CFU per day. The results showed that after 8 weeks of treatment, probiotic mixture VSL#3 improves rectal bleeding and induces remission in relapsing UC patients.[54] Sood *et al.* also used probiotic mixture VSL#3 to treat patients with mild-to-moderately active ulcerative colitis, and reported that probiotic mixture VSL#3 is safe and effective in achieving clinical responses and remissions in patients that were given 3.6×10^{12} CFU twice daily for 12 weeks.[55]

While in CD treatment, rare trials used probiotics singly without pharmacotherapy. Besides, the trial length ranges from 3 months to 2 years, much longer times than those used in clinical UC treatment. Table 5 lists some recent clinical trials using probiotics, but most of them yielded disappointing results.

Overall, the effects of probiotics to improve disease symptoms appear to be acceptable (but not outstanding). Although there has been a rapid increase in the number of publications on probiotic use in clinical therapy, the principle of bacterial strain selection and adequate dosage recommendation needs more empirical research. Further investigation into the mechanisms underlying probiotic action on GI diseases can be of enormous value to develop novel selection criteria that is more effective for specific types of diseases.

Table 4   Ulcerative colitis clinical trials evaluating the effect of probiotic therapy

Subjects	Probiotics	Dose (CFU per day)	Duration	Ref.
40	*L. reuteri* ATCC 55730	1×10^{10}	8 weeks	56
41	*B. breve* Yakult	3×10^{9}	1 year	57
144	VSL#3 probiotic mixture	3.6×10^{12}	8 weeks	54
147	VSL#3 probiotic mixture	3.6×10^{12} (twice)	12 weeks	55
120	*B. longum*	2×10^{9}	4 weeks	58
90	*E. coli* Nissle	1×10^{8}	2–8 weeks	59
57	*E. coli* Nissle	1×10^{11}	12 weeks	60

Irritable bowel syndrome

IBS is a symptom-based diagnosis characterized by recurrent abdominal pain and alteration of bowel habits accompanied with discomfort, flatulence, bloating, as well as defecatory dysfunction.[66] The IBS etiology, which is proposed to result from a dysfunctional interaction between the GI microbiota and the mucosal immunity system, is partially like IBD. However, the intensity and distribution of the inflammatory process and location are quite different. IBS does not induce severe inflammation, ulcers or other damage to the digestive tract.

Over the past few years, growing evidence has accumulated showing that small intestinal bacterial overgrowth (SIBO) plays an imperative role, due to the observation of bacterial quantitative changes in the indigenous flora in IBS patients.[67] Furthermore, it has been reported that eradicating SIBO *via* antibiotics treatment showed a significant improvement in the symptoms of IBS patients. Since the decreasing quantity of intestinal bacteria *via* antibiotics is a mainstream therapy for IBS, it raises the possibility of probiotics being used to help relieve symptoms.

Yoon *et al.* conducted a trial investigating whether probiotics treatments are effective in IBS patients, and could alter the composition of microflora.[68] Their findings showed that after 4 weeks of treatment IBS symptoms were substantially relieved in the probiotics group (68.0%) compared to the placebo group (37.5%). In addition, fecal analysis revealed that *B. lactis*, *L. rhamnosus* and *S. thermophilus* increased considerably. Another clinical trial focused on IBS patients with constipation (especially those with delayed transit) was carried out by Agrawal and his research team.[69] Patients consumed milk fermented by *B. lactis* DN-173 010, which relieved distension and accelerated gastrointestinal transit, thus improving constipation symptoms. Table 6 lists some recent studies of probiotic clinical use on IBS patients. Although probiotic screening criteria for IBS remains vague, antibiotics or probiotics treatment with either appear to be effective for ameliorating the symptoms of IBS patients.

Clostridium difficile infection

C. difficile infection (CDI) is the principal cause of GI disorder-derived infectious diarrhea. It has been reported that *C. difficile* is an opportunistic pathogen which can be found in many healthy individuals. In general, both patients suffering from GI disorders and infants with underdeveloped immune systems have an increased risk of CDI.[73] Oral administration of antibiotics (such as metronidazole and vancomycin) is the most commonly used method for CDI, but cases of therapeutic failures are usually reported without clear reasons.

IMT has become a novel, promising treatment for patients who have failed standard treatment strategies. IMT involves the administration of a suspension (yogurt, milk, or saline) of healthy donors' feces into the CDI patients' intestinal tract, with the aim of remodelling the homeostasis of the intestinal microbiota. IMT has not been universally advocated as a standard CDI therapy in part because of poor documentation of safety assessments.[74] Regardless of these concerns, this approach has been employed in a growing number of patients with suboptimal response to standard therapy.

A systematic review conducted by Gough *et al.* revealed that in 317 patients treated across 27 case series and reports, the treatment of IMT resolved 92% of cases.[75] Kassam *et al.* conducted a systematic review and meta-analysis, which also indicated that IMT holds considerable promise for recurrent CDI treatment.[76] In their investigation, 245 out of 273 CDI patients (90%) reached clinical resolution, and significant adverse effects caused by IMT treatment were uncommon. Nevertheless, both the systematic studies on IMT pose the

Table 5   Crohn's disease clinical trials evaluating the effect of probiotic therapy

Subjects	Probiotics	Dose (CFU per day)	Duration	Ref.
35	*B. longum*	2×10^{11}	3–6 months	61
75	*L. rhamnosus* GG	2×10^{10}	2 years	62
70	*L. johnsonii*, LA1	1×10^{10}	3 months	63
28	*E. coli* Nissle	5×10^{10}	1 year	64
11	*L. rhamnosus* GG	2×10^{9}	6 months	65

Table 6 Irritable bowel syndrome clinical trials evaluating the effect of probiotic therapy

Subjects	Probiotics	Dose (CFU per day)	Duration	Ref.
49	LacClean Gold-S® probiotic mixture	5×10^9	4 weeks	68
214	*L. plantarum* 299v	1×10^{11}	4 weeks	70
16	*L. plantarum* MF 1298	1×10^{10}	3 weeks	71
120	*E. coli* Nissle	$2.5–25 \times 10^9$	12 weeks	72
41	Yogurt (*Bifidobacterium lactis* DN-173 010)	1.25×10^{10}	27 days	69

same problem. Firstly, most case studies are lacking randomized, placebo-controlled experiment design and long-term follow-up reports; secondly, the route of administration, the criteria for being a feces donor, and the dose of feces given have not yet been well-standardized.

Because of the relative paucity of mechanisms illustrated specifically for IMT, there is limited, fragile evidence to develop optimal protocols of IMT to be a standard part of clinical therapy, with safety being a particular concern. Although numerous case studies for IMT therapy showed positive results, still some challenges must be overcome before this therapeutic tool can be extensively performed.

Probiotics in treating oncological diseases

Since the integrity of GI microflora is highly essential to human health and its composition changes with age, some aspects of its integrity and composition are thought to be greatly relevant to the carcinogenic processes in the host.[77–79] The most frequently occurring and most well-researched cancers are colorectal cancer and *Helicobacter pylori*-dependent gastric cancer.

According to the Center for Disease Control and Prevention (USA), colorectal cancer is the third most common carcinoma in the United States and the second most common diagnosed cancer in the European Union. Many epidemiological studies have revealed that dietary factors and GI microflora play vital roles in colorectal carcinogenesis. It attracts overwhelming interest in the potential protective and preventive role of probiotics. The occurrence of cancer begins with accumulative cell mutations and develops over many years in a multistep and complicated process. Due to the complexity of colorectal cancer initiation, promotion and progression, as well as exposure to different carcinogens in the gut, solutions to a single carcinogenic factor could not exert a significant effect on such multifaceted diseases. So far there has been lack of studies showing probiotic interventional treatment to be clinically effective on colorectal cancer patients, even though both *in vitro* and *in vivo* evidence supports the potential anticarcinogenic action of probiotics.

Helicobacter pylori is a Gram-negative, microaerophilic bacterium. It has been found in patients suffering from chronic gastritis and gastric ulcers, two diseases which were demonstrated relative to the development of stomach cancer.[80] Individuals infected with *H. pylori* have been reported as a risk factor and one of the major causes of gastric cancer. Several lines of study have shown that probiotics are very potent for the improvement of *H. pylori* infection.[81] However, does elimination of *H. pylori* infection reduce the occurrence of gastric cancer? To date, empirical evidence for gastric cancer prevention by the elimination of *H. pylori* is still lacking. The results of meta-analysis on the correlation between gastric cancer prevention and *H. pylori* eradication remain inconsistent and it is hard to make definitive statements.

Future perspectives on probiotics

Rapidly growing studies on the characteristics of GI microflora in human health and disease have been particularly influential in contributing insight into the therapeutic use of probiotics under specific diseases. In addition to GI diseases, which were conventionally thought to be highly associated with probiotics, there is increasing knowledge pointing to the fact that aberrant GI microflora leads a wide range of diseases. In the last decade, many advances have been made in the area of GI microflora-related diseases, with important perspectives towards documentation of the specific probiotic strains improving various aspects of a host's metabolic capacity, and physiological, or even psychological state.

Until now, clinical use of probiotics has posed several challenges and problems: Firstly, is vegetative bacteria in fermented milk or lyophilized resting bacteria in capsules the best approach to probiotic delivery? Although lyophilized probiotics have low-caloric, portable advantages, and a long shelf-time, the process of lyophilization could lead to metabolic injury to probiotics, and thus alter their colonization abilities which are coupled with nutritional requirements. Also, the lyophilized probiotics need more than half a day to reach the log phase, the required growth time being much longer than that of vegetative probiotics. Furthermore, research teams seldom perform acid-bile resistance tests and inhibition zone assays using lyophilized resting probiotics. Whether lyophilized resting (non-spore forming) bacteria or vegetative bacteria exhibit greater tolerance to acid-bile adversity warrants future investigation.

In terms of probiotic application in metabolic diseases, Massey (2001) suggested that adequate consumption of calcium, potassium and magnesium, the three minerals found in large amounts in milk or its products (*e.g.* yogurt and cheese), at least in part improves hyperglycemia, hyperlipidemia and hypertension.[90] Furthermore, serum levels of magnesium were inversely correlated with fasting serum insulin, plasma HDL cholesterol, systolic and diastolic blood pressure. It is worth

noting that dairy foods contribute up to sixteen percent of the daily intake of magnesium in the food supply. Another viewpoint reported by Ranadheera *et al.*, proposed that it seems likely that the probiotic carriers (*e.g.* milk, cheese and ice cream) exert a considerable protective effect during the digestion process, due to the observation that some probiotic culture starters are sensitive to the gastrointestinal condition, but usually exist in high quantities in feces.[91] For that reason, evaluation of gastrointestinal tolerance *in vitro* is not a satisfactory model predicting *in vivo* survivability of probiotics accompanied by the food matrix.

Secondly, what is an appropriate dosage and what is their duration of use in the body? Over the past years of clinical trials on different diseases, there has been a lack of standardized or conclusive dosage indication. In addition to the a recommended dosage, the exact time interval of probiotic uptake is also a rough question which varies from case to case. Gill and Guarner suggested daily consumption is probably the best manner to maintain probiotic effective amounts.[82] Harvard Women's Health Watch suggested probiotic uptake that ranges from1 billion to 10 billion colony-forming units for two to several days per week is enough to achieve efficiency. Another tricky problem of clinical applications is concerned with probiotic retention time in human bodies. Although the ability to adhere to human epithelial cells is an important criterion for probiotics selection, Bezkorovainy reported that the majority of probiotic adherence to the gut, in general, is transient and does not permanently colonize as a new member of the indigenous microflora.[83] However, it is believed the probiotics tend to exert their functions and benefits when they proliferate, metabolize and react with host's cells during their passage through the GI tract.

Thirdly, assessing the presence of fecal probiotic bacteria after oral administration was not adequate to predict the probiotic functionality. The currently available method is to measure the fecal gene content or the colony-forming units of lactic acid bacteria. Supposing the prerequisite that most probiotics do not permanently colonize in the gut is satisfied, it is reasonable to assume that the quantity of fecal probiotics, which were orally administrated, has an inverse correlation with the strength of their adherence ability. Such assumption faces technical limitations because until now there has been lack of fundamental procedures for detecting the extent of probiotic proliferation in the gut.

A number of human studies have shown the results of probiotic clinical use as to the positive, at least in part, effect on improving the undesirable symptoms of numerous diseases. Despite the fact that there is encouraging evidence that particular strains of probiotics are both generally recognized as safe (GRAS) and able to elicit certain functional benefits on humans, the functionality of other strains cannot be deduced from such specific consequences without empirical evidence. However, in addition to documentation of specific strains, future research is required to establish a dosage recommendation and a minimum daily amount required to exert functional benefits, in laying the groundwork for clinical use.

Database

This systematic review was conducted using Cochrane Database of Systematic Reviews (CDSR) and Database of Abstracts of Reviews of Effect (DARE). We searched the human controlled trials comparing probiotic application on different diseases within ten years.

Acknowledgements

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