

Using probiotics and prebiotics to improve gut health

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Recent molecular-based investigations have confirmed the species diversity and metabolic complexity of the human gut microbiota. It is also increasingly clear that the human gut microbiota plays a crucial role in host health, both as a source of infection and environmental insult and, conversely, in protection against disease and maintenance of gut function. Although little is known about the health impact of the dominant groups of gut bacteria it is generally accepted that bifidobacteria and lactobacilli are important components of what might be termed the beneficial gut microbiota. The microbiota management tools of probiotics, prebiotics and synbiotics have been developed and, indeed, commercialized over the past few decades with the expressed purpose of increasing numbers of bifidobacteria and/or lactobacilli within the gastrointestinal tract.

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▼ A complex community of microorganisms inhabit the gastrointestinal tract throughout its length. The colon is the main site of microbial colonization and, typically, the indigenous microbiota are considered to be made up of more than 500 different species of bacteria. Recent molecular studies have confirmed this view of microbial diversity within the gut [1]. The gut microbiota plays an important role in both human health and disease [2]. The main function of the gut microbiota, from the host's point of view, is to prevent colonization by potentially pathogenic microorganisms. It does so efficiently by outcompeting invading pathogens for ecological niches and metabolic substrates. Microbial metabolism also serves as an important source of energy for the gut wall, providing up to 50% of the daily energy requirements of colonocytes by fermentation of carbohydrates to organic acids, mainly butyrate. The gut microbiota acts as an important modulator of the immune system, not only educating the naïve infant immune

system but also serving as an important source of noninflammatory immune stimulators throughout life in healthy individuals. However, these health-promoting aspects of the gut microbiota are not infallible and can be overcome by pathogens specifically evolved for gastrointestinal infection (e.g. *Salmonella* spp., attaching and effacing *Escherichia coli* strains and *Campylobacter jejuni*). Similarly, the defence mechanisms afforded by a healthy gut microbiota might be overcome when compromised by chemotherapy (especially antibiotics) or chronic disease [e.g. colon cancer and inflammatory bowel disease (IBD)]. This realization has led to the development of foods specifically designed to fortify the gut microbiota.

Introducing the concept of probiotics

A probiotic has been defined as 'a live microbial food ingredient that is beneficial to health' [3]. Probably the most studied probiotics belong to the genera lactobacilli and bifidobacteria. These genera have a considerable safety record both within the fermented foods industry, where they have been used for many years, and, more recently, in probiotic foods. Probiotic therapy has been investigated for its effectiveness against a range of gastrointestinal diseases and disorders.

Probiotics in relief of lactose maldigestion

About two-thirds of the world's adult population suffer from lactose maldigestion, with the prevalence particularly high in Africa and Asia. In Europe, lactose maldigestion varies from about 2% in Scandinavia to about 70% in Sicily [4]. Symptoms include loose stools, abdominal bloating, pain, flatulence, nausea and borborygmi. Individuals with lactose

Table 1. Effectiveness of probiotic therapy against diarrhoea

Diarrhoea	Probiotic strain	Outcome of treatment	Ref
Infantile diarrhoea	<i>Lactobacillus rhamnosus</i> GG	Reduced duration of diarrhoea	[6]
	<i>Lactobacillus reuteri</i>	Reduced duration of diarrhoea	[58]
	<i>Bifidobacterium bifidum</i> and <i>Streptococcus thermophilus</i>	Prevented rotavirus diarrhoea	[7]
	<i>Lactobacillus rhamnosus</i> and <i>L. reuteri</i>	Ameliorated acute diarrhoea and reduced period of rotavirus shedding	[59]
	<i>L. rhamnosus</i> and <i>L. reuteri</i>	Reduced duration of diarrhoea in nonhospitalized children with mild gastroenteritis	[59]
Antibiotic-associated diarrhoea	<i>Bifidobacterium longum</i>	Decreased duration of erythromycin-induced diarrhoea	[60]
	<i>B. longum</i> and <i>Lactobacillus acidophilus</i>	Reduced incidence of clindamycin-induced diarrhoea	[61]
	<i>L. acidophilus</i> and <i>Lactobacillus bulgaricus</i>	Reduced incidence of ampicillin-induced diarrhoea	[62]
	<i>L. rhamnosus</i> GG	Decreased duration of erythromycin-induced diarrhoea	[63]
	<i>L. rhamnosus</i> GG	Reduced incidence of diarrhoea	[64]
	<i>Enterococcus faecium</i>	Decreased diarrhoea induced by antitubercular chemotherapy	[65]
	<i>Ent. faecium</i>	Reduced incidence of diarrhoea	[66]
	<i>Streptococcus boulardii</i>	Reduced incidence of diarrhoea owing to β -lactamins or tetracycline	[67]
Relapsing <i>Clostridium difficile</i> colitis	<i>L. rhamnosus</i> GG	Improves/terminates colitis	[68]
	<i>L. rhamnosus</i> GG	Eradicated <i>C. difficile</i> -associated diarrhoea	[69]
Traveller's diarrhoea	<i>L. acidophilus</i> , <i>B. bifidum</i> , <i>L. bulgaricus</i> , <i>S. thermophilus</i>	Reduced frequency but not duration of diarrhoea	[70]
	<i>L. rhamnosus</i> GG	Decreased incidence of diarrhoea	[71]
	<i>S. boulardii</i>	Reduced incidence of diarrhoea	[72]

maldigestion can tolerate lactose present in yoghurt to a much greater degree than the same amount of lactose in raw milk [5]. Two different, though not exclusive, mechanisms of action have been put forward to explain this finding. Yoghurt and probiotic lactic acid bacteria contain high levels of lactase, which is released within the intestinal lumen when these bacteria are lysed by bile secretions. Lactase then acts on the ingested lactose, thus relieving maldigestion symptoms. The reduced intestinal transit time of yoghurt might also allow slower digestion of lactose, so reducing the symptomatology.

Use of probiotics to combat diarrhoea

A range of probiotic strains has been evaluated for their anti-diarrhoeal capabilities, with varying degrees of success (Table 1). In acute infantile diarrhoea, often the result of infection with rotavirus, *Lactobacillus rhamnosus* GG has repeatedly been shown to reduce the duration of diarrhoea

by about 50% [6]. The mechanisms of action have not been fully elucidated, but might involve fortification of the mucosal integrity and/or stimulation of the immune response, for example through increased antirotavirus-specific immunoglobulin (Ig) A. *Bifidobacterium bifidum*, given in conjunction with *Streptococcus thermophilus* in standard milk formula, has also been shown to reduce the incidence of rotaviral diarrhoea [7]. However, evidence for a preventative effect of *L. rhamnosus* GG against rotaviral diarrhoea is equivocal, with feeding studies showing both a reduction in diarrhoeal incidence and no effect above placebo levels [5,6,8].

Diarrhoea occurs in about 20% of patients who receive antibiotics [5]. The antibiotics might directly affect the indigenous gut microbiota by compromising colonization resistance and favouring the growth of pathogenic microorganisms, for example *Clostridium difficile* and *Klebsiella oxytoca*. Several probiotic strains have been shown to

Table 2. Human studies with probiotics in treatment of inflammatory bowel disease (IBD)

Disease	Probiotic strain	Outcome of treatment	Ref
UC	<i>Escherichia coli</i> Nissle 1917	Maintenance of remission in UC (as effective as mesalazine, the standard treatment)	[12]
UC (not placebo controlled)	VSL#3	75% of patients given VSL#3 remained in remission for 12 months. No side effects	[73]
Pouchitis	VSL#3 pouchitis	Reduced risk of relapse recurring compared with placebo	[14]
Pouchitis	VSL#3	More effective than placebo in preventing pouchitis	[74]
Crohns' disease (active, moderate disease)	<i>Streptococcus bouardii</i>	Reduction in bowel movements and decrease in disease activity compared with placebo	[75]
Crohn's disease (in remission)	<i>S. bouardii</i>	Probiotic plus mesalamine reduced incidence of relapse compared to mesalamine alone	[13]
Crohn's disease	<i>E. coli</i> Nissle 1917	Significant reduction in rate of relapse compared with placebo	[76]
Crohn's disease	VSL#3	Reduced risk of relapse in postoperative Crohn's disease patients compared with patients given mesalazine	[15]

reduce the incidence and duration of antibiotic-associated diarrhoea (Table 1). Probiotics are also effective in reducing the side effects of 'triple therapy' with antibiotics used to eradicate *Helicobacter pylori* from the stomach. *L. rhamnosus* GG reduces the incidence of diarrhoea, nausea and taste disturbance in patients receiving rabeprazole, clarithromycin and tinidazole for *H. pylori* eradication [9]. Similar results have also been observed with other probiotics including *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, bifidobacteria and *Streptococcus bouardii*.

Several volunteer studies have been performed to determine the efficacy of probiotics in reducing the incidence of traveller's diarrhoea. Oksanen *et al.* [10] investigated the ability of *L. rhamnosus* GG to prevent diarrhoea in 820 volunteers travelling to two resorts in Turkey. In only one of the holiday destinations was there a significant reduction in the incidence of diarrhoea among travellers taking the probiotic. This study highlights the mechanistic problems associated with prophylactic trials on the effects of probiotics against traveller's diarrhoea, not least because traveller's diarrhoea is caused by a diverse, ever-changing range of microbial pathogens, including pathogenic *E. coli*, *Salmonella*, *Campylobacter* and *Shigella* strains as well as viruses. It is unlikely that a single probiotic strain will inhibit such a broad spectrum of pathogens *in vivo*. This might account for the many probiotic trials targeting traveller's diarrhoea that have failed to show any positive impact after treatment with particular probiotic strains [5,11].

Probiotics for the treatment of IBD

Probiotics have also been studied for their ability to improve the symptomatology of more chronic disease states, such as IBD and colorectal cancer (CRC). IBD refers to a group of disorders of unknown aetiology that are characterized by chronic or recurrent mucosal inflammation. An immunological reaction to some members of the gut microbiota is thought to play a role in disease onset or maintenance. Probiotic administration, either through regulation of the inflammatory response or modulation of gut microbiota composition and/or activity might bring about relief in IBD symptoms or maintain remission from symptoms. The well-defined, nonpathogenic strain *E. coli* Nissle 1917 has proven more effective in preventing relapse in Crohn's disease patients compared with a placebo (Table 2) [12]. *S. bouardii* has shown some success in relieving the symptoms of active Crohn's disease (i.e. reducing stool frequency and disease activity) and in reducing the risk of relapse [13]. VSL#3 is a mixture of four lactobacilli (*L. acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei* and *Lactobacillus plantarum*), three bifidobacteria (*Bifidobacterium breve*, *Bifidobacterium infantis* and *Bifidobacterium longum*) and *S. thermophilus*. The mixture has proven effective in reducing the recurrence of chronic relapsing pouchitis. VSL#3 (at 6 g/day) significantly reduced relapse recurrence (15%) compared with placebo (100%) over a 9 month period [14] and was also effective in preventing the occurrence of pouchitis in patients with

ileo-pouch anal anastomosis for ulcerative colitis (UC). Campieri *et al.* [15] showed that VSL#3 significantly reduced the risk of relapse in Crohn's disease patients post-operatively compared with the control group who were given mesalazine. The information on the mechanisms of probiotic activity in the treatment of IBD has been derived mainly from studies with animal models of disease. Plausible mechanisms of action include probiotic interaction with mucosal regulatory T cells and regulation of cytokine transcription factors within the mucosa in response to invasive bacteria [16].

Impact of probiotics on CRC

Approximately three-quarters of all incidences of CRC are sporadic and increase with age. It is probable that diet and its interaction with the gut flora, together with reduced protection from the ageing microbiota, are contributing factors. Although many faecal microorganisms can produce carcinogens and tumour promoters from dietary constituents, studies have not identified which species are responsible. Bifidobacteria and lactobacilli do not produce toxic or carcinogenic metabolites. However, probiotic bacteria have been investigated for their ability to beneficially modulate biomarkers of CRC. Several enzyme activities expressed by gut bacteria (particularly species of clostridia and bacteroides) have been implicated in the conversion of dietary constituents into toxic or carcinogenic compounds, for example β -glucuronidase, β -glycosidase, azoreductase, nitroreductase, IO 'hydratase-dehydrogenase' and nitrate/nitrite reductase. Many probiotics have been shown to reduce the level of these detrimental faecal enzyme activities. In humans, however, it still remains to be proven that this actually reduces the risk of developing CRC [17]. Pool-Zobel *et al.* [18] investigated the ability of different probiotic strains (*L. acidophilus*, *Lactobacillus gasseri*, *Lactobacillus confuses*, *B. longum*, *B. breve* and *S. thermophilus*) to protect against DNA damage in rats. All showed dose-dependant protection with the exception of *S. thermophilus*. Probiotic strains (e.g. *L. acidophilus*, *B. longum* and *L. rhamnosus* GG) have also reduced the incidence of colonic tumours in rats dosed with colonic carcinogens or cooked food mutagens [17]. Human epidemiological studies suggest that probiotics delivered as fermented dairy products, usually yoghurt, might reduce the risks of large adenomas in the colon [17]. Intervention studies in human volunteers have shown promising, although sometimes equivocal, results on the impact of probiotic supplementation on biomarkers of CRC (e.g. faecal water genotoxicity, urinary mutagenicity and proliferation of rectal mucosal crypts) in both healthy patients and those with colon adenomas [17].

Impact of probiotics on immune function

The microbiota is an important constituent of the intestine's defence barrier because it induces and maintains specific immune responses and hyporesponsiveness to antigens. Furthermore, it is known that certain bacterial species in the gastrointestinal tract can liberate low molecular weight peptides that trigger the immune system [19]. Such tolerance induction and antigenic stimulus matures the gastrointestinal associated lymphatic tissue, such that it is ready to produce IgA in response to an antigenic stimulus (i.e. in the presence of *E. coli* toxin). This stimulation begins at birth and it has been reported that children born vaginally have more circulating IgA cells than those born through Caesarian delivery [19]. A recent study by Chiang *et al.* [20] described a *Bifidobacterium lactis* HN019 strain that can enhance nonspecific immune functions, namely leucocyte (lymphocytes and phagocytes) proliferation, enhanced phagocyte production and proinflammatory cytokine production. Several studies have shown that feeding *B. lactis* HN019 to healthy volunteers, including the elderly, resulted in an increase of peripheral blood leucocytes and natural killer cells that were active in tumour killing and viral destruction [21,22]. Through modulation of the immune response, *L. rhamnosus* GG has proven effective in prevention of early atopic disease in high-risk children [23]. Rosenfeldt *et al.* [24] demonstrated the therapeutic nature of a combination of *L. rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 122460 in the management of atopic dermatitis in children. The results showed that 56% of children treated with probiotics had less severe eczema compared with 15% of children in the placebo group. *L. rhamnosus* GG has also been shown to downregulate the immunoinflammatory response in individuals with milk hypersensitivity, while acting as an immunostimulator in healthy individuals [25].

Use of probiotics in less well defined gut disorders

Irritable bowel syndrome (IBS) affects 8-22% of the population, with women being most affected. The causes are diverse, but are often related to a depletion of beneficial gut bacteria. To date, human feeding studies in IBS patients have yielded mixed results. O'Sullivan and O'Morain [26] found that *L. rhamnosus* GG had little effect on IBS symptoms whereas *L. plantarum* 299V had a measurably beneficial effect [27]. The ability of probiotics to impact on IBS remains to be proven satisfactorily and there is a need for further intervention studies with well-defined groups of IBS patients, larger cohorts and, possibly, a broader range of probiotic strains.

Gastroenteritis is commonly associated with autistic spectrum disorders and there is some evidence that an altered gut microbiota might even play a role in autistic pathology [28]. Probiotic therapies could hold promise, not only in the relief of gastrointestinal symptoms associated with autism but also in normalizing the autistic gut microbiota in terms of its composition and the profile of microbial metabolites produced, some of which are thought to play a psychoactive role in autism. Indeed, there is much circumstantial evidence from clinical practice and carers of autistic individuals that probiotic intake does have some impact on autistic symptomatology [29]. Appropriate intervention studies using probiotics are needed to establish any impact on the disease.

Mechanisms of probiotic activity

The mode of action of probiotic strains is likely to be multifactorial and, from existing evidence, appears to be strain specific. Enhancement of colonization resistance and/or direct inhibitory effects against pathogens is likely to be important in situations in which probiotics have reduced the incidence and duration of gastroenteritis. Probiotic strains have inhibited pathogenic bacteria both *in vitro* and *in vivo* through several different mechanisms. These include production of directly inhibitory compounds (e.g. bacteriocins), reduction of luminal pH through short chain fatty acid production (which could themselves be directly inhibitory to certain pathogens), competition for nutrients and adhesion sites on the gut wall, modulation of the immune response and regulating colonocyte gene expression (e.g. expression of mucin genes) [2,30,31]. Applying probiotics to stimulate immune function, especially in individuals with underdeveloped or dysregulated immune function, appears to be sound, considering the positive outcomes of feeding studies targeting viral infections, IBD and allergic diseases. Crucial to our future understanding of how probiotics work is the application of high resolution molecular techniques (e.g. transcriptomics measured using DNA microarrays) to elucidate the crosstalk between probiotics and the mucosa cells. It is still unclear which mechanism or, more probably, which spectrum of mechanisms, is used by probiotics within the human gut microbiota to bring about improved health. Further human feeding studies are required to confirm probiotic efficacy in specific disease states such as IBD, colon cancer and gastroenteritis. However, considering the fact that probiotic activity is likely to be strain specific and that these disease states are of multifactorial aetiology, such studies should be mechanistically driven, building on data from *in vitro* and animal studies with specific probiotic strains showing specific modes of action against defined pathological targets.

Introducing the concept of prebiotics

A prebiotic is defined as 'a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health' [32]. Bacterial genera targeted for selective stimulation are the indigenous bifidobacteria and lactobacilli.

Modulation of the gut microbiota using prebiotics

Prebiotics of proven efficacy that are commercially available are fructooligosaccharides (FOS) and inulin, lactulose and galactooligosaccharides. The ability of these oligo-saccharides to alter the gut microbiota towards a more beneficial composition, with increased numbers of bifidobacteria in particular, has been shown reproducibly in human feeding studies upon both traditional microbiology and direct molecular analysis (K.M. Tuohy *et al.*, unpublished) [2,33]. An interesting observation from such studies has been that the degree of bifidogenesis seen in healthy individuals correlates inversely with pretreatment population levels [34], suggesting the greatest benefit in those individuals with low levels of bifidobacteria (i.e. patients with chronic gastrointestinal disease and the elderly) [35]. Although there is no daily recommended dose of prebiotics, doses of 4-20 g/day (K.M. Tuohy *et al.*, unpublished) have shown efficacy. Roberfroid *et al.* [34] suggested that a minimum daily dose of 4 g/day of inulin or FOS would be needed to observe an increase in gut bifidobacteria. Prebiotic doses higher than 20 g/day might induce some side effects, such as increased flatulence or abdominal bloating [K.M. Tuohy *et al.*, unpublished]. However, prebiotics appear to have few side effects at lower doses and, as existing food components, for example in bananas, onions and artichokes, have a good safety record. Many other potential prebiotics are currently under investigation, including xylooligosaccharides, lactitol, soyoligosaccharides, pectooligosaccharides, glucooligosaccharides, isomaltooligosaccharides and gentiooligosaccharides (K.M. Tuohy *et al.*, unpublished) [33].

Prebiotics in infant health and nutrition

The microbiota of breast-fed infants differs from that of infants fed milk formula. Typically, the gut microbiota of breast-fed infants is dominated by bifidobacteria, whereas in formula-fed infants a more diverse microbiota develops, with higher numbers of *Bacteroides* spp., *Clostridium* spp. and the *Enterobacteriaceae* [36]. This predominance of bifidobacteria in breast-fed infants is usually correlated to a lower risk of intestinal infection. Besides the numerous maternal immune antibodies transmitted through breast-milk from mother to child, human milk oligosaccharides

might also contribute directly to the natural defence against infection by promoting a proliferation of intestinal bifidobacteria and lactobacilli [37]. The composition and structure of human milk oligosaccharides cannot be reproduced by the food industry, therefore prebiotics are being considered for fortification of infant formulas (K.M. Tuohy *et al.*, unpublished). Moro *et al.* [38] fed infants a cow milk formula supplemented with FOS and galactooligosaccharides (GOS). After 28 days of feeding, the numbers of faecal bifidobacteria and lactobacilli were significantly increased compared with the placebo group, although faecal pH was lower in infants given the prebiotic-fortified formula.

Prebiotics and colon cancer

Inulin, FOS, lactulose and galactooligosaccharides have all been shown to have a positive effect on biomarkers of CRC (K.M. Tuohy *et al.*, unpublished) These prebiotics reduced the activity of microbial enzymes involved in the production of toxins and carcinogens as well as reducing the concentration of these metabolites in faeces [17,18]. Lactulose can directly protect against DNA damage in animal models challenged with colonically active carcinogens [39]. Conversely, in a human feeding study, lactulose failed to reduce faecal water genotoxicity, highlighting the problems in monitoring biomarkers of CRC in healthy individuals [40]. Inulin and FOS have reduced the number and size of precancerous lesions as well as tumour incidence in carcinogen-treated rats [41]. Such studies illustrate that prebiotics might have the potential to reduce CRC risk and might even alter CRC progression [41]. The mechanisms of prebiotic action against CRC remain to be elucidated, but probably involve changes in gut microbiota in terms of bacterial numbers and activity, more direct effects on mucosal gene expression (e.g. through production of butyrate) and stimulation of the immune response.

Prebiotics and IBD

Butyrate can maintain remission in IBD patients by promoting mucosal cell proliferation and accelerating the healing process in animal models and human studies. Thus, the use of dietary fibre and prebiotics has been investigated as a means of stimulating butyrate production in the colon of UC patients. Germinated barley foodstuff containing glutamine-rich protein and hemicellulose-rich fibres has alleviated the symptomatology in both animal models of UC and patients with UC [42]. The mode of action is thought to be three-fold: decreasing stool frequency, increasing the concentration of butyrate within the gut and increasing the numbers of bifidobacteria and eubacteria.

Germinated barley fibres have proven efficacious at delivering butyrate to the colonic mucosa, a process that is

difficult to achieve by direct administration of butyrate orally or rectally. The presence of glutamine, a preferential substrate for colonocytes, could fortify the mucosal barrier, preventing bacterial translocation through the colonic epithelium and subsequent mucosal damage. Although not a prebiotic in the strictest use of the term, because germinated barley fibre is more complex than existing prebiotics and is likely to have a broader fermentation spectrum within the colon, this fibre clearly holds promise for the development of a functional food that specifically targets IBD. The better-defined prebiotic inulin also increases colonic butyrate and reduces inflammation and disease severity in animal models of colitis [43].

Prebiotics and human metabolism

Prebiotics have been suggested to modify serum triglyceride levels and cholesterol. However, owing to the complexity of human lipid metabolism, comprehensive investigations are difficult to undertake and have often given conflicting results [11,44]. Data for the consumption of FOS show either no effect or a slight decrease in circulating triacylglycerols and plasma cholesterol concentrations, whereas higher molecular weight inulins have shown more success in lowering triglyceride levels [45], suggesting that these prebiotics have no detrimental effect on individuals with minor hypercholesterolaemia or hypertriglyceridaemia. Pereira and Gibson [46] have reviewed the possible mechanisms of action of probiotics and prebiotics on lipid metabolism. The same authors examined the ability of a range of probiotic strains to assimilate cholesterol *in vitro*. *Lactobacillus fermentum* KC5b proved particularly effective at removing cholesterol from batch fermentations [47].

Prebiotics, specifically inulin and FOS, have been linked to an enhancement of mineral absorption in the large bowel [48]. Stimulation of calcium and magnesium absorption has been demonstrated in ovariectomized rats fed FOS. In a placebo-controlled study using a stable isotope of calcium, FOS improved calcium intake in women at the late menopause phase [49]. A significant increase in calcium absorption was observed in adolescent girls who were given a drink fortified with FOS and inulin (4g/day) and a daily supplement of calcium (1.5g/day) [50]. An increase in magnesium absorption has been observed in humans and animals after the consumption of prebiotics [51].

The synbiotic approach

A synbiotic can be defined as 'a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of

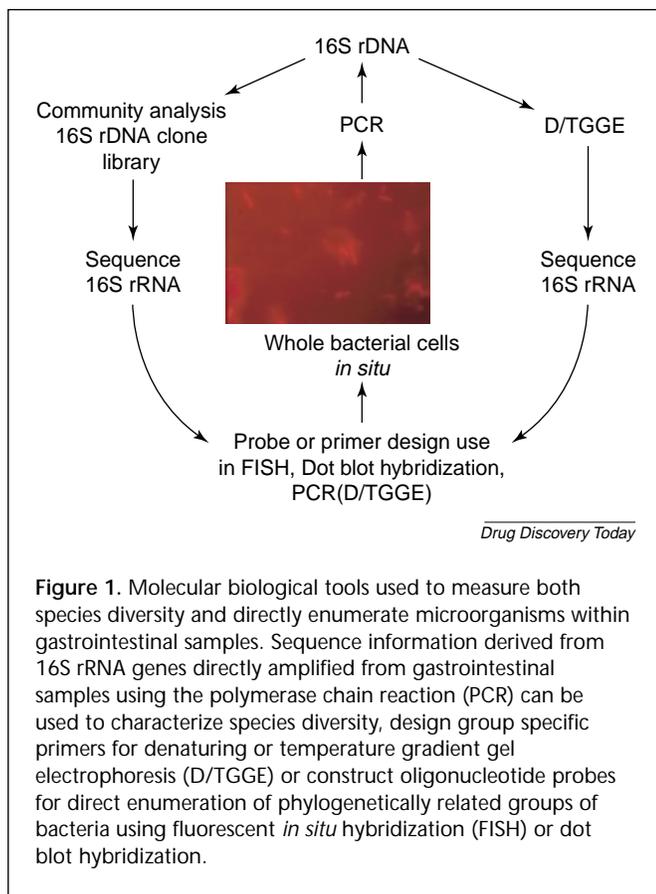


Figure 1. Molecular biological tools used to measure both species diversity and directly enumerate microorganisms within gastrointestinal samples. Sequence information derived from 16S rRNA genes directly amplified from gastrointestinal samples using the polymerase chain reaction (PCR) can be used to characterize species diversity, design group specific primers for denaturing or temperature gradient gel electrophoresis (D/TGGE) or construct oligonucleotide probes for direct enumeration of phylogenetically related groups of bacteria using fluorescent *in situ* hybridization (FISH) or dot blot hybridization.

one or a limited number of health-promoting bacteria, and thus improving host welfare' [32]. Synbiotics aim to enhance the survival and activity of proven probiotics *in vivo* as well as stimulating indigenous bifidobacteria.

Few studies have been carried out in humans on the effectiveness of synbiotics. Bouhnik *et al.* [52] monitored the effect of a synbiotic mix containing inulin and *Bifidobacterium* spp. in healthy volunteers. Although an overall increase in faecal bifidobacterial numbers was observed, the authors concluded that no additional increase in the numbers of bifidobacteria was observed solely due to the prebiotic component. A fermented milk product consisting of yoghurt starter strains and *L. acidophilus* plus 2.5% FOS decreased total serum cholesterol levels, as well as decreasing low density lipoprotein (LDL)-cholesterol and the LDL/high density lipoprotein (HDL) ratio [53]. More recently, Kießling *et al.* [54] showed that long-term (7 weeks) consumption of synbiotic yoghurt (*L. acidophilus* 145, *B. longum* 913 plus FOS) led to a significant improvement in LDL/HDL cholesterol ratios in 29 healthy women. Further convincing evidence for the enhanced performance of synbiotics compared with either their probiotic or prebiotic moieties taken alone has been forthcoming from animal models of CRC. Synbiotic

products containing *B. longum* and lactulose or inulin reduced the incidence and size of aberrant crypt foci in rats challenged with the carcinogen azoxymethane [17,18]. Similarly, Femia *et al.* [55] showed that rats fed Synergy 1 (a mixture of high and low molecular weight inulin) or Synergy 1 plus the probiotic strains *L. rhamnosus* GG and *B. lactis* BB12 developed fewer colonic tumours upon azoxymethane challenge than rats fed the probiotic strains alone. Synbiotic products have the potential for enhanced health promotion over either probiotics or prebiotics alone but require further investigations in human feeding studies.

Future developments in pro- and prebiotics

Numerous human feeding studies have shown that the human gut microbiota can be modulated with probiotics, prebiotics and synbiotics to increase the numbers and activity of bifidobacteria and lactobacilli. All three microbiota management tools have also shown some positive health outcomes against specific disease conditions. However, there is a gap in our knowledge linking the elevated levels of bifidobacteria and lactobacilli to specific health effects and there is a limited understanding concerning the mechanisms of probiotic activity *in vivo*. Modern high resolution molecular techniques based on the phylogenetic information encoded by the 16S rRNA gene are now being applied to characterize the gut microbiota within different disease states. Similarly, such techniques allow phylogenetically relevant measurement of microbiota changes in response to different dietary regimes [1]. Figure 1 illustrates how such techniques can be employed to characterize microbiota composition and monitor population changes without the need for microbiological culture, thus bypassing the inherent limitations of lack of selective growth media and unculturable bacteria. Similarly, measurement of *in vivo* activity is crucial in the development of efficacious probiotic strains against particular diseases, especially considering the strain specificity shown by probiotics in human feeding studies. Although prebiotics have been shown repeatedly to increase numbers of bifidobacteria at the genus level *in vivo*, little information is available on the strain or species specificity of different prebiotics within the human gut. It is probable that because probiotics show strain specificity in their health-promoting capabilities, different species of indigenous bifidobacteria will also vary in their ability to promote gastrointestinal health. The development of novel, or second generation, prebiotics is likely to concentrate on species specificity and on the delivery of prebiotics into the proteolytic environment of the distal colon, the site of origin of both CRC and UC [33]. In Europe there is

currently a concerted effort to tackle some of the gaps in knowledge concerning both the medical efficacy and mechanistic principles of microbiota management using probiotics, prebiotics and synbiotics. The PROEUHEALTH (<http://www.vtt.fi/virtual/proeuhealth/>) cluster of EU-funded collaborative projects aims to identify the mechanisms through which probiotics, prebiotics and synbiotics can improve host health. Using a combination of *in vitro* systems, animal models of disease and multicentred human feeding studies the cluster aims to develop efficacious microbiota management tools specifically targeting IBD, CRC and improved gastrointestinal health in the elderly. The cluster also aims to develop and apply molecular methodologies to study the crosstalk between the gut microbiota and host cells and develop the technological capabilities for the production of novel and safe probiotics and prebiotics [56].

Conclusion

The interest in gut flora modulation has generated data whereby human wellbeing can be enhanced and the risk of disease onset reduced. New molecular techniques that allow an accurate assessment of the flora composition has resulted in improved strategies for underpinning mechanisms of effect. This is a crucial step forward for this area, which, in the past, has suffered from a lack of mechanistic input into human and *in vitro* trials. Given the lack of directed therapy for many clinical disorders of the gut, and the expense involved, both probiotics and prebiotics can offer alternative options. New advances that use the synbiotic effect, target distal colonic activity and include improved functionality (e.g. antiadhesive effects against gut pathogens and vaccine delivery), as well as a wider range of delivery systems, will further open up the possibilities involved.

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