The Role of Prebiotics and Probiotics in Human Health

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Abstract

There has recently been a significant increase in research on the potential health benefits associated with probiotics and prebiotics. Some effects attributed to selected probiotics or prebiotics have been proved by clinical trials, while others have been acquired on the basis of in vitro tests which need to be replicated in vivo in order to be validated. Clinical reports in the literature for the application of probiotics have been done for the treatment of infectious diseases including viral, bacterial or antibiotic associated diarrhea, lowering of serum cholesterol, decreased risk of colon cancer, improved lactose digestion, and altered intestinal microbiota. However, information on probiotic species, a specific strain-therapeutic application, and adequate dosages, is not yet sufficient to allow rational consumption. Moreover, probiotic oligosaccharides are poorly understood in regard to their fermentation profiles and dosages required for health effects. The present review summarizes some of the literature in regard to clinical or therapeutic trials of probiotics and prebiotics.

Bacteria of the Human Gastrointestinal Tract

The microflora in the Gastrointestinal (GI) tract of human infants begins establishment immediately after delivery. Gram-negative anaerobic bacteria together with Gram-positive and Gram-negative anaerobic bacteria can be recovered from fecal specimens within 3 days after birth [1]. The vaginal and fecal flora of the mother, and also the environment (air and food, for instance) are important sources of bacteria [2]. After approximately 2 years of age the microbial composition resembling that of an adult becomes established [3]. Furthermore, it has been found that changes in the diet can modify the microbial composition in the colon [4]. For example, infants fed breast milk have mainly Bifidobacteria in their gut, but infants fed formula have more complex microflora which are predominantly Bacteroides, Bifidobacteria, Clostridia and Streptococci [3,5].

In the GI tract the large intestine is the most colonized area and can harbor up to 10^{12} bacteria per gram of waste contents [6,7] with hundreds of species represented [7]. The large intestine contains mostly strict anaerobes and they may be categorized as bacteria that are either harmful or beneficial [5]. The pathogenic effects of harmful bacteria include diarrhea, systemic infections, liver damage and carcinoma. In contrast, the beneficial bacteria promote the host’s health by stimulating the immune system, synthesizing vitamins, inhibiting the growth of harmful bacteria and improving digestion and absorption of essential nutrients [3]. Furthermore, the microflora in the large intestine derives a proportion of their energy from dietary components that have not been degraded in the upper intestinal tract and reach the large intestine [8]. These dietary components consist mainly of polysaccharides, oligosaccharides, proteins, peptides and glycoproteins that are fermented by the gut bacteria and subsequently produce short chain fatty acids (SCFA) as their major end products; the majority of these SCFA are acetate, propionate and butyrate [9].

Probiotics

Probiotics are live microorganisms which when consumed may confer a health benefit to the host [10]. Certain species of Lactic Acid Bacteria (LAB) and Bifidobacteria spp, are commonly used in the manufacture of probiotic products because of their well-known beneficial effect to host health and they are Generally Recognized as Safe (GRAS) [11-13]. Many studies have shown that probiotics can stimulate the immune system, decrease serum cholesterol, alleviate lactose intolerance, decrease diarrheal incidence, control infections, act as antibiotics, suppress tumors and protect against colon/bladder cancer [14].

Role of probiotics in lactose intolerance

For most mammals, including humans, lactase activity declines after weaning; however, in some humans, lactase activity persists at a high level throughout adult life due to the development of lactase persistence [15]. This lactase persistence among the population of the world varies depending on ethnicity and habit of consuming dairy products; consequently, the northern European population has as little as 2% of the population deficient in lactase [16]. The lactase deficiency is prevalent in Hispanic people (80%), black and Ashkenazi Jewish people (60 to 80%) and almost 100% of Asian and Native American Indian people [15].

In persons lacking lactase, when lactose reaches the large intestine, it is metabolized by the colonic microflora to produce methane, carbon dioxide, and hydrogen and altering the osmotic balance in the colonic lumen, causing symptoms such as cramping, diarrhea, flatulence, and abdominal bloating [17]. However, a number of human studies have shown that high-lactose milk products supplemented with starter cultures containing Lactococcus and/or Bifidobacteria can be tolerated by lactose-intolerant individuals, possibly because these fermented products contain the microbial β-galactosidase which functions in the small intestine to support lactose hydrolysis [18]. Additionally, it has been demonstrated that in mice Streptococcus thermophilus or Lactobacillus casei subsp defens is able to hydrolyze lactate during transit through the gut [19]. Independent of easing lactose intolerance, probiotics also seem to relieve some gastrointestinal complaints such as diarrhea, systemic infections, liver damage and carcinoma [14].

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as flatulence or diarrhea most probably by their influence on the gut microflora [20,21].

**Probiotics effects on diarrheal diseases**

**Infectious diarrhea caused by bacteria or viruses:** The World Health Organization (WHO) estimates 8.1 million deaths occur yearly in children (<5 years of age) with diarrhea accounting for 14% of those deaths [22]. In addition to causing mortality, diarrhea elicits serious long-term effects with multiple episodes and persistent diarrhea affecting growth, nutrition, and cognition [23]. Rotavirus is the most common cause of diarrhea in children [24]. The beneficial effects of certain probiotic strains on preventing rotavirus-caused diarrhea have been studied. In one study, human infants aged 5 to 24 months who were admitted to a chronic medical care hospital were randomized to receive a standard infant formula or the same formula supplemented with *Bifidobacterium bifidum* (1.9×10⁶ CFU/g) and *Streptococcus thermophilus* (1.4×10⁹ CFU/g) for 14 days. The results demonstrated significantly lower numbers of children experiencing diarrhea who received the supplemented formula compared to un-supplemented control groups [25]. Other beneficial effects recorded from clinical studies include shortening the duration of diarrhea by as much as 1.5 days and less shedding of rotavirus [26,27]. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Pediatric Infectious Diseases recommend that only probiotic strains with proven clinical efficacy and in appropriate dosages be used as an adjunct to rehydration therapy for the management of children with acute diarrhea [28]. Proven clinical efficacy was determined from formal systematic reviews of the literature; treatments were highly recommended if there was strong evidence from more than one systematic review of well-designed randomized control trials that a probiotic was more effective than a placebo [28]. It was determined that probiotics *L. reuteri* (ATCC 55730), *L. acidophilus LB*, *S. boulardii* and a mixture of *S. thermophilus*, *L. acidophilus* and *L. bulgaricus* significantly reduced the duration of diarrhea when compared with placebo, particularly in rotavirus gastroenteritis [28]. Effects of probiotics were also determined to be dose dependent dose-dependent, that is the effects were greater for doses higher than 10⁹ to 10¹⁰ colony-forming units [28]. The World Gastroenterology Organization (WGO) included *L. casei* DN-114 001 in their evidence-based recommendation and pointed out that timing of administration (at the very first sign of illness) is considered highly important [29].

**Antibiotic associated diarrhea:** Many patients taking antibiotics that disturb the gastrointestinal flora experience diarrhea as a side effect [30,31]. Symptoms range from mild and self-limiting to severe, and this antibiotic-associated diarrhea (AAD) is an important reason that many patients refuse to take antibiotics or curtail their treatment regimen [32]. Many studies indicate the ingestion of certain probiotic strains, primarily *Lactobacilli* either alone or in combination with other genera, before and during antibiotic treatment reduces the frequency, duration and severity of antibiotic induced diarrhea [33].

**Lowering of cholesterol**

There have been several mechanisms proposed for the purported cholesterol lowering effects of probiotics, including deconjugation of bile acids by bile-salt hydrolase enzymes of probiotics [34], assimilation of cholesterol by probiotics [35], co-precipitation of cholesterol with deconjugated bile [36], cholesterol binding to cell walls of probiotics [37], incorporation of cholesterol into the cellular membranes of probiotics during growth [38], conversion of cholesterol into coprostanol [39] and production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics [40].

Numerous studies on the effects of probiotics on cholesterol have used animals as models. Shinnick et al. [41] studied the effect of processing on the ability of oat fiber to lower plasma and liver cholesterol concentrations in rats. Rats were fed diets containing 6% dietary fiber as cellulose, oat bran, high fiber oat flour or one of four processed high fiber oat flours for 3 wk; all diets also contained 1.0% cholesterol and 0.2% cholic acid. All of the oat products significantly lowered plasma and liver cholesterol without depressing food intake or weight gain. A study was conducted to determine the effect of glucomannan, chitosan, or an equal mixture of the two on cholesterol absorption and fat and bile acid excretion in rats [42]. Total liver cholesterol was significantly reduced in all groups compared with the control group. Cholesterol absorption was significantly reduced from 37.5% in the control group to 20.2% in the glucomannan group, 18.2% in the combination and 9.4% in chitosan. Mice deficient in the LDL receptor have also been widely used as a model to mimic human atherosclerosis by feeding them Western diets with or without insulin-sensitizing agents; however, the time-course of atherosclerotic lesion development and distribution of lesions at specific time-points are yet to be established [43]. Lin et al. [44] conducted a study aimed to investigate whether the combination of plant sterol esters with soy protein or soy isolavones would have extra cholesterol-lowering effects. Hamsters were fed diets containing casesin (control), plant sterol esters, intact soy protein, soy isolavones, plant sterol esters plus soy protein, or plant sterol esters plus soy isolavones for 5 wk. All diets contained 0.08 g cholesterol/100 g diet. The plant sterol esters and the soy protein diets significantly lowered the plasma total cholesterol concentration by 13% and 9%, respectively as compared to the control, whereas the isolavone diet had no effect. The combination of plant sterol esters and soy protein decreased plasma total cholesterol by 26%. In conclusion, the combination of PSE and soy protein more dramatically lowers plasma lipids than the individual ingredients [44]. Guinea pigs [45] and pigs [46] have also been used as models for humans in studies of cholesterol lowering drugs. Because these animals share some attributes with humans in terms of cholesterol and bile acid metabolism, plasma lipoprotein distribution, and regulation of liver cholesterol enzymes [47] and digestive anatomy and physiology [46] they are useful experimental models for research. Therefore the cholesterol lowering effects seen in these animal studies suggest a similar potential in humans.

Although numerous studies have demonstrated convincing cholesterol-lowering effects of probiotics in both animals and humans, controversial results have surfaced. Hatakka et al. [48] reported that the administration of *L. rhamnosus* LC705 (10⁸ CFU/g per capsule; two capsules daily) for 4 weeks did not influence blood lipid profiles in thirty-eight men with mean cholesterol levels of 6.2 mmol/L (240 mg/ dl). In another study involving forty-six volunteers (aged 30 to 75 years old), Simons et al. [49] found that the consumption of *Lactobacillus fermentum*, (2×10⁹ CFU per capsule; four capsules daily) did not result in any lipid profile changes after 10 weeks. Lewis and Burmeister [50] conducted a randomized, placebo-controlled double blind and crossover designed study to determine the effect of *Lactobacillus acidophilus* on human lipid profiles. In the study, eighty volunteers 20 to 65 years of age with a baseline total cholesterol of more than 5.0 mmol/L (greater than 193 mg/dL) and a mean Body Mass Index of 27.8 kg/m² consumed two capsules containing freeze-dried *L. acidophilus* (3×10⁸ CFU/2 capsules) three times daily for six weeks, and crossed over for another six weeks after a 6-week washout period. The authors
found that *L. acidophilus* capsules did not significantly change plasma total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides of the subjects.

Even *in vivo* trials using human subjects are also affected by external factors such as experimental design, lack of adequate sample size for statistical purposes, failure to control for other nutrient intake and energy expenditure during the experiments, and different strains and dosages of probiotics [51,52]. There is a need for large-scale clinical trials with controlled dosing, determination of viability, definition of strains and other critical variables to provide the necessary scientific evidence required to determine efficacy of the ever-increasing use of probiotics.

**Cancer Prevention**

Yogurt and other fermented milk products have been promoted as cancer preventing, especially for colon cancer [53]. Epidemiological studies suggest that colon cancer is associated with a high-fat diet because of the large international variations in rate are strongly correlated with apparent per capita consumption; the high fat diet potentially stimulates bile acid turnover and can lead to an increase of bile acids in the colon which affects the metabolism of the bacterial flora [54]. The bile acids sodium cholate and sodium chenodeoxycholate were studied as promoters of colon carcinomas by comparison of conventional rats to germ free rats and these researchers concluded that indigenous microflora in the intestine could produce enzymes such as glucuronidase, nitroreductase and azoreductase which can convert pro-carcinogens into carcinogens [54]. This has prompted research on the use of probiotic bacteria to reduce the risk of colon cancer. However, the factors involved in the initiation and promotion of colon cancer are separated in time from actual tumor development, making it difficult to find definitive indicators of usefulness of probiotics. Brady et al. [55] analyzed animal studies over a 10 year period that looked most directly at the relationship between probiotic consumption and colon cancer development and found that apparently probiotics do have an inhibitory effect on the development of precancerous lesions and tumors in animal models. Several mechanisms have been proposed for the anticancer effects of probiotics including preventing the growth of bacteria that produce enzymes responsible for converting pro-carcinogens into carcinogens [56,57], inhibition of tumor growth by metabolites of the *Lactobacilli* stimulating the immune system [58], and production of tumor necrosis factor by macrophages [59]. Goldin and Gorbach [56] recruited 21 people for a study designed to investigate the effect of oral supplements of *Lactobacillus acidophilus* on the fecal bacterial enzymes beta-glucuronidase, nitroreductase, and azoreductase, which can catalyze procarcinogen conversion to carcinogen. The sequence of feeding studies and fecal enzyme assays was the same for all subjects and were highly statistically significant, but were observed only during the period of *Lactobacilli* feeding. During the final control period, after *Lactobacilli* feeding, fecal enzyme levels returned to normal after 4 wk. Fujiwara et al. [57] investigated the fate of *L. gasseri* SBT2055 (LG2055SR) and the influence of its oral administration on the composition and metabolism of the intestinal microflora. They confirmed that *L. gasseri* became established in the human intestine fecal and noted that populations of *Staphylococcus* and fecal contents of p-cresol were reduced [57]. A double-blind trial was conducted in 138 human patients with carcinoma of the bladder after surgery to evaluate the prophylaxis of recurrence by an oral *L. casei* preparation, wherein the *L. casei* preparation showed a better prophylactic effect than placebo [58]. Roller et al. [59] fed probiotics (*L. rhamnosus* GG and *Bifidobacterium lactis* Bb12), prebiotics (inulin enriched with oligofructose), and synbiotics (combination of probiotic and prebiotic) to rats for 4 wk as supplements to a high fat diet. Functions of immune cells isolated from peripheral blood mononuclear cells, spleen, mesenterial lymph nodes and Peyer’s patches were investigated. The synbiotic supplement increased secretory immunoglobulin A production in the ileum compared with controls fed the high fat diet alone, and decreased the oxidative burst activity of blood neutrophils compared with rats fed probiotics alone. The prebiotic supplement enhanced the production of interleukin-10 in Peyer’s patches as well as the production of IgA in the cecum, compared with controls [59].

For probiotics to be effective they must be alive in the food or supplement as well as being able to survive the harsh conditions of the GI tract while still maintaining activity [60]. A multitude of factors affect viability and survivability including the strain of bacterium, pH of the food or supplement, nutrients in the food or supplement and heat treatment [61-66]. A myriad of methods have been developed to increase survivability and viability of the probiotics ranging from pre-exposure to sublethal stresses, use of immobilized cell technology, microencapsulation, genetic modification, combining different synergistic strains, to incorporation of nutrients and prebiotics to the matrix [64,67-70].

**Prebiotics**

Prebiotics are employed to promote both beneficial bacteria which are already established in the colon as well as externally administered probiotic bacteria. The original definition of prebiotics was that they are food ingredients which are indigestible in the upper GI tract and reach the colon to beneficially influence the host by selectively promoting the growth and/or activity of certain bacteria in the colon [3]. Given the large number of bacterial strains present in the GI tract, some of which are non-cultivable, the definition was revised to “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health” [71,72]. Consumers often take in moderate levels of prebiotics naturally from many fruits and vegetables including leeks, Jerusalem artichokes, chicory, onion, garlic, banana, and asparagus, but the levels of prebiotics in these food sources are generally too low to exhibit any significant effect on the composition of intestinal microflora [73]. Thus, prebiotics are commercially extracted and concentrated from fruits and vegetables through the hydrolysis of polysaccharides from dietary fibers or starch, or through enzymatic generation. Prebiotics are mixtures of indigestible oligosaccharides, except for inulin which is a mixture of fructooligo- and polysaccharides [73,74]. All currently known prebiotics are carbohydrates, and there are many different carbohydrates marketed world-wide as prebiotics, the only four that are well supported by good quality data from human trials are the fructans (inulin and fructo-oligosaccharides), galacto-oligosaccharides, and the synthetic disaccharide, lactulose.

**Fructans**

A fructan is composed of fructose polymers which are generally linked to the moiety of a terminal glucose [75]. Fructans are resistant to enzymes in the digestive tract of humans and pass through the upper portion of the human GI tract, reach the colon and are fermented by colonic microflora producing SCFA [76].
Inulin: Inulin is a naturally occurring storage carbohydrate commonly found in leeks, onions, wheat, asparagus, garlic, Jerusalem artichoke and chicory [77,78]. Most of the inulin commercially produced is from Jerusalem artichokes and chicory [79]. The effect of daily intake of 10 g inulin on fasting blood lipid, glucose and insulin levels in healthy middle-aged men and women with moderately raised total plasma cholesterol and triacylglycerol levels was studied in a double-blind randomized placebo-controlled parallel study in which subjects received either inulin or placebo for a period of 8 weeks [78]. Fasting blood samples were collected before the supplementation period and at weeks 4 and 8, with a follow-up at week 12. Compared with baseline values, insulin concentrations were significantly lower at 4 weeks in the inulin group, and there was a trend for triacylglycerol values to be lower in the inulin group at 8 weeks returning to baseline concentrations at week 12 [78]. Nair et al. [80] reviewed the literature on functional aspects of inulin and reported that several animal and human studies have shown inulin to promote optimal digestive health, positively influence lipid metabolism, decrease the risk of osteoporosis by increasing calcium absorption, as well as reduce the risk of colon cancer, breast cancer, and tumor growth.

**Fructo-oligosaccharides**: Fructo-oligosaccharides (FOS) are composed of a mixture of oligosaccharides consisting of glucose linked to fructose units by β-(1,2) links with a degree of polymerization (DP) between 1 and 5. They occur naturally in several plants such as asparagus, wheat, Jerusalem artichokes, and rye. Onion is especially rich in FOS which ranges from 25 to 40% of dry matter [81]. The commercial production of FOS is mainly based on two processing methods which are either a continuous process using immobilized cells in calcium alginate gel or enzyme on an insoluble carrier or a batch conversion of sucrose by fungal fructosyltransferase [82]. In the human colon the FOS are completely fermented mostly to lactate, SCFA (acetate, propionate and butyrate), and gas [83]. The FOSs stimulate *Bifidobacterial* growth and suppress the growth of potentially harmful species in the colon [83]. Other effects of FOS include a decrease in fecal pH, an increase in fecal or colonic organic acids, a decrease in fecal bacterial enzymatic activities and a modification in fecal neutral sterols [83]. They have been demonstrated to enhance magnesium absorption in humans and, in animal models, have demonstrated reduction in colon tumor development due to enhancement of both colon butyrate concentrations and local immune system effectors [83].

**Galacto-oligosaccharides**

Galacto-oligosaccharides (GOS) is a collective term for a group of carbohydrates composed of oligo-galactose with some lactose and glucose, and which are produced commercially from lactose by β-galactosidase [84]. There are oligosaccharides that resemble GOS naturally found in human milk which may be one of the factors that protect breast fed infants from gastrointestinal pathogenic bacteria [85]. Several animal studies, mainly in rats, evaluated GOS that protect breast fed infants from gastrointestinal pathogenic bacteria [86]. The effect of feeding a diet containing GOS with or without *Bifidobacterium breve* (administered in the drinking water) was investigated in rats colonized with a human fecal microflora [86]. The GOS or GOS plus *B. breve*, given for 4 weeks, induced increases in fecal concentration of total anaerobic bacteria, *Lactobacilli* and *Bifidobacteria*, and decreases in numbers of enterobacteria; fecal pH was significantly reduced, as were the activities of beta-glucuronidase and nitrate reductase [86]. Rats inoculated with a human fecal flora and fed a diet containing GOS exhibited non-specific induction of beta-galactosidase, beta-glucoisidase and alpha-glucosidase activities and increased CH₄ excretion, a decrease in cecal pH and total SCFA concentration as compared to control diet [87]. Morishita et al. [88] studied the effect of 5% GOS on the population of *Clostridium perfringens* during the development of primary intestinal microflora in gnotobiotic mice. *B. breve* increased and *C. perfringens* decreased in the feces of the GOS-fed animals, but not in the controls [88]. There have also been several human trials of GOS with different methods and study designs which have generated rather mixed results. While several studies failed to show any significant changes in fecal microbiota of volunteers [89-91], others did show a response [92-94]. In one study exactly controlled experimental diets were supplied to 3 intervention groups in a parallel design of 2 consecutive 3-wk periods during which each participant consumed a run-in diet followed by an intervention diet that differed only in the amount of GOS: 0 (placebo), 7.5, and 15 g/d; breath samples and fecal samples were collected at the end of both the run-in and intervention periods [89]. The number of *Bifidobacteria* increased after both placebo and GOS ingestion, but the differences between the increases were not significantly different. Additionally, GOS did not significantly affect bowel habits, stool composition, the concentration of short-chain fatty acids or bile acids in feces, the concentration of ammonia, indoles, or skatoles in feces, fecal pH or the composition of the intestinal microflora [89]. A feeding trial was conducted on healthy human volunteers to study the effect of GOS-containing syrup (60% GOS) alone or combined with the probiotic strain *B. lactis* Bb-12 on selected components of the fecal flora, and the effect of GOS supplementation on colonization of *B. lactis* Bb-12 [90]. Mean numbers of *Bifidobacteria* increased slightly in all study groups during the feeding period and there was good survival of *B. lactis* Bb-12 through the gastrointestinal tract, but no differences in the prevalence or numbers of isolates with *B. lactis* Bb-12 genotype could be observed between groups suggesting that GOS-containing syrup did not enhance the survival or persistence of *B. lactis* Bb-12 in the gut [90]. A culture-independent approach based on genus-specific PCR and denaturing gradient gel electrophoresis (DGGE) was used to monitor qualitative changes in fecal *Bifidobacterial* communities in the same type of human feeding trial [91]. The DGGE profiles revealed that administration for two weeks of GOS and/or *B. lactis* Bb-12 (8 g and 3×10⁸ CFU/day, respectively) did not affect the qualitative composition of the indigenous *Bifidobacterium* population, while *B. lactis* Bb-12 transiently colonized the gut [91]. Gopal et al. [92] studied the effects of GOS and *B. lactis* HN019 on the microbial composition of the gastrointestinal tract of human subjects using a randomized, double blind, placebo-controlled study design. Volunteers either consumed a reconstituted milk containing 2.4 g of GOS/day, reconstituted milk containing 3×10⁹ CFU of *B. lactis* HN019/ day, or reconstituted milk without additions. Subjects receiving either GOS or probiotic bacteria had a significant increase in the fecal counts of both *Lactobacilli* and *Bifidobacteria* [92]. Other studies using human volunteers have also found GOS to be bifidogenic [93,94].

Due to the stability of GOS at high temperature and in acidic environments, they are used in many food applications such as infant formulas, dairy products, sauces, soups, breakfast cereals, snack bars, ice creams, beverages, bakery products, animal feeds, and as sugar replacements to increase texture and mouthfeel of foods, and act as bulking agents [95]. Also, the mixture of 90% GOS and 10% inulin are used in infant formulas to simulate human milk [96,97].

**Lactulose**

Lactulose is a synthetic disaccharide which was used originally as a laxative [98]. Lactulose has also been shown to increase *Lactobacilli*...
and *Bifidobacteria* and significantly decrease *Bacteroides* in mixed continuous fecal culture [99]. In some clinical trials lactulose has been demonstrated to have potential for use as a prebiotic. Terada et al. [100] studied the effect of dietary supplementation with lactulose (3 g/d for 2 wk) on the fecal flora and fecal bacterial metabolism in healthy human volunteers. During the intake of lactulose, the number of *Bifidobacteria* increased significantly, whereas the numbers of *Clostridium perfringens* and *Bacteroides* decreased; fecal indole, skatol and phenols, and fecal P-glucuronidase, nitroreductase and azoreductase activities were also decreased significantly [100]. In another study involving human volunteers, lactulose (2×10 g/d) increased probiotic bacteria and decreased putrefactive bacteria and potential pathogens [101]. In another human feeding trial a dose of 10 g per day, half the pharmacological dose, was fed to healthy adult volunteers and *Bifidobacteria* showed a statistically significant increase and a simultaneous decrease in *Clostridia* as compared to the control group with placebo [102]. A single administration of lactulose significantly decreased urinary nitrogen-excretion in a dose-dependent way in healthy volunteers, and long-term administration of lactulose produced a significant reduction of the urinary nitrogen-excretion accompanied by a significant increase in the fecal nitrogen-output; a significant rise in the *Bifidobacterium* population was also observed [103]. In spite of these promising results lactulose is not yet widely used as a prebiotic, although it remains an established product in the medical market.

**Emerging and Novel Prebiotics**

Several other oligosaccharides have been proposed as prebiotics that do not have sufficient data from human studies to support the claim. There are other carbohydrates also currently being studied for their prebiotic potential that have data mostly from *in vitro* studies or animal studies but do not yet have human data to support them.

**Xylo-oligosaccharides**

Xylo-oligosaccharides (XOS) are chains of xylose molecules linked by β1–4 bonds [104,105] which are produced enzymatically by hydrolysis of xylan from birch wood, oats, or corn cobs [105]. They have been studied using *in vitro* batch culture systems where they have proven to be very selective for *Bifidobacteria* [106,107], and the prebiotic potential has also been confirmed in animal studies [108-110]. Lecerf et al. [111] performed a randomized, parallel, placebo-controlled, double-blind study of XOS in healthy humans and found that it increased the fecal concentrations of *Bifidobacterium* and butyrate and activities of α-glucosidase and β-glucuronidase, while decreasing the concentrations of acetate and p-cresol, showing a clear prebiotic effect.

**Resistant starch**

Resistant starch is the term used for the fraction of starch that escapes digestion in the upper GI tract and that reaches the colon to be fermented by the colonic microbiota [112]. A few animal studies on the probiotic properties of resistant starch have been carried out and in general show an increase in *Bifidobacteria* and SCFA in rats [113-115], human-flora-associated rats [116], and pigs [117]. The effect of prolonged feeding of resistant potato starch on butyrate production in rats was studied and it was determined that butyrate production was promoted by long-term ingestion from the cecum towards the distal colon, suggesting a slow adaptive process within the digestive tract. Another rat study resistant starch increased the rate of fermentation accompanied by a decrease of pH in cecum, colon, and feces; also bile acids became bound to the resistant starch and thus were not reabsorbed, resulting in a higher turnover through the large bowel [114]. Silvi et al. [116] studied the effect of resistant starch on human gut microflora and associated parameters in human flora-associated (HFA) rats, colonized with microflora populations from UK or Italian subjects. In both the UK and Italian flora-associated rats, numbers of *Lactobacilli* and *Bifidobacteria* were increased 10 to 100-fold, and there was a concomitant decrease in enterobacteria [116].

**Pectic oligosaccharides**

Pectin is a complex galacturonic acid-rich polysaccharide which occurs naturally in the cell walls of higher plants and acts as a cement-like material for the cellulosic components of the plant cell wall [118]. The major polysaccharides forming pectin include homogalacturonan (HGA), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II) which are believed to covalently link together [118].

Enzymatic or physical methods can be used to manufacture pectic oligosaccharides. Enzymatic hydrolysis of citrus and apple pectins in membrane reactors produces oligosaccharides of 3-4 kDa molecular weight [119]. A nitric acid hydrolysis of citrus peel produces low molecular weight arabino- or galacto-oligosaccharides [120]. Both of these materials have been evaluated in fecal batch cultures and have both been shown to promote growth of *Bifidobacteria* [121,122].

**Conclusions**

There is adequate evidence of benefit of probiotics in specific circumstances, such as acute diarrhea of viral or bacterial origin and AAD. When using or prescribing probiotics, consideration must be given to the probiotic formulation, including live, dead, compounded preparations or their products, the effective dose to use and the type of disease targeted. It is not possible to extrapolate specific actions or doses of a given probiotic and generalize these properties to other dosages or strains of probiotic bacteria. The United States Food and Drug Administration does not currently regulate probiotic products, providing no government oversight for quality control, and the actual number of viable organisms in commercial products may be quite different from what is being advertised. In summary, future large-scale clinical trials controlling dosing, viability and other critical variables will be crucial to provide the necessary scientific evidence required to determine efficacy of the ever-increasing use of probiotics.

Oligosaccharides are gaining increasing recognition as prebiotics capable of modulating the colonic microbiota in humans and animals. The currently defined prebiotics have a wide range of degrees of robustness of data to support their prebiotic status, and there is a need for more large scale, well designed human trials with clearly defined outcomes using modern molecular microbiological methods. There is much data that supports the role of prebiotics in dietary intervention in several disease conditions, including colon cancer, acute infections, and inflammatory bowel disease.

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