Probiotics: determinants of survival and growth in the gut[1–3]

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ABSTRACT Bifidobacteria and lactobacilli are purportedly beneficial to human health and are called probiotics. Their survival during passage through the human gut, when administered in fermented milk products, has been investigated intensely in recent years. Well-controlled, small-scale studies on diarrhea in both adults and infants have shown that probiotics are beneficial and that they survive in sufficient numbers to affect gut microbial metabolism. Survival rates have been estimated at 20–40% for selected strains, the main obstacles to survival being gastric acidity and the action of bile salts. Although it is believed that the maximum probiotic effect can be achieved if the organisms adhere to intestinal mucosal cells, there is no evidence that exogenously administered probiotics do adhere to the mucosal cells. Instead, they seem to pass into the feces without having adhered or multiplied. Thus, to obtain a continuous exogenous probiotic effect, the probiotic culture must be ingested continually. Certain exogenously administered substances enhance the action of both exogenous and endogenous probiotics. Human milk contains many substances that stimulate the growth of bifidobacteria in vitro and also in the small intestine of infants; however, it is unlikely that they function in the colon. However, lactulose and certain fructose-containing compounds, called prebiotics, are not digested in the small intestine but pass into the cecum unchanged, where they are selectively utilized by probiotics. Beneficial effects may thus accrue from exogenously administered probiotics, often administered with prebiotics, or by endogenous bifidobacteria and lactobacilli, whose metabolic activity and growth may also be enhanced by the administration of prebiotics. *Am J Clin Nutr* 2001;73(suppl):399S–405S.

KEY WORDS Probiotics, prebiotics, bifidobacteria, lactobacilli, intestinal tract, diarrhea

INTRODUCTION

Although there are numerous publications purporting that probiotics are active in the gut after ingestion, others have questioned such claims and the beneficial effects that probiotics are said to confer on their hosts. "There is little evidence that they [probiotics] divide or carry out any metabolic activity on their way through. Thus, the notion that they would have any effect on the host in the presence of a finely tuned ecosystem consisting of hundreds, well-adapted species seems irrational," stated Wilson (1) in regard to the colon. Reflecting such doubts, O'Sullivan et al (2), in a review article titled "Probiotic bacteria: myth or reality?" stated "Although there are numerous probiotic products on the market, there is a lot of skepticism regarding their beneficial effects." The colon is certainly a host to a stable and "finely tuned" ecosystem, consisting of some 1×10^{11}–10^{12} microorganisms (3). The ecosystem of the small intestine is, however, less stable and "more susceptible to modifications than that of the colon" (4). It would then follow that exogenously administered probiotics would have no difficulty influencing small intestinal microflora in a meaningful way. Thus, it is the colon that remains, so to speak, the bone of contention.

The aim of this article is to provide a brief overview of the evidence in support of the hypothesis that exogenously administered probiotics (mostly lactobacilli and bifidobacteria) may survive their passage through the stomach and the small intestine and affect bacterial ecology and metabolism in the colon. An important aspect of this issue is to examine the various determinants and factors that allow for probiotic passage through the gut and enhancement of their metabolic activity. In this context, the effect of a group of nondigestible fructose-containing compounds, which enhance the metabolic activity of endogenous colonic probiotics (mostly bifidobacteria) to give results similar to those of administered probiotics, is also reviewed. We begin with the various types of diarrhea, originating in both the large and small intestines, where the beneficial effects of probiotics have been best documented. The beneficial effects of probiotics in such cases bear witness to their survival in the gut and to their ability to influence the nature of intestinal ecosystems.

PREVENTION AND TREATMENT OF DIARRHEA IN INFANTS AND ADULTS

Diarrhea is caused by pathogenic bacterial or viral overgrowth in either the small or large intestine. For example, *Clostridium difficile* induces diarrhea in adults and rotavirus induces diarrhea in children. There are several mechanisms through which these agents cause diarrhea, but the end result in all cases is the accumulation and then expulsion of fluid from the intestinal tract, resulting in loss of body fluid and electrolytes. Some potential causes of diarrhea involving the ecosystems of both the small and large intestines, mechanisms of pathogenic action, and effective

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TABLE 1
Potential causes, mechanisms of pathogenic action, and effective probiotic treatments of diarrhea[1]

Causative microorganism	Site of microbial overgrowth and effect	Mechanism of pathogenic action	Effective probiotic and reference
Rotavirus	Small intestine	Destruction of villus cells	*Bifidobacterium bifidum* and *Streptococcus thermophilus* (5) *Lactobacillus* GG (6)
Clostridium difficile	Colon	Enteropathogens and cytotoxins	*L.* GG (7–9) *B. bifidum* (10)[2]
Escherichia coli (travelers' diarrhea)[3]	Small intestine	Attachment and enterotoxins	*L.* GG (11)
Salmonella spp.	Small intestine	Invasion	*L.* GG (12)
Shigella spp.	Small intestine and colon	Invasion and toxins	*L.* GG (12)

[1] Unless otherwise stated, the subjects were humans.
[2] In gnotobiotic mice.
[3] Traveler's diarrhea usually caused by *Escherichia coli*.

probiotic treatments are listed in **Table 1**. Probiotics have also proved to be effective therapeutic agents in cases in which the exact cause of the diarrhea was not identified. Thus, *Lactobacillus* GG, administered in yogurt, was quite effective in controlling erythromycin-induced diarrhea (13).

Some related, though nondiarrheal, situations involving the effects of probiotics on bacterial overgrowth are also noteworthy. In patients with chronic kidney failure, there is often a bacterial overgrowth in the small intestine, resulting in high blood dimethylamine and nitrosodimethylamine concentrations. These toxic compounds were significantly lower in patients treated with 2 strains of *Lactobacillus acidophilus*, resulting in a significantly better quality of life for these patients (14). Of public health importance, *Campylobacter jejuni* shedding in broiler chicks was all but eliminated by the administration of *L. acidophilus* (15). *C. jejuni* is often the cause of food poisoning in humans.

Note that few authors of studies of the clinical effects of probiotics speculated about the mechanisms that might explain these observations (16). For instance, the mechanism by which the duration of rotavirus-induced diarrhea is reduced by *L.* GG may be the elicitation of a local immune response (6). The beneficial clinical outcomes observed in the above-mentioned, well-controlled studies indicate that the probiotic doses and regimens that were used strongly influenced the behaviors of the ecosystems of both the large and small intestines.

DOES THE ADMINISTRATION OF PROBIOTICS ALTER THE COMPOSITION AND METABOLISM OF THE INTESTINAL MICROFLORA?

The intestinal microflora within a given individual are remarkably stable, although major differences may exist among different persons (17–19). Nevertheless, administration of probiotics to either newborns or adults results in certain changes in the microbial profiles and metabolic activities of the feces. Admittedly, such changes are minor; yet, when applied to pathologic situations, they are often sufficient to beneficially alter the course of disease. In most situations, probiotic administration results in an increase in fecal counts of bifidobacteria and lactobacilli, a decrease in fecal pH, and a decline in those bacterial enzyme activities that are associated with the development of colon cancer.

In newborns, the colonic microflora can be modified by including probiotics in feeding formulas. Because a largely bifidobacterial flora were observed in breast-fed infants, who show a greater resistance to various infectious diseases than do bottle-fed infants (20), the desire arose to generate a predominantly bifidobacterial flora in bottle-fed infants. In a 7-d trial, the stool of infants fed an artificial formula containing an inoculum of *Bifidobacterium bifidum* was compared with that of bottle-fed infants who were fed an artificial formula with no added bifidobacteria, and breast-fed infants. The breast-fed and *B. bifidum*–fed infants had bifidobacteria in their stools, whereas bottle-fed infants did not. The fecal pH of both the breast-fed and the *B. bifidum*–fed infants was nearly identical (5.30 and 5.38, respectively), whereas the pH of the bottle-fed infants was 6.83 (21). In a 2-mo, well-controlled study in which *B. bifidum* was also incorporated into an artificial formula, the fecal pH was the same in both breast-fed and *B. bifidum*–fed infants, whereas it was significantly higher in control infants fed an artificial formula to which no bifidobacteria had been added (22). One month into the study, colonic colonization by bifidobacteria was significantly higher in the *B. bifidum*–fed infants than in the control infants, but not significantly different from that of the breast-fed infants.

A similar scenario was evident in adults. In volunteers with a median age of 31.5 y, *Bifidobacterium longum* administration (as a pharmaceutical) resulted in higher fecal bifidobacterial and lower clostridial counts, lower fecal pH, and lower fecal ammonia concentrations (23). In another study, in 64 females with a mean age of 24 y, *L.* GG administration resulted in *L.* GG recovery in the feces and a decline in fecal β-glucuronidase, nitroreductase, and glycocholic acid hydrolase activities. Urinary excretion of *p*-cresol, a product of colonic *Bacteroides fragilis*, also decreased. The fecal enzyme activities remained low as long as the probiotic was being administered (4 wk) and returned to reference concentrations when administration of the probiotic was discontinued (24). The decrease in fecal β-glucuronidase and azoreductase activities was observed by others after administration of *L. acidophilus* (25). Similar results were obtained in mice fed *L. acidophilus* and whose intestinal microflora had previously not contained any lactobacilli (18).

The metabolic viability of administered probiotics in the intestinal tract was evaluated in adult volunteers by measuring the amount of exhaled hydrogen. As expected, *B. longum*–fed individuals exhaled more hydrogen than did placebo-fed subjects (19). That endogenous bifidobacteria are the major actors in colonic bacterial metabolism in breast-fed infants was tested by incubating their feces with 3-[^{13}C]glucose. Bifidobacteria, but not other bacteria, generate $^{13}CH_3{}^{13}COOH$ via their bifidus pathway. As

expected, most if not all the acetate produced was of this type (26); this promising technique has not been used in vivo.

FACTORS THAT AFFECT THE SURVIVAL OF INGESTED PROBIOTICS IN THE GASTROINTESTINAL TRACT

Having established that some ingested probiotics can affect the composition and behavior of intestinal microflora, it is of interest to explore some factors that determine the survival of probiotics while in transit. Such studies have been performed in vivo and in vitro. In one such study, 2 strains of *Bifidobacterium* (species not identified) were exposed to stomach-like acidity for 90 min. In one strain, growth was inhibited by only 0.5 log units, whereas growth declined by 4 log units in the other strain. Similar differences were observed in vivo in intubated human subjects after these 2 strains were administered in fermented milk (27). In another study, it was shown in vitro that the viability of an unspecified bifidobacterial species remained unchanged at a pH of 3 for 180 min, declined slowly at a pH of 2, and was zero after 60 min at a pH of 1 (28). One of the more extensive in vitro studies used 6 *L. acidophilus* and 9 *Bifidobacterium* strains, which were maintained at a pH of 1.5–3.0 for ≤3 h. Viability, as expected, depended on the pH, the length of the exposure to acid, and the species and strains used. The hardiest organisms were *L. acidophilus* strains 2401, 2409, and 2415; *B. longum* strain 1941; and *Bifidobacterium pseudolongum* strain 20099 (29).

In the small intestine, the most serious obstacle to probiotic survival is bile salts. In vitro studies of the resistance of probiotics to bile salts can be divided into 2 types: survival and growth studies. The former is exemplified by a study in which *Lactobacillus* and *Bifidobacterium* strains were maintained at bile concentrations of 0–1.5% for ≤3 h. The bacterial suspensions were then plated and the colonies were counted. Survival varied among the various strains and depended on bile concentration and exposure times. Among the bifidobacteria, *B. longum* 1941, *Bifidobacterium infantis* 1912, and *B. pseudolongum* 20099 were the hardiest, whereas strains 2404 and 2415 were the hardiest of the *L. acidophilus* strains (29). Shorter incubation times (40 min) were used in another study: little if any lysis was observed among *L. acidophilus* strains in the presence of 0.3% oxgall; however, leakage was observed because the β-galactosidase activity of the cells increased (30).

Growth experiments in the presence of bile salts are associated with another variable: the appearance of unconjugated bile acids in the medium. Deconjugation of bile salts is carried out by bile salt hydrolases, which are present in both lactobacilli and bifidobacteria. Unconjugated bile acids are better bacterial lysing agents than are conjugated bile acids. *L. acidophilus* strains 2405 and 2401 were the most resistant to 0.3% oxgall in these experiments. Of the bifidobacteria, *B. infantis* 1912 and *Bifidobacterium adolescentis* 1920 were most resistant. Maximum deconjugation of taurocholic acid by both bifidobacteria and lactobacilli was observed after 12–14 h of growth, but there was no apparent correlation between the extent of deconjugation and growth inhibition (31). Others also observed growth differences among *L. acidophilus* strains in the presence of 0.3% oxgall, but no attempt was made to correlate this with respective bile salt deconjugation activities (30). In a study designed to simulate passage of probiotics through the small intestine into the colon, several American Type Culture Collection species were grown for 24 h in the presence of 0.6–3.0 g glycocholic acid/L and then were transferred twice to fresh media containing no bile salts. Growth resumed to maximal extent after the second transfer, and normal bifidobacterial enzyme profiles were recovered (32). Growth inhibition of several murine *L. acidophilus* strains by taurocholic acid was correlated with their bile salt hydrolase activities (33).

The in vitro experiments described above showed that many variables can determine the degree to which probiotics survive passage through the upper gastrointestinal tract: the degree of stomach acidity, the length of exposure to acid, the concentration of and length of exposure to bile salts, the level of bile salt hydrolase activity, and other as yet unspecified properties of the probiotics themselves. Nevertheless, many probiotic strains can withstand the rigors of passage through the upper gastrointestinal tract and enter the colon in a viable state in sufficient amounts to affect its microecology and its metabolism.

Several studies endeavored to quantitate the degree of probiotic survival during passage through the gastrointestinal tract. Experiments involving human intubation and sampling of bifidobacteria (strains unspecified) from the cecum showed that these probiotics, when given in fermented milk, survive to the extent of 23.5% ± 10.4% of the administered dose (28). With the use of known probiotic species and strains, it was determined that the delivery of *B. bifidum* and *L. acidophilus* to the cecum was ≈30% and 10% of the administered dose, respectively (34). This same group reported on the construction of an artificial model for the human gastrointestinal tract, with the various compartments containing fluids found in vivo. It was determined that the organisms most resistant to stomach acid were *B. bifidum* and *Lactobacillus bulgaricus*, with a half-life of ≈140 min. *Streptococcus thermophilum* and *L. acidophilus* had a half-life of ≈40 min. The pH of the stomach compartment varied from 5.0 at ingestion to 1.8 at 80 min after ingestion. The half-life for gastric emptying was 70 min and for ileal emptying was 160 min. Delivery of *B. bifidum* and *L. acidophilus* to the cecum was ≈20% and 10%, respectively, in the presence of physiologic bile salt concentrations and 50% and 30%, respectively, at low bile salt concentrations. These values were comparable with those observed in vivo.

THE ISSUE OF COLONIZATION

It is generally agreed that to permanently establish a bacterial strain in the host's intestine, the organism must be able to attach to intestinal mucosal cells (2). Moreover, many pathogens cannot exert their deleterious effects on the gut unless they become so attached (35) and the beneficial action of probiotics has been explained by their purported ability to interfere with the adherence of pathogens to intestinal mucosal cells (36). However, do probiotics themselves attach to intestinal cells and thus proceed to colonize the gut? In vitro studies using tissue cultures suggest that the answer is yes and that probiotics do (37–39) interfere with the adherence of pathogens, such as *Salmonella typhimurium*, to Caco-2 cells (40). However, is this also true in vivo? Currently available evidence suggests that it is not. For instance, the recovery rate of an antibiotic-resistant strain of *Bifidobacterium* in the feces was determined after it was administered to human volunteers (41). The recovery rate was 29.7% ± 6.0% of the ingested dose, which is consistent with the percentage survival during probiotic passage through the gastrointestinal tract (*see* above). When administration of this strain was stopped, it was no longer recovered in the feces. The authors concluded that "administered *Bifidobacterium* sp. do not colonize the human colon." The same

results were obtained by Kullen et al (42), who fed a unique *Bifidobacterium* strain to human volunteers and then examined fecal bifidobacterial flora. While the feeding continued, total bifidobacterial excretion increased (including the administered strain) but this strain disappeared from the feces after the feeding was discontinued. The conclusions were that although bifidobacteria can survive the passage through the gastrointestinal tract, they do not colonize the gastrointestinal tract to a significant extent and that colonization may be unnecessary to achieve positive results in probiotic therapy. In support of this conclusion, Fujiwara et al (43) found that bifidobacteria produce a 100 000-kDa protein, which prevents the adhesion of pathogenic *Escherichia coli* to their normal receptors in the intestinal tract. Direct competition of the probiotic with *E. coli* for adhesion sites may thus not be necessary to achieve the desired results.

The highly effective probiotic *L.* GG is said to colonize the human intestinal tract (12). However, a review of the literature indicates that this notion is based on the ability of this probiotic to adhere to Caco-2 and other enteric cells in vitro (44). When *L.* GG in fermented milk was fed to volunteers, it appeared in their feces, but after its administration was stopped, it disappeared from the feces of 67% of the subjects within 7 d (45). The same was true in premature infants fed milk formulas containing *L.* GG (46). Thus, despite *L.* GG being of human origin, there is little if any evidence that it can permanently establish itself (ie, colonize) in the intestines of the general population.

Colonization studies in animals have been more revealing. Murine *Lactobacillus* ssp. were permanently reestablished in mice that had been freed of lactobacilli by antibiotic therapies and whose intestinal microflora were otherwise normal (47); various tissues of the gastrointestinal tract were cultured and lactobacilli were found in normal amounts (48). Germ-free mice are also susceptible to permanent colonization by *B. longum* (49). In farm animal production, it is desirable to identify those probiotic species and strains that can colonize the animals' intestinal tracts and provide health benefits (50). For this purpose, several adhering strains of *Lactobacillus* were isolated from different sections of the gastrointestinal tract of young pigs. These strains adhered to intestinal epithelial cells in tissue culture and were resistant to stomach acid and normal porcine feed (51, 52). Such species-specific probiotic strains that have adhesion capabilities are being strongly advocated for use in animal husbandry. One recent application of this principle has been in the area of poultry farming. Avian strains of *L. acidophilus* and *Streptococcus faecium* in combination with *Salmonella* antibodies were sprayed on hatched chicks and added to their drinking water, resulting in a marked reduction in *S. typhimurium* counts in their gastrointestinal tracts. Such sprays are now commercially available (53) and could improve food safety for the general population.

Should it become desirable to permanently colonize the human intestinal tract with an exogenous probiotic, it is reasonable to suggest that a human-specific probiotic with potent intestinal mucosal cell adhesion properties be chosen. Selection of such strains on the basis of this criterion may be insufficient. It may be necessary to culture surgical or biopsy specimens to select suitable probiotic strains. Until this is accomplished, we have to be content with recognizing that certain ingested probiotics do survive their passage through the gastrointestinal tract and that they "are excreted from the colon to the feces without overall multiplication or death" (41). Nevertheless, during such passage, these probiotics continue to be metabolically active, thus providing health benefits to their hosts.

FACTORS THAT AFFECT THE ACTIVITY OF ENDOGENOUS PROBIOTICS

Bifidobacteria and lactobacilii are normal components of the intestinal flora throughout the life cycle. The fecal microbial flora of breast-fed infants consist largely of bifidobacteria, whereas other organisms predominate in bottle-fed infants (20, 54, 55). The prevalence of bifidobacteria and the resultant decrease in fecal pH are associated with lower rates of morbidity and mortality in breast-fed infants (*see* 56 for a review), which has resulted, not without good reason, in the perception that a probiotic-rich intestine and low fecal pH are beneficial in adults as well (57–59).

In the search for reasons for the colonic differences between breast-fed and bottle-fed infants, investigators focused on differences in the compositions of cow milk and human milk. It was found that human milk has a higher lactose content, a lower buffering capacity, and lower protein, phosphate, and residue contents than does cow milk. Because of the lower buffering capacity of human milk, lactic and acetic acids produced by endogenous bifidobacteria could lower the pH of the colon contents to ≈5, thus preventing the growth of pathogens and many organisms normally found in adults and in bottle-fed infants (60). Other investigators suggest that human milk contains bifidus factors, which stimulates the growth of bifidobacteria. This notion originated from the work of Gyorgy (61), who showed in 1953 that the growth of *B. bifidum* var. *pennsylvanicus* was stimulated in vitro by human but not cow milk. The growth factors were apparently a group *N*-acetylglucosamine–containing compounds that were required for the construction of the bifidobacterial cell wall. For many years thereafter, *B. bifidum* var. *pennsylvanicus* was used as an indicator organism in bifidobacterial research, resulting in the isolation of numerous growth-promoting compounds (62). However, as new *Bifidobacterium* species were identified, it became clear that the growth requirements of *B. bftidum* var. *pennsylvanicus* were an exception rather than the rule, and although all *Bifidobacterium* species require complex biologic substances for growth in vitro, such growth factors were peptide- rather than carbohydrate-based and could be supplied by many biologic substances other than human milk. Even cow milk contained growth enhancers, such as κ-casein (enzymatic digest) (63, 64) and whey proteins (65). Some of these growth promoters were apparently cysteine-containing peptides (64, 66).

Are the various complex biologic materials (eg, κ-casein and cow milk whey proteins), which were shown to be good bifidobacterial growth promoters in vitro, useful in stimulating the growth and metabolic activity of endogenous or exogenously administered bifidobacteria and possibly lactobacilli? Perhaps this is true in the small intestine but most likely not in the colon because such materials would have been digested and absorbed in the small intestine and would not have reached the cecum. Nevertheless, it is possible that bifidobacterial growth-promoting factors may be generated endogenously through the intestinal exfoliation process and the availability of mucin. Pig gastric mucin is known to be an excellent bifidobacterial growth promoter (63).

Another component of human milk, which was advocated recently as a formula supplement for infants, is lactoferrin. Its concentration in human milk is manyfold greater than that of cow milk. It is an iron-binding protein similar to transferrin (67),

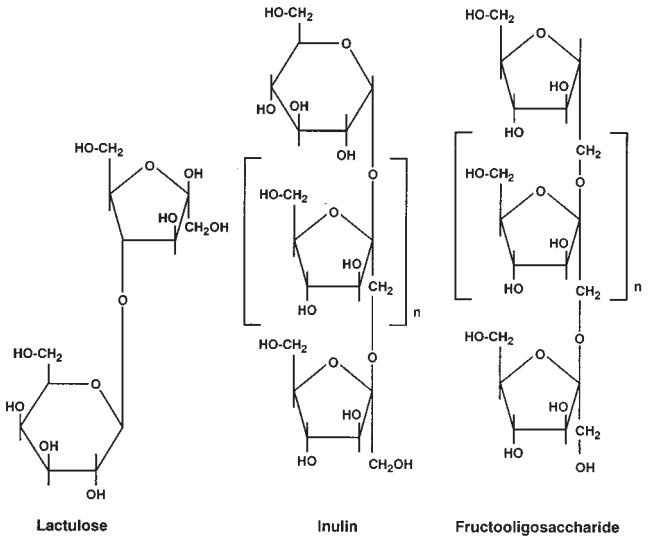

FIGURE 1. Chemical structures of the prebiotics lactulose, inulin, and fructooligosaccharide.

and its antimicrobial activity has been well documented also (68). Early work on the antimicrobial activity of lactoferrin was based on in vitro bacterial growth inhibition by iron-unsaturated lactoferrin, but later it became clear that peptides of lactoferrin were bactericidal without any relation with iron binding (69, 70). Lactoferrin is thus an antibacterial substance in its own right, but does it have anything to do with stimulating the growth of bifidobacteria? It may in the role of an iron provider because bifidobacteria do require iron for growth. *Bifidobacterium breve* was shown to obtain iron from iron-saturated lactoferrin or the C-terminal fragment of lactoferrin (71). Lactoferrin and its fragments do, to some extent, survive their passage through the gastrointestinal tracts of infants (72, 73). On the other hand, the iron required for bifidobacterial growth can also be supplied by free ferrous iron, which is likely to exist in the anaerobic environment of the colon (74). Lactoferrin is better known as a bacterial inhibitor than as a promoter, although the opposite is true for mammalian cells in culture (75). The most promising approach for enhancing the role of endogenous probiotic organisms in the gut is the use of prebiotics. Prebiotics are simple, naturally occurring or synthetic sugars that are normally indigestible in the human gut but that are used by certain colonic bacteria, especially bifidobacteria, as a carbon source for growth and metabolism (76). Of these sugars are the widely used lactulose and various fructose oligosaccharides and polysaccharides (**Figure 1**). Those sugars that are naturally occurring, such as inulin, are considered to be a part of dietary fiber (77). Inulin has 1 molecule of glucose and ≤60 molecules of fructose and is thus considered to be an "extended-sucrose" molecule. Shorter extended-sucrose molecules containing ≤4 fructose units have been synthesized and are often called neosugars (78).

Lactulose has been used clinically to provide symptomatic relief in severe liver disease (79). Specifically, it lowers blood ammonia concentrations and prevents the development of hepatic encephalopathy. Because bifidobacteria and other colonic organisms metabolize lactulose, colonic contents become acidic, converting NH_3 to NH_4^+, which serves to draw the NH_3 from the blood to the colon. NH_4^+ is then excreted in the feces.

Fructooligosaccharides, when incorporated into the human diet, alter both the microbial flora and the metabolic activity of the colon. Subjects receiving 15 g fructooligosaccharides or inulin per day had higher hydrogen and methane outputs in their breath than did subjects fed sucrose. Fecal bifidobacterial counts increased almost 10-fold, whereas those of bacteroides, coliforms, and cocci decreased. Fecal short-chain fatty acid concentrations (eg, acetic, propionic, and butyric acids) did not change significantly (80). Raffinose ingestion, a naturally occurring sugar consisting of one molecule each of glucose, galactose, and fructose, resulted in a decrease in fecal pH, an increase in the short-chain fatty acid content, and an increase in *Lactobacillus* ssp. counts in rats (81). Other more exotic synthetic sugars, such as oligoglucosyl inositols, are also of interest as potential prebiotics (82). A combination of probiotics and prebiotics (called symbiotics) are now being used in medical practice.

CONCLUSIONS AND PERSPECTIVES

Probiotics, perhaps in combination with prebiotics, may become an important means of preventing and treating disease. In fact, several types of diarrhea have been successfully treated with probiotics. This practice, however, may represent only the "tip of the iceberg" because the potential benefits of probiotic therapy promise to be almost limitless. Research to fully realize this potential must focus on the following areas:

1) the identification of strains of *Bifidobacterium* and *Lactobacillus* that can withstand passage through the gastrointestinal tract better than do known species (ie, withstand gastric acidity and the effects of bile salts);
2) the identification of probiotic species and strains that are effective against specific disease processes or for the prevention of disease;
3) the investigation of mechanisms of probiotic action; and
4) the identification of additional compounds that will enhance the growth of probiotic organisms (eg, the development of more effective and safer prebiotics and selection or development of strains that will adhere to the intestinal mucosal cells in the population at large to allow for true colonization and growth).

Thus, an ideal probiotic would be one that can survive passage through the gastrointestinal tract, establish itself permanently in the small intestine and colon, and provide a specific health benefit for the host by eliciting an immune response; secretion, production, and synthesis of compounds such as short-chain fatty acids, lactic acid, and bacteriocins; or another appropriate mechanism. As a source of energy, this probiotic would selectively utilize a prebiotic, would be safe, and would have few, if any, side effects.

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