

Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut^{1,2}

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ABSTRACT The microbiota of the human large intestine influences health and well-being. Whereas it has long been accepted that gut bacteria play a role in host pathogenesis, current opinion is that certain microflora components can have beneficial effects on gastroenteritis resistance, blood lipids, antitumor properties, lactose tolerance, and gastrointestinal immunity. It is postulated that in the infant gut an elevated bifidobacterial count may be associated with health advantages that breast-fed infants may have over formula-fed infants. Whereas beneficial aspects of the human gut flora still need definitive confirmation and mechanistic explanations, there is now interest in modulating the composition of gut flora such that a potentially more remedial community exists. This may be achieved through the targeted use of dietary supplementation. This article provides an overview of how probiotics, prebiotics, and synbiotics may contribute toward nutritional modulation of the gut microecology, with emphasis on the neonatal intestine where appropriate. The use of modern molecular methods, as an essential step forward for assessing the validity and accuracy of the modulatory approach, is also discussed. *Am J Clin Nutr* 1999;69(suppl):1052S–7S.

KEY WORDS Human gut flora, probiotics, prebiotics, synbiotics, molecular techniques, neonates

INTRODUCTION

Many factors affect the composition of the large-intestinal microbiota in humans. These include the age, susceptibility to infections, nutritional requirements, and immunologic status of the host and the pH, transit time, interactions between flora components, and presence and availability of fermentable material in the gut. Of these, it is probably the amount and type of growth substrate that has the most influential role. Dietary residues that are undigested in the upper gastrointestinal tract, as well as endogenous materials like mucins, sloughed epithelial cells, and bacterial lysis products, contribute to the pool of metabolizable substrates. Their contribution to the fermentation process was discussed by Macfarlane and Gibson (1). Diet may exert a major influence on gut bacterial populations and their development. In this context, possible differences between the microbiota of breast-fed and bottle-fed infants are notable (2).

The main fermentable dietary substrates in the adult gut are carbohydrate-based materials such as dietary fibers, resistant

starches, oligosaccharides, food sweeteners, and other nonabsorbed sugars. There is a lesser contribution from nitrogen-based materials like proteins and amino acids and some dietary lipids may also reach the colon in a metabolizable form. In the infant gut, the form of the milk substrate can have important effects on the composition of gut flora.

The gastrointestinal tract of newborns is inoculated primarily by organisms originating from the mother's vagina and feces and from the environment. For newborns delivered by cesarean birth this latter factor is of particular importance. Bacterial populations develop during the first day of life. Not surprisingly, facultative anaerobic strains such as *Escherichia coli* and streptococci initially exist in highest numbers (3, 4). These bacteria may subsequently create a highly reduced environment that allows the growth of strictly anaerobic species. Soon after delivery, the infant may be weaned from breast milk to formula. Differences in the fecal flora of breast-fed and bottle-fed infants exist and have been associated with a lower risk of gastrointestinal infection in breast-fed infants. Although the data are conflicting, there is some evidence that the microflora of breast-fed infants is dominated by populations of bifidobacteria (5–9), an observation first promoted in the early work of Tissier (10). In contrast, formula-fed infants are thought to have a more complex flora with no one bacterial genus showing a numerical predominance. The high incidence of bifidobacteria has been cited as one possible explanation for the purported health advantages. Although the ecosystem development is undoubtedly influenced by both host and environmental factors, some postulations directly attribute this development to the feeding regime as follows:

- 1) Oligosaccharides, including N-acetylglucosamine (11), glucose, galactose, and fucose oligomers or certain glycoproteins (12), which form a significant proportion of human breast milk, may be specific growth factors for bifidobacteria.
- 2) Low protein content and reduced buffering capacity of human milk may allow elevated growth of bifidobacteria (13, 14). The nature, type, absorption, and quantity of milk proteins present in the feed may also exert an effect.

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- 3) Certain compounds, including lactoferrin and some lipids, inhibit microorganisms (15–17).
- 4) Certain bacteria may stimulate immunoglobulin molecules such as secretory immunoglobulin A (18).

After weaning, a community resembling the adult flora becomes established (at >2 y of age). Assuming that differences in the type of food given to an infant can affect gut flora, the opportunity for gut flora manipulation arises in bottle-fed infants.

Infants delivered abdominally have far fewer lactobacilli in the early stages of life than those delivered vaginally (19), and the hygienic conditions in hospitals may prevent the full transfer of microorganisms to abdominally delivered newborns. Such deficiencies have led to the consideration that dietary supplements may influence the flora composition, and possibly the health status, of infants. The use of probiotics, prebiotics, and synbiotics may all be feasible.

PROBIOTICS

Although many different definitions of a probiotic have been proposed, the most widely used, scientifically valid, and therefore accepted version is that of Fuller (20, 21), ie, a live microbial food supplement that beneficially affects the host animal by improving its intestinal microbial balance. For human adult use, this includes fermented milk products as well as over-the-counter preparations that contain lyophilized bacteria. The microorganisms involved are usually lactic acid producers such as lactobacilli and bifidobacteria. An effective probiotic should 1) exert a beneficial effect on the host, 2) be nonpathogenic and nontoxic, 3) contain a large number of viable cells, 4) be capable of surviving and metabolizing in the gut, 5) remain viable during storage and use, 6) have good sensory properties, and 7) be isolated from the same species as its intended host. Postulated health advantages associated with probiotic intake are the 1) alleviation of symptoms of lactose malabsorption, 2) increase in natural resistance to infectious diseases of the intestinal tract, 3) suppression of cancer, 4) reduction in serum cholesterol concentrations, 5) improved digestion, and 6) stimulation of gastrointestinal immunity (20–25). Although these effects have usually been related to adult intake, probiotics have also been administered to infants. The design and results of volunteer trials are often variable, however there may be some rationale for the approach.

In an early study by Robinson and Thompson (26), a *Lactobacillus acidophilus* supplement given to formula-fed infants was thought to improve weight gain. Other studies directed the use of probiotics to specific clinical disorders. In a well-designed study, Isolauri et al (27) showed that oral rehydration that included a strain of *L. casei* promoted recovery from acute diarrhea in children. Results from a lactulose-mannitol permeability test showed that no mucosal disruption occurred. The duration of diarrhea was reduced from 2.4 d in a placebo group to 1.4 d in the intervention group. A rotaviral infection was thought to be the etiologic agent in most of the cases. *L. casei*, given in conjunction with live oral rotavirus vaccine to infants, caused an elevated response in rotavirus-specific immunoglobulin M-secreting cells, and improved antirotavirus immunoglobulin A seroconversion (28). Oral administration of *L. acidophilus* has also been shown to be effective against bacterially induced gastroenteritis (29, 30). Gonzalez et al (22) used a mixture of both lactobacillus species as bacteriotherapy against infantile diarrhea caused by *E. coli*, salmonella, and shigella.

Bifidobacteria have also been used in microbial food supplements for infants, both individually (31, 32) and in combination

with lactobacilli (33). Tojo et al (34) observed that oral administration of *Bifidobacterium breve* may be effective against campylobacter-induced enteritis in children. Encouraging results have also been proposed for the use of bifidobacteria in rotaviral infections (23). Randomly assigned infants <24 mo of age who were admitted to a chronic medical care facility received standard infant formula or formula supplemented with *B. bifidum* and *Streptococcus thermophilus*. Thirty-one percent of patients in the control group, but only 7% of those in the supplemented group, developed acute diarrhea. Moreover, 39% of the control subjects, but only 10% of the supplemented group, shed rotavirus in stools at sometime during the 17-mo study (35). Other applications of bifidobacterial probiotics in infants have been directed toward reducing the growth of *Candida albicans* (36) and the incidence of enterocolitis (37).

Often, studies of probiotics in infants have given equivocal data (24). This may be related to the ready extrapolation of data from poorly controlled trials that did not include credible scientific control. However, it is also likely that the survival of the probiotic was compromised in the supplement product before ingestion, and even more so in the host after ingestion. The bacteria are confronted by many physicochemical effects that may adversely influence culture viability. These include gastric acid and secretions of the small intestine such as bile salts and pancreatic enzymes. Moreover, in the large intestine, the bacteria must compete effectively with a complex and metabolically active indigenous flora. For this area of research to gain improved scientific credibility, it would help if the orally fed organisms could be recovered in a reliable and quantifiable state in fecal material. This necessitates the use of advanced molecular-based methods (discussed below) and has only recently been applied to the use of probiotics in infants (38). One alternative to the survivability problems may lie in the use of prebiotics for gut modulation in infants.

PREBIOTICS

A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity, or both of one or a limited number of bacterial species already resident in the colon (25). For a food ingredient to be classified as a prebiotic, it must 1) neither be hydrolyzed nor absorbed in the upper part of the gastrointestinal tract; 2) be a selective substrate for one or a limited number of potentially beneficial commensal bacteria in the colon, thus stimulating the bacteria to grow, become metabolically activated, or both; and 3) be able as a consequence to alter the colonic microflora toward a more healthier composition.

Although any food ingredient that enters the large intestine is a candidate prebiotic, it is the selectivity of the fermentation in the mixed culture environment that is critical. At present, most searches for prebiotics are directed toward the growth of lactic acid-producing microorganisms. This is due to their purported health-promoting properties. However, it may be that future developments in the study of prebiotics may include aspects of their effect on pathogenic flora components. A possible example of the latter is the ability of cellobiose to attenuate virulence in *Listeria monocytogenes* (39).

Lactulose was used some 40 y ago as a prebiotic infant formula food supplement to increase numbers of lactobacilli in infant intestines (40). However, the specificity of this substrate for enhancing these microorganisms has not been validated effectively from a molecular biology approach.



If, as some investigators have indicated, bifidobacteria form the predominant bacterial genus in breast-fed infants, then prebiotics in infant formulas directed toward these organisms may be of some use. In adults, consumption of fructooligosaccharides resulted in the numerical predominance of bifidobacteria in feces (41, 42). Similar effects were seen when galactooligosaccharides were fed to rats colonized with human fecal flora (43). Future research should be directed toward determining the realistic health consequences of microflora manipulation in this manner.

Bifidobacteria are reasonable target organisms for prebiotics. *B. infantis* and *B. breve* are thought to be predominant in infants (2), whereas *B. adolescentis* and *B. longum* are prevalent in adults (44, 45). Some reported advantages of bifidobacterial proliferation in the human large gut are listed in **Table 1**. In this context, the application of molecular diagnostic methods, such as species-specific DNA probing or strain-specific genetic fingerprinting, will in the future facilitate not only more precise quantitative monitoring, but also highly reliable, discriminating, and accurate population analysis techniques. Therefore, it may be that prebiotics targeting change at the species level, rather than the genus level, are more appropriate. In this context, it is recognized that the types of bifidobacteria found in breast-fed and bottle-fed infants vary (56).

SYNBIOTICS

Another possibility in microflora management procedures is the use of synbiotics, in which probiotics and prebiotics are used in combination (25). The live microbial additions (probiotics) may be used in conjunction with specific substrates (prebiotics) for growth (eg, a fructooligosaccharide in conjunction with a bifidobacterial strain or lactitol in conjunction with a lactobacillus organism). This combination could improve the survival of the probiotic organism, because its specific substrate is readily available for its fermentation, and result in advantages to the host that the live microorganism and prebiotic offer. Probiotics, prebiotics, and synbiotics that may be suitable for human consumption are listed in **Table 2**.

MOLECULAR APPROACHES FOR ASSESSING BACTERIAL DIVERSITY AND POPULATION CHANGES

There are many issues surrounding the use of microflora management techniques. These include the identification of health advantages, the preferred approach to use, and, in the case of infants, the definitive assessment of differences in microbiota between breast-fed and formula-fed infants. It is essential that modern methods be applied to the research questions that arise. A central requirement for the study of gut flora manipulation,

TABLE 1

Reported health advantages associated with bifidobacteria in the adult and infant human gut¹

Inhibition of pathogen growth
Immunomodulatory activity
Restoration of gut flora after antibiotic therapy
Production of digestive enzymes
Positive effects on antibiotic-associated diarrhea
Repression of rotaviruses

¹From references 7, 20, 21, 25, 31, 34, 35, 37, and 46–55.

TABLE 2

Examples of common probiotics, prebiotics, and synbiotics¹

Probiotics
Lactobacilli
<i>L. acidophilus</i>
<i>L. casei</i>
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i>
<i>L. reuteri</i>
<i>L. brevis</i>
<i>L. cellobiosus</i>
<i>L. curvatus</i>
<i>L. fermentum</i>
<i>L. plantarum</i>
Gram-positive cocci
<i>Lactococcus lactis</i> subsp. <i>cremoris</i>
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>
<i>Enterococcus faecium</i>
<i>S. diaacetylactis</i>
<i>S. intermedius</i>
Bifidobacteria
<i>B. bifidum</i>
<i>B. adolescentis</i>
<i>B. animalis</i>
<i>B. infantis</i>
<i>B. longum</i>
<i>B. thermophilum</i>
Prebiotics
FOS (eg, oligofructose and neosugar)
Inulin
GOS
Lactulose
Lactitol
Synbiotics
Bifidobacteria + FOS
Lactobacilli + lactitol
Bifidobacteria + GOS

¹Some still under evaluation. FOS, fructooligosaccharides; GOS, galactooligosaccharides.

pertinent to probiotics, prebiotics, and synbiotics, is the precise qualitative and quantitative monitoring of population changes.


The human gut contains a great diversity of bacteria (>400 species). Traditionally, descriptive and diagnostic bacteriology has been based on phenotypic characterization of the organisms, such as cellular form (eg, Gram staining and cell morphology) and function (eg, biochemical reactions). The phenotypic analysis of bacteria is, however, dependent on our ability to isolate and culture organisms, and many of the tests routinely used suffer from unreliability (eg, because of poor test reproducibility, metabolic plasticity of the organisms, or operator subjectivity) and poor discrimination. As a result, the descriptive and taxonomic frameworks of gut anaerobes (and indeed many other groups of organisms) are artificial and unstable, and the identification of these organisms is often fraught with difficulties. Systematic bacteriology has undergone a revolution in recent years with the advent of 16S ribosomal RNA (rRNA) sequence analysis (57). 16S rRNA is an immensely powerful molecular chronometer and for the first time has permitted the construction of an all-embracing phylogenetic, evolutionary framework for bacteria ranging from kingdoms and major phylogenetic domains to individual species. In addition to providing a powerful means of marshaling the great natural diversity of bacteria, the rapid accumulation of gene sequence data

is revolutionizing our perception of bacterial diversity and the discovery of new taxa. Furthermore, 16S rRNA sequence data provides information essential for the development of molecular-based tests for identifying specific bacterial populations directly, in their natural environment, without the need to cultivate. By exploiting different “clocks” or regions of conservation within the 16S rRNA sequence data, it is possible to identify sequence idiosyncrasies, so-called signatures, that are characteristic of different taxa and can act as targets for gene probes. Numerous probing strategies exist, of which quantitative dot blot hybridization and whole-cell in situ hybridization are the most appropriate, to use phylogenetically based rRNA and rDNA probes for quantification. Quantification of a specific 16S rRNA (eg, characteristic of a genus or species) compared with total 16S rRNA can be obtained by using dot blot hybridizations of a directly isolated nucleic acid mixture with universal and specific oligonucleotide probes. In a pioneering study, this approach was first applied to the monitoring of population changes in the rumens of cattle (58). By contrast with in situ identification and enumeration, with whole-cell hybridization, morphologically intact microorganisms harboring a certain rRNA sequence are specifically detected using fluorescently labeled rDNA hybridization probes (59). Identification at the single-cell level by using whole-cell hybridizations can provide more detailed information than quantitative dot blot hybridizations (eg, spatial distributions of organisms in situ). Although whole-cell hybridizations have not to date been used extensively in the monitoring of colonic microbial populations, advances in instrumentation such as the use of laser confocal scanning microscopy, will greatly improve the applicability of this technique. Langendijk et al (60) described 16S rDNA gene probes specific to the genus *Bifidobacterium* that enabled the exclusive detection of bifidobacteria in fecal samples through whole-cell in situ hybridization. The use of whole-cell in situ hybridizations will soon revolutionize the routine qualitative and quantitative monitoring of gut anaerobes and alleviate laborious cultivation and purification tasks, which are currently prerequisites for phenotypic identification. The approach will be extremely valuable for future research in prebiotics, both in improved validity and in the development of gene probes for use in extensive volunteer trials.

When using live microbes (eg, bifidobacteria or lactobacilli) as dietary adjuncts, an inherent problem is the difficulty of detecting and enumerating the specific probiotic in the gut or feces (eg, to assess survival). In particular, it is essential to be able to distinguish between the probiotic and endogenous strains of the same species in the host. Several molecular diagnostic approaches can be used to address this problem. In principle, 16S rRNA probing strategies can be used to monitor organisms at the strain level if strain-specific signatures within the 16S rRNA are present. Kok et al (38) reported the use of such an approach for the specific detection and analysis of a probiotic bifidobacterium strain in infant feces. Strain-specific 16S rRNA gene-targeted oligonucleotide primers were developed that allowed the specific detection and differentiation of the probiotic strain from the endogenous flora via the polymerase chain reaction. A potential problem with this approach, however, is the purported specificity of the probes. Although some 16S rRNA gene sequence microheterogeneity (ie, sequence variation) is evident in bacterial species, it is generally minor. Consequently, the number of strains within a particular species will invariably exceed the number of 16S rRNA variants, and the probes are therefore unlikely to be strain-specific. Thus, to use this approach it is essential to first show that an identical 16S rRNA variant (as the probi-

otic) is not present in the indigenous flora of the subject. An alternative approach to polymerase-chain-reaction detection is to use DNA fingerprinting to distinguish between strains of ingested and endogenous flora. For example, Kullen et al (61) recently used 16S rDNA restriction fragment length polymorphism analysis to examine the fate of ingested bifidobacteria through the gastrointestinal tract. As with the aforementioned scenario, evaluating the specificity of the probiotic 16S rDNA fingerprint is crucial to the success of this approach. In this respect, because the discriminatory level of 16S rDNA restriction fragment length polymorphism is somewhat limited, other DNA fingerprinting methods with higher resolution, such as ribotyping or pulse-field gel electrophoresis (62), will undoubtedly prove to be more appropriate. An alternative to the use of gene probes or DNA fingerprinting to distinguish between ingested and endogenous organisms is to use genetically modified probiotic organisms. For example, green fluorescent protein (the source of fluorescent-light emission in the jellyfish *Aequorea victoria*) technology provides a robust means of genetically labeling organisms (63). Ultraviolet-irradiated, green-fluorescent-protein-labeled strains emit intense green fluorescence, thereby permitting direct cellular microscopic observation and enumeration. Such technology could facilitate the direct monitoring of probiotic strains in mixed-culture environments such as the gut and feces, eliminating the necessity for cultural bacteriology. In particular, this approach would directly lend itself to addressing important issues such as probiotic colonization and survival in the gut after termination of dietary supplementation.

CONCLUSION

It is possible to manipulate the composition of the gut microbiota in infants and adults through dietary supplementation. The improved validity that a molecular approach to gut microbiology offers will accelerate this potential and allow for definitive assessment of flora changes. However, it is probably more important that the health advantages of microflora modulation are addressed, and this requires a mechanistic approach. Some interesting data have arisen from the use of probiotics to reduce diarrhea and gastroenteritis in infants. Hypothetically, the mechanisms involved may include 1) a reduced gut pH through stimulation of the lactic acid-producing microflora (32), 2) direct antagonistic effects on pathogens (64–66), 3) competition for binding and receptor sites that pathogens may occupy either by probiotics (46) or prebiotics (47), 4) improved immune function and stimulation of appropriate immunomodulatory cells (27, 28), and 5) competition for available nutrients and other growth factors. Moreover, there is some contention as to whether a flora high in bifidobacteria exists in breast-fed infants. This could be resolved by the application of high fidelity molecular approaches to more definitively assess the microflora composition. 

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