Probiotics That Modify Disease Risk

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ABSTRACT Probiotics are defined as live bacterial preparations with clinically documented health effects in humans. Probiotics have specific properties and targets in the human intestinal tract and intestinal microbiota. Each probiotic strain, independent of its genus and species is unique and, thus, the properties and the human health effects of each strain have to be assessed in a case-by-case manner. Understanding the mechanisms by which probiotics influence the normal intestinal microbiota and counteract aberrancies in microbiota would facilitate the use of probiotics for both dietary management and reduction in risk of specific diseases. Development of intestinal microbiota is an important factor affecting the health of the newborn. Recent studies suggest that specific bacterial components, especially the bifidobacteria, have a key impact on development of a healthy balanced infant microbiota. The composition of infant and child intestinal microbiota may become aberrant and thus influence the development of diarrheal, inflammatory, and allergic diseases. Based on this understanding, positive health effects of probiotics have been reported in the management of diarrheal, inflammatory, and allergic diseases in infants. Most recently, a reduction in risk of atopic diseases followed early administration of specific probiotics. J. Nutr. 135: 1294–1298, 2005.

KEY WORDS: • probiotic • intestinal microbiota • health effects

Probiotics have been defined as bacterial preparations that impart clinically verified beneficial effects on the health of the host when consumed orally (1). Most probiotics are currently either lactic acid bacteria or bifidobacteria, but new species and genera are being assessed for future use. The selection of effective probiotics was based on general properties of probiotics.

However, mechanisms of probiotic action are multifaceted, and each probiotic may have specific functions affecting the host. Thus, it may be necessary to redefine these criteria and to acquire new standards to allow the development of probiotics for specific functions and targets (2). The focus of this report is to characterize current knowledge of probiotics and to define the steps that need to be taken to further develop probiotics with properties that would reduce the risk of diarrheal, inflammatory, and allergic diseases in infants.

Intestinal microbiota: basis of probiotic action

The intestinal colonization that follows birth represents the host’s earliest contact with microbes (3). The indigenous gut microbiota plays an important role in the generation of immunophysiologic regulation in the gut by providing crucial signals for the development of the immune system in infancy and by interfering and actively controlling the gut associated immunologic homeostasis later in life (4,5). The intestinal microbiota forms a natural defense barrier against harmful microbes in the environment. Thus, early establishment of a healthy microflora provides the first key step in long-term well-being later in life.

The microbiota of a newborn develops rapidly after birth and is initially strongly dependent on the mother’s microbiota, the mode of birth, and the birth environment, subsequently, it is influenced by feeding practices and the environment of the child. Most microbiota succession studies have been based on culture-dependent methodologies, limiting our understanding of intestinal microbiota species composition (6). Recent studies using molecular biology have indicated that the microbiota of infants develops rapidly during the first week and remains unstable for the first year of life, becoming more stable afterward. This initial stage of microbiota establishment could be a key moment for establishing a healthy microbiota in the individual through microbiota modulation. Later in life, the
stability of the microbiota makes its permanent modification difficult.

Lactic acid bacteria account for <1% of the total microbiota in infants, but bifidobacteria make up 60 to 90% of the total fecal microbiota in breast-fed infants (7,8). The composition of bifidobacteria microbiota in infants was first clarified by Benno and Mitsuoka (9). Usually, bifidobacteria appear after birth, and, within a week, they have been reported as the dominant bacterial group, with *Bifidobacterium breve* and *Bifidobacterium bifidum* being the most common species in healthy infants. More recent molecular studies have identified *Bifidobacterium infantis*, *Bifidobacterium longum* and *B. breve* as the species most often found in infants (10). The greatest differences between breast-fed and formula-fed infants appear to be in lactic acid bacteria colonization and species of bifidobacteria present. In breast-fed infants, *Lactobacillus gasseri* was the most common species measured by cell culture (9). More recent molecular studies indicate that the *Lactobacillus acidophilus* (sensu lacto) group organisms are the most common lactobacilli in both breast-fed and formula-fed infant feces (7,11). Regarding bifidobacteria, *B. breve* has been reported as the most common species present in breast-fed infants (9), while other authors found that *B. infantis, B. longum*, and *B. bifidum* predominate in breast-fed infants (12).

We have yet to define the specific bacteria of the developing intestinal microbiota that most influences infant health. It is of overriding interest to improve our knowledge of species composition within a healthy microbiota. Specific deviations in intestinal microbiota may predispose the infant to allergic disease. Such aberrancies include decreased numbers of bifidobacteria and an atypical composition of bifidobacteria (13). Aberrancies in the clostridium content and composition also have been reported to be important (13–16). Compositional differences among clostridia and their relation to bifidobacterial composition and concentration need to be assessed carefully. Aberrant microbiota may also be predisposing factors for both inflammatory gut diseases and rotavirus diarrhea (17). A more thorough knowledge of intestinal microbiota composition will provide a basis for future probiotic development and for the search for new strains for human use.

**Probiotics, intestinal microbiota, and health**

One of the main selection criteria for probiotics has been competitive exclusion of pathogens. Probiotics compete directly or indirectly the adhesion of pathogens on stero-specific receptors on gastrointestinal (GI) surface (18). They can also influence the development of intestinal microbiota in infants. The outcome of the microbiota development and competitive exclusion would depend on the specificity of the bacteria and bacterial adhesions for the receptors and the relative concentration of the competing bacteria. The effective dosage of a probiotic is thus determined by the relative affinity for the receptor sites.

The rationale for modulating gut microbiota with probiotics is the demonstration that intestinal microbiota is important to health (19). Specific probiotic bacteria modulate the intestinal and systemic immune responses (2,20–22). Activation of immunologic cells and tissues requires close contact of the probiotic with the immune cells and tissue on the intestinal surface (23). Lactobacilli and bifidobacteria mainly colonize the small and the large intestine, respectively; interestingly, when given as probiotic supplements, both were able to modify immunologic reactions related to allergic inflammation, but lactobacilli were ineffective in protection against cow's milk allergy (2,23–25). In this aspect, preferential binding of probiotics on the specific antigen-processing cells (macrophages, dendritic, and epithelial cells) (26,27) may be even more important than the location of adhesion. We have also shown that the cytokine stimulation profiles of different bifidobacteria strains vary (16), and strains isolated from healthy infants mainly stimulate noninflammatory cytokines. Probiotic properties also vary in this respect. *Lactobacillus casei* has been reported to induce IL-12 and TGFβ from murine dendritic cells, whereas *Lactobacillus reuteri* causes IL-10 production and downregulates the effects of L. casei (28). Oral administration of *Lactobacillus rhamnosus* GG resulted in elevated serum IL-10 concentrations in atopic children, indicating that specific probiotics may have anti-inflammatory effects in vivo. Such effects may be mediated through changes in intestinal microbiota, especially through modification of the bifidobacteria microbiota (16,29,30).

An increasing number of clinical and experimental studies demonstrate the importance of constituents within the intestinal lumen, in particular the resident microbiota, in driving the inflammatory responses in these diseases. Probiotic bacteria may counteract the inflammatory process by stabilizing the gut microbial environment and the permeability barrier of the intestine and by enhancing the degradation of enteral antigens and altering their immunogenicity. Another explanation for the gut-stabilizing effect could be improvement of the immunological barrier of the intestine, particularly intestinal IgA responses. Probiotic effects may also be mediated via control of the balance between pro- and anti-inflammatory cytokines (1,15,25,29).

Specific strains of the gut microbiota also have been shown to contribute to a TH cell population that maintains a disease-free state of the gut. These microbiota-host interactions and the specific properties of the strain should be taken into account when selecting candidate probiotics. This would allow the target-specific selection of probiotics (Table 1).

**Optimal characteristics for probiotics**

**Tolerance to upper-GI environment.** The effects of GI conditions, such as pH, bile, and digestive enzymes, on the survival (31) and the adhesion properties (32) of probiotic bacteria have been documented. Various bacteria show different levels of tolerance to GI conditions. For example, the adhesion of *L. rhamnosus* GG on mucus was reduced to one-tenth, whereas the adhesion of *Lactobacillus johnsonii* La1 was reduced to one-third after pretreatment with amylase, pepsin, bile, and pancreatin (32). Such properties should be studied for all candidate probiotics.

**Adhesion.** Adhesion on intestinal surface lengthens the retention time of a probiotic, and it is particularly important in the small intestine because of the short residence time of intestinal material in it.

*L. rhamnosus* GG, which has been reported to adhere and to colonize the small intestine, was effective in shortening rotavirus diarrhea in infants (33,34). *Lactobacillus bulgaricus*, which could not adhere to and colonize intestine, had no effect on infant diarrhea. Similarly, a highly adhesive strain, *Bifidobacterium lactis* Bb12, is effective in preventing and treating acute diarrhea in infants (35). Recent information on the genome of *B. longum* indicates that the strain has specific gene sequences that promote adherence to intestinal mucosa, especially in colon (36). The gene may predispose some strains and species
to inhabit specific target sites in the intestinal tract of infants, and these properties should be carefully characterized for each candidate strain.

The ability of probiotics to adhere to intestinal mucus glycoprotein likely reflects the persistence of a probiotic in the intestine but may not necessarily be related to their capacity to successfully adhere to intestinal tissue (37). A probiotic bacterium that binds strongly on mucin glycoprotein would compete with pathogens for adhesion on the mucus surface but may have a high turnover rate on the mucosal surface because of continuous dislodgement from the intestinal surface, along with the mucus that they bind to. Conversely, a probiotic bacterium that penetrates the mucus layer may adhere to the epithelial surface (37).

**Specificity to target sites.** Once consumed, probiotics pass through the entire GI tract, and new candidate probiotics selected from members of the normal microbiota are therefore likely to have the prerequisite survival and specificity, depending on the location from which they were isolated. An effective probiotic must reside at the desired target sites sufficiently long and at sufficient concentrations to elicit probiotic effects. Moreover, adhesion, and even temporary multiplication, of probiotic bacteria at the target sites would result in an enhanced concentration of probiotics at the optimal places of action, achieving the desirable responses even at a lower dosage (37–39).

**Growth and metabolic activity.** Without adhesion on intestinal mucosa, the concentration of probiotic bacteria would be diluted to an insignificant level after a meal or a drink. It is not clear whether all probiotics can grow in the intestinal environment. No commercial probiotics have been reported to be able to establish themselves permanently in the human intestine; this suggests that, even if there is cell division, the specific growth rates are not fast enough to replenish detached probiotic cells on the intestinal surface (1). Long-term colonization studies are needed to further understand microbiota effects after early probiotic administration.

Multiplication in the intestinal tract would increase the size of a probiotic population and the concentration of its metabolites, thereby increasing their ability to alter GI bacteria. We have yet to see growth of probiotics in the GI tract. Some probiotics attach to intestinal mucosa and can be recovered in biopsies much longer than in feces (40,41). Thus, adherence studies need to complement fecal recovery assessment, preferably in biopsies. The importance of viability is underscored in reports where immune-enhancing effects during probiotic treatment of rotavirus diarrhea were observed only with viable probiotics (42). Another aspect of viability is metabolite production. Acid and peroxide production by bacteria are linked to growth, but secondary metabolites that are nongrowth linked may be produced when cells are not multiplying. These metabolites may have a role in locally modulating GI microbiota.

### Studies on management and prevention of diseases

Acute gastroenteritis is a significant problem around the world. Probiotics have been successfully administered for the treatment of rotavirus diarrhea in infants and children, initially in Finland with studies using *L. rhamnosus* strain GG, and later confirmed in several studies, including a multicenter study in Europe (34). Rotavirus diarrhea was also prevented by *B. lactis* Bb12 (35), and several other lactic acid bacteria and bifidobacteria probiotics have been tested in human clinical studies.

During the last 2 decades, the prevalence of atopic disease has increased in industrialized countries (43). The development of intestinal microbiota in early infancy appears to be a critical factor in the establishment of normal gut barrier functions and in the modulation of immune system development in the neonate. Differences in the composition of the intestinal microbiota of allergic and healthy infants have been reported (42,44), and differences in microbiota composition may precede the development of some allergic diseases (13,14).

The modulation of the intestinal microbiota by probiotics can be a useful tool for both the dietary management and the prevention of some allergic diseases. A double-blind, placebo-controlled trial has shown that administration of *L. rhamnosus* GG prenatally to mothers and during the first months of life to infants with high risk of atopic disease significantly reduced the prevalence of atopic eczema (24,45). Supplementation of extensively hydrolyzed whey formula with *L. rhamnosus* GG or with *B. lactis* Bb12 is more effective than unsupplemented formula in alleviating atopic eczema in infants (21). However, *L. rhamnosus* GG administration did not benefit adolescents suffering from pollen allergy (46). *B. breve* has been reported to prevent atopic diseases (47). These results suggest that probiotics exert their effects on allergy during the development of the immune system in early infancy.

Preliminary reports indicate that probiotic treatment may reverse some of the immunological disturbances characteristic of Crohn’s disease (48,49). In addition, probiotic intervention reduces disease activity and increases intestinal permeability in pediatric patients with Crohn’s disease (50). In adults, however, *Lactobacillus* GG failed to prevent recurrence of Crohn’s disease, as measured endoscopically, during 1 y of follow-up (50). A recent study suggests that treatment with a non-pathogenic *Escherichia coli* maintains remission in ulcerative colitis (51).

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**TABLE 1**

<table>
<thead>
<tr>
<th>Target for probiotic action</th>
<th>Selection criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleviation of lactose maldigestion symptoms</td>
<td>High lactase producing strongly site specific adhesion LAB</td>
</tr>
<tr>
<td>Intestinal inflammation</td>
<td>Site specific adhesion properties, anti-inflammatory cytokine expression, mucosal properties to alleviate permeability disorder and gut microbiota abnormality</td>
</tr>
<tr>
<td>Alleviation or food allergy symptoms, reducing the risk of atopic diseases</td>
<td>Adherence to small intestine, induction of local TGFβ production, proteolytic properties</td>
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<tr>
<td>Reducing the risk of colon cancer</td>
<td>Target specific adhesion to distal or proximal colon, mucosal butyric acid production, competitive exclusion of inflammatory bacteria, toxin binding, and promotion of nontoxigenic mucosal microbiota</td>
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</table>
CONCLUSIONS

In addition to the traditional selection criteria for probiotics, it is clear that new selection criteria are needed to fulfill anti-allergic potential. The normal microbiota for infants who remain healthy need to be characterized for several years. Infants who later develop allergic diseases should have any microbiota aberrancies identified. At the same time, bifidobacteria and lactobacilli in healthy infants should be characterized and assessed for their influence on normalizing microbiota aberrancies. Within this scheme, it is possible to identify new probiotic candidates for future clinical trials. Such strains should be carefully characterized before application in human studies.

The development of intestinal microbiota is of major importance to the health of the newborn. The concentration and the composition of bifidobacteria are more important than lactic acid bacteria during early intestinal colonization. These factors form the basis for selecting probiotics from among those currently available, suggesting bifidobacteria as the first option and specific lactic acid bacteria that may stimulate intestinal bifidobacteria as the second option. Qualitative effects of new probiotics on intestinal microbiota should be understood before their introduction into infant foods. Knowledge of intestinal microbiota development, nutrition, immunity, and specific diseases should be carefully combined with information of the genome of potential probiotic strains to find new probiotics with disease risk modifying properties.

LITERATURE CITED


gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from the faeces. Appl. Environ. Microbiol. 68: 3401–3407.