Probiotic Agents and Infectious Diseases: A Modern Perspective on a Traditional Therapy

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(See the editorial commentary by Salminen and Arvilommi on pages 1577–8)

There is an increasing scientific and commercial interest in the use of beneficial microorganisms, or “probiotics,” for the prevention and treatment of disease. The microorganisms most frequently used as probiotic agents are lactic-acid bacteria such as *Lactobacillus rhamnosus GG* (LGG), which has been extensively studied in recent literature. Multiple mechanisms of action have been postulated, including lactose digestion, production of antimicrobial agents, competition for space or nutrients, and immunomodulation. We have reviewed recent studies of probiotics for the treatment and control of infectious diseases. Studies of pediatric diarrhea show substantial evidence of clinical benefits from probiotic therapy in patients with viral gastroenteritis, and data on LGG treatment for *Clostridium difficile* diarrhea appear promising. However, data to support use of probiotics for prevention of traveler’s diarrhea are more limited. New research suggests potential applications in vaccine development and prevention of sexually transmitted diseases. Further studies are needed to take full advantage of this traditional medical approach and to apply it to the infectious diseases of the new millennium.

Humans live in close association with vast numbers of microorganisms that are present on the skin, in the mouth, and in the gastrointestinal (GI) tract. These commensal microbes have coevolved with humans and demonstrate a high degree of interdependence with them. The human body contains 10 times as many protective indigenous microorganisms as it does eukaryotic cells. The greatest concentration of commensal organisms is found in the GI tract, which has >400 m² of surface area; this constitutes the second largest surface area of the body after that of the respiratory tract. The GI tract harbors a rich flora of >500 different bacterial species, some of which have important health functions, which include stimulating the immune system, protecting the host from invading bacteria and viruses, and aiding digestion [1–4]. The gut flora is acquired rapidly after birth, remains relatively stable throughout the life, and is essential for human homeostasis. Intestinal microbes seem to drive the development of the gut-associated lymph tissue during the neonatal period. The challenge facing this mucosal immune system is to discriminate between pathogens and benign organisms by stimulating protective immunity without excessive inflammatory response that may disrupt the integrity of the GI mucosa. However, it is apparent that the mucosal immune system is in a constant state of activation, or physiological inflammation, because of this massive antigenic challenge. This stimulation plays an important role in host defense against pathogens [3–6].

Several factors may decrease our resistance to disease and may predispose us to infectious, inflammatory, degenerative, and neoplastic conditions. The use of antibiotics, immunosuppressive therapy, and irradiation, among other means of treatment, may cause alteration in the composition and effect of the gut flora. Therefore, the introduction of beneficial bacterial species into the
GI tract may be a very attractive option to reestablish the microfloral equilibrium and prevent disease [6, 7].

“Probiotic bacteria” are defined as dietary supplements of living microorganisms found in the normal flora with low or no pathogenicity, but with positive effects on the health of the host. They can resist the rigors of the human digestive system and improve the balance of the gut flora [8–10]. The most commonly used probiotics come from 2 genera, Lactobacillus and Bifidobacterium. Probiotics have several mechanisms of action, which are described in the Mechanisms of Action section. All of these mechanisms are caused by bacterial interference, in which the presence of a microorganism limits the pathogenic potential of another microorganism [9, 11, 12]. However, even closely related probiotic strains may have completely different clinical effects.

We have reviewed the current understanding of probiotics and their potential use in the management of several infectious diseases, with emphasis on diarrheal diseases. A brief overview of the mucosal microflora and its development during childhood is also useful to understand how probiotic agents are selected and developed.

MUCOSAL MICROFLORA AND MUCOSAL IMMUNITY: AN OVERVIEW

A healthy and functional GI tract is necessary to maintain good health. The human intestine contains a complex, dynamic, and diverse society of nonpathogenic bacteria, which may be differentiated into native inhabitants and transient flora. Native inhabitants include organisms that colonize or fill specific niches in the human body. These microorganisms colonize the mucosal surface of the oral cavity, the upper respiratory tract, much of the GI tract, and the urogenital tract. Transient flora include those microorganisms that colonize the body from the external environment and can persist if some niches are not filled with native flora [4, 13, 14].

The normal full-term infant has the essential components of the mucosal immune system and the potential to respond to antigenic stimulation. The GI tract is sterile at birth. Indigenous microflora colonize the mucosal surfaces of infants during an ecological succession of organisms that differ from the adult microflora. The composition of the colonic bacterial species can be influenced by factors such as mode of parturition and type of oral formula. Infants born by vaginal delivery become colonized with microorganisms from the birth canal of the mother and the environment, including coliforms, streptococci, and gram-positive, non–spore-forming anaerobic rods. Breast-fed infants have an increased number of Bifidobacteria but rarely have Clostridium colonizing the GI tract. However, formula-fed infants have large numbers of Lactobacilli, Bacteroides, and Clostridium and relatively few Bifidobacterium spe-

Cies. The microflora become similar in both breast-fed and non–breast-fed infants when food supplements are started, and Bacteroides and anaerobic gram-positive cocci prevail. After the infant reaches 2 years of age, conversion to normal adult flora begins, and populations of Bacteroides and anaerobic cocci increase until they equal or exceed those of Bifidobacterium. The number of gram-negative anaerobes increases to adult levels, whereas coliform, clostridial, and streptococcal populations decrease to the levels found in healthy adults. Illness, infection, stress, and changes in diet, climate, and medication may lead to changes in this physiological succession process [2, 4, 5, 10, 15, 16].

The requirement for the intestinal mucosal immune system to discriminate between potential pathogens and the enormous diversity of dietary antigens to which it is exposed has resulted in the evolution of a unique and distinct intestinal immune system. Interactions between microflora with the host immune system begin when the microflora develop in the neonate and continue throughout life [3, 5, 15, 16]. Mucosal surfaces are specialized in 2 seemingly opposing functions: tolerance to environmental antigens (food antigens and probiotics) and immunity to mucosal pathogenic microorganisms. Long-term exposure to luminal antigen in the intestine induces an immunologic unresponsiveness known as oral tolerance [17–19]. The intrinsic mechanisms of oral tolerance are not fully understood. However, the major mechanisms are believed to operate through clonal deletion, clonal anergy of antigen-specific T cells, or immune deviation. Class I–restricted CD4 T cells and cytokines may mediate this inhibitory mechanism with immunosuppressive functions, such as IL-10, transforming growth factor-β, or both. The acquisition of tolerance may also result from the stimulation of antigen-specific CD8 T cells that in turn produce inhibitory cytokines. Microflora seem to have a crucial role in tolerance induction because germ-free animals are defective with regard to oral tolerance [2, 3, 17–21].

In contrast to immune tolerance, clearly protective immune responses also occur in the GI tract. The local immunoglobulin response of the intestinal mucosa is highly skewed toward the production of IgA. T cells from the lamina propria have primarily a memory phenotype (CD45RO) with increased activation of lymphokines and cytokine production under normal physiologic conditions. Lamina propria lymphocytes seem to have the potential for both Th-1 (IL-12, IFN-γ) and Th-2 (IL-10, IL-4, IL-5) types of cytokine production. These cells may be replaced by further differentiated cells that express either Th-1 or Th-2 response [20–25]. Intracellular infections localized in macrophages are potent inducers of cellular immune responses through the initial strong induction of IL-12 and IFN-γ production. Extracellular pathogens, such as intestinal parasites, are strong inducers of IL-4 and IL-10 production [3, 20, 21, 24].
The mucosal flora have the capacity to limit the growth of, or to kill, certain transient microbial pathogens that enter their habitat, a process called “microbial interference.” However, the mechanisms are not yet well understood [6, 7, 10, 26, 27]. Nutritional competition is established as an important mechanism in animal models, and suppressive factors, such as bacteriocins and toxicity of end metabolic products, have also been implicated. Bacteriocins are proteins that produce intraspecies antagonistic effects, that is, antibiotics produced by endogenous flora [27–29]. The primary factor in the establishment of microflora on mucosal surfaces is mucosal adhesion, which eventually results in the formation of bacterial biofilms. Adhesion helps organisms to survive peristalsis, or voiding motion, depending on the host site [6, 7, 28]. Survival is the main goal on sites where small numbers of species usually predominate (e.g., lactobacilli in the vagina), whereas competition is the main goal in tissue surface ecosystems that support heavy bacterial growth and a large variety of species (e.g., in the intestine and the oropharynx) [4, 6, 7, 10, 11, 29].

PROBIOTIC: BACKGROUND, CONCEPT, AND TAXONOMY

In ancient times, the benefits and health potential of foods containing live bacteria were recognized, and fermented foods were quite common. During the beginning of the 20th century, Metchinkoff [30] proposed a scientific rationale for the beneficial effects of bacteria in yogurt and attributed the long life of Bulgarian peasants to their intake of yogurt containing Lactobacillus species. In the 1930s, Minoru Shirota, a Japanese medical microbiologist, proposed that many diseases could be prevented if an optimal gut microflora was maintained. He selected beneficial strains of lactic acid bacteria that could survive passage through the digestive system and used them to develop fermented milk products [15]. Since then, multiple antimicrobial properties of probiotics have been suggested as potential protective factors in the digestive system against microorganisms such as Escherichia coli [31, 32], Salmonella [33] and Listeria species [34], and Helicobacter pylori [35]. Several areas of potential use of probiotics have been proposed in the past 50 years, including the prevention and treatment of diarrheal diseases in adults and children, prevention of vaginitis and urinary tract infection in adults, food allergy prevention, and antitumor action in the gut, bladder, and cervix [7, 11, 29, 36, 37]. Studies of the metabolic, antitumor, immunomodulatory, and GI benefits of probiotic agents have been recently reviewed [38].

The word “probiotic” is derived from the Greek, meaning “for life.” Probiotics are live bacteria that can resist the rigors of the human digestive system, compete with pathogens, and that help to improve the gut flora balance [9, 11, 29]. There are several basic characteristics of bacteria that may be effective probiotics. They should be preferably of human origin, innocuous, able to withstand processing conditions, and able to survive transit through the gut and colonize mucosal surfaces. They also should act against pathogens by means of multiple mechanisms and elicit minimal resistance to their effects. The onset of beneficial effects should be rapid in comparison with the time required for a vaccine to be fully protective. Optimally, they should function with or without antibiotics [7, 9–11, 29].

There are several commercially available supplements containing viable microorganisms with probiotic properties, either in lyophilized form or as fermented food products. The most commonly used probiotics are lactic acid bacteria (LAB) and nonpathogenic, antibiotic-resistant, ascospore yeasts, principally Saccharomyces boulardii. Strains of LAB used as probiotics include species of Lactobacillus, Enterococcus, and Bifidobacterium (table 1) [11, 29, 39]. Probiotic bacteria with desirable properties and documented clinical effects include L. rhamnosus strain GG (LGG; ATCC53503), L. acidophilus (NCFB1478), L. casei Shirota strain, L. johnsonii L11, L. reuteri, and B. lactis Bb12 [40]. LGG, a variant of L. casei species rhamnosus, is by far the most extensively studied probiotic organism in adults and children [41].

The taxonomy and nomenclature of probiotic LAB is still changing. It is no longer based only on morphological, physiologic, and biochemical criteria, but also on molecular-based phenotypic and genomic characteristics [29, 39, 42]. DNA tech-

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<td>Enterococcus faecalis</td>
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<td>Streptococcus thermophilus</td>
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nology, gene probes, and randomly amplified polymorphic DNA PCR are now useful tools to evaluate the dynamics of probiotics throughout the GI tract and the quality of the products containing probiotics. For classification of strains at the species level, investigators have used either fingerprinting or numeric analysis of biochemical reactions as phenotypic methods and used either pulsed field electrophoresis or restriction enzyme analysis as genomic methods. A polyphasic taxonomy that combines these methods is essential for quality assurance, as required by governmental law and by the consumer [39].

Currently, there is a renewed interest in probiotics worldwide. However, many of the probiotic cultures used in dairy products lack the appropriate species designation, do not contain a listed species, contain extra species, or vary in concentration of microorganisms [39, 43]. Validated in vitro models are now available for rapid screening of these bacteria and for quality assurance [39]. Each strain should be always clearly identified and deposited into an international culture collection. There are several studies in which the strain identification is incomplete or the strain information is available only for the manufacturer, and as a consequence, no firm conclusions can be drawn from these results.

**MECHANISMS OF ACTION**

The main mechanisms whereby probiotics exert protective or therapeutic effects are not fully elucidated, but several mechanisms have been postulated. Scientific evidence is based on a limited number of in vivo studies and deductions from well-founded in vitro studies that have involved adults and children with intestinal disorders [29, 39]. Strain information should be reported with each clinical and microbiological study because even closely related probiotic strains may have different clinical effects. This information may have important implications both for assessing the studies and for planning future studies.

Partial lactose digestion and stimulation of the intestinal mucosal lactase activity has been postulated as a mechanism against some types of diarrhea. Therefore, during episodes of acute gastroenteritis or recurrent abdominal distress, disaccharidase activity in the small intestine may affect the transport of monosaccharides and produce osmotic diarrhea. Lactobacilli used in the fermented milk industry have active β-galactosidase to decrease the lactose concentration in dairy products, which may affect the severity of osmotic diarrhea due to organisms such as rotavirus [9, 10].

LAB also produce antimicrobial substances, such as organic acids, fatty free acids, ammonia, hydrogen peroxide, and bacteriocins in vitro. These metabolites are used to extend the shelf life of food and to suppress spoilage and food-borne pathogens in dairy products [26, 28, 29, 36]. An example of a probiotic with these properties is *L. casei* strain GG, isolated by Gorbach et al. [28] and recently reclassified as “LGG” [6, 39]. LGG was found by natural selection, and its unique colonial morphology makes it easy to identify in a mixed culture of other lactobacilli and streptococci [41]. It has the ability to produce a low-molecular-weight antibacterial substance that inhibits both gram-positive and gram-negative enteric bacteria in the intestinal flora of mice [28, 29]. Probiotics can also use enzymatic mechanisms to modify toxin receptors and block toxin-mediated pathology. For example, *S. boulardii* degrades *Clostridium difficile* toxin receptors in the rabbit ileum [44, 45] and blocks cholera-induced secretion in rat jejunum by the production of polyamines [46].

Probiotic agents also prevent colonization of pathogenic microorganisms by competitive inhibition for microbial adhesion sites. LGG and *L. plantarum* have been shown to competitively inhibit the attachment of enteropathogenic *E. coli* 0157H7 to HT-29 human colonic cancer cells [32]. *S. boulardii* has been shown to decrease in vitro attachment of *Entamoeba histolytica* trophozoites to erythrocytes [47].

Several studies have demonstrated adjuvant-like effects on intestinal and systemic immunity by oral administration of different probiotics. Kaila et al. [48] found enhancement of specific serologic antibody response to rotavirus, as measured by IgM and IgA, during the acute stage of diarrhea in Finnish children who were receiving LGG. In 2 more recent studies, the same authors showed increased numbers of IgA-specific antibody secreting cells to rotavirus in most patients with rotavirus diarrhea who received viable LGG at the convalescent stage [49, 50]. Healthy adult volunteers who received fermented milk that contained *L. acidophilus* and *Bifidobacterium* species for 3 weeks showed significantly higher serum IgA to *S. typhi* Ty21a upon receiving oral typhoid vaccine than did persons in a control group who received no fermented food [51]. However, we did not find systemic antibody response to LGG in a study of Peruvian children who had received oral administration of this product for 10 days [52].

Probiotics may also affect nonhumoral immunity. Increased macrophage phagocytic activity against several intracellular bacteria has been detected in mice after oral or parenteral administration of *L. casei* [53, 54]. Oral administration of *S. boulardii* has been associated with complement and reticuloendothelial activation in humans [55]. Both in vitro production of γ-IFN, IL-12, and IL-18 by human blood lymphocytes and enhanced capacity to produce γ-IFN have been reported in response to different lactic bacteria strains [56]. A recent publication showed that mucosa-associated lactobacilli (mainly *L. paracasei*) could be potent stimulators of IL-12, thereby enhancing cell-mediated immunity if they are able to translocate over the gut barrier and interact with the gut mucosal immune system [57]. However, IL-12 may downregulate the Th-2 response, decreasing IL-4 and IgE production. This response
would explain the potential role of probiotics in allergy prevention [58, 59].

SAFETY, ADVANTAGES, AND DISADVANTAGES

Probiotics have been used for many years with relatively few safety problems. Adverse effects were not found in a large population receiving LGG in Finland [60], and preterm infants who received the same probiotic agent did not have side effects [61]. Some cases of sepsis or endocarditis caused by lactobacilli were reported in immunosuppressed patients who had multiple risk factors for translocation and systemic dissemination of organisms that are usually considered nonpathogenic, but none of these infections were related to lactobacilli used in probiotic food products [62, 63]. S. boulardii given orally has been well tolerated in different populations, including patients with AIDS and Crohn’s disease [64, 65]. However, disseminated fungemia in a 1-year-old infant with severe chronic diarrhea [66] and a few cases of fungemia that have affected patients with catheter-related infection have been reported among persons who received S. boulardii. All of these resolved during antifungal therapy [65]. Finally, there is special concern about the use of enterococci as probiotics because of possible acquisition of multiple antibiotic resistance, including resistance to vancomycin [7, 10, 36, 64]. Strains that harbor resistance plasmids should not be used either as human or animal probiotics.

Although most probiotics seem safe, available information is limited. Most studies with these products have been done in Europe, where probiotic products differ from those used in the United States. Definition of the pharmacodynamic profiles and viability of organisms in many commercially produced probiotic preparations are lacking [7, 9, 10, 64].

POSSIBLE INDICATIONS IN INFECTIOUS DISEASES

The use of probiotics to control certain infections and to re-establish the human bacterial ecology has started to gain acceptance. Factors that have spurred this renewed interest include the alarming rise of antimicrobial resistance and the application of molecular techniques to select probiotic agents and assess their clinical impact. However, to date there are few well-conducted clinical trials to clearly demonstrate the benefits of probiotics in humans, and additional high-quality clinical trials are still needed to make specific recommendations. Recent studies that have definitively improved our understanding of probiotics, and their potential use in infectious diseases, are discussed in this section.

Infectious diarrhea in children. Several recent clinical studies have attempted to establish the value of probiotics in the prophylaxis and treatment of diarrheal disease in children. LGG has demonstrated the most significant benefits in patients with acute gastroenteritis caused by rotavirus, which is still the most important pathogen for pediatric diarrheal disease worldwide. These studies have been well conducted and performed in several countries by several investigators with both academic and industrial support. In comparison, the data for L. reuteri and Bifidobacterium lactis (earlier B. animalis) are mainly based on a few clinical studies.

Most therapeutic trials that have evaluated probiotics in pediatric patients with diarrhea have shown better results in populations with higher socioeconomic status and higher rates of rotaviral diarrhea. Isolauri et al. [48–50, 67–69] have conducted several double-blinded placebo controlled trials involving Finnish infants and young children with rotavirus diarrhea, which showed significant reduction of disease duration in those receiving different LAB compared with placebo groups. One of these studies showed a reduced duration of episodes of diarrhea among young children who received L. reuteri (duration, 1.7 days vs. 2.9 days for control patients) [68]. Guarino et al. [70] also reported shortened duration of diarrhea and decrease in rotavirus shedding with LGG, which confirms the findings of prior clinical studies. Besides the therapeutic effects, these findings may have considerable implications regarding decreasing the rate of nosocomial rotaviral infection in infants with a high risk for gastroenteritis [71]. The most recent study from a multicenter European group showed that the simultaneous administration of hypotonic oral rehydration solution and LGG to children (age range, 1 month to 3 years old) with acute diarrhea was safe, and it reduced the duration of diarrhea and resulted in both less chance of protracted diarrhea and shorter duration of hospital stay [72]. However, no improvement in duration of diarrheal disease was demonstrated in a controlled trial from Canada in 1972 with a commercially available probiotic preparation (10^8 lyophilized bacteria comprised of 50%–60% Streptococcus thermophilus, 35%–45% L. acidophilus, and 5% L. bulgaricus) in 94 children with acute diarrhea [73].

In the few published studies performed in developing countries, the early administration of LGG in addition to oral rehydration therapy resulted in faster correction of acidosis and shorter duration of diarrhea, but not in persons with bloody diarrhea [74–76]. The addition of heat-killed L. acidophilus to oral standard rehydration therapy in Thailand was effective in the treatment of children with acute gastroenteritis, reducing the duration of diarrhea [77].

Fewer studies have been done to evaluate prophylaxis of pediatric diarrhea with probiotics. In the United States, a double-blinded, placebo-controlled trial, conducted during a 17-month period among 55 children (5–24 months of age) from a long-term care facility, showed a beneficial effect in the group that received B. bifidum (35.8 × 10^8 organisms per 100 kcal)
and *S. thermophilus* (2.69 × 10⁸ organisms per 100 kcal) compared with the placebo group (2 vs. 8 episodes of diarrhea; *P* = 0.035). Rotavirus caused the most diarrheal disease, and its excretion was lower among patients in the group that received the supplement [78]. Another study involved 287 healthy children (mean age, 18 months; range, 7–32 months) from 12 day care centers in Paris during a 6 month-period [79]. Children received 1 of 3 dairy products: standard yogurt, milk fermented by yogurt and LGG casei, or jellied milk as placebo. No difference in the incidence of diarrheal disease was found between groups, but the duration of episodes of diarrhea was shorter among patients in the *L. casei* group than it was among patients in the jellied milk group (4.3 days vs. 8.0 days, respectively; *P* = 0.009). The study did not examine either etiology of diarrheal disease or intestinal colonization with probiotics.

Our group evaluated the prophylactic effect of LGG against diarrhea during a 15-month period among 204 undernourished children from Peru in a randomized, placebo-controlled trial [80]. The duration and etiology of episodes of diarrhea were similar in both groups of patients, but adenovirus was more common among patients in the placebo group. We demonstrated a modest, but statistically significant, prophylactic effect in non–breast-fed children in the toddler age group, and we also demonstrated transmission of LGG from some of the children who were receiving it daily to family contacts. Conversely, no difference in rates of incidence of diarrhea by treatment group was seen among breast-fed infants and children.

**Diarrheal disease in adults.** Few controlled trials have been conducted in adults with infectious diarrhea. A double-blinded, randomized trial that studied the effect of a preparation containing *Enterococcus SF* 68 strain in patients with acute infectious gastroenteritis showed less severe diarrhea with a shorter duration among patients in the probiotic group than that among patients in the placebo group [81]. However, a study conducted with 23 healthy volunteers who received a product that contained *L. acidophilus* and *L. bulgaricus* did not show differences in attack rate, incubation period, or duration of illness when they were challenged with enterotoxigenic *Escherichia coli* [82]. Several studies of the preventive effect of lactic bacteria in patients with traveler’s diarrhea have conflicting results, and reported benefits are modest. No protective effect could be demonstrated among 282 British soldiers who traveled to Belize and received a probiotic product that contained *L. acidophilus* strain LA or *L. fermentum* strain KLD [83], or among 820 Finnish travelers who received LGG before traveling to Turkey [84]. Hilton et al. [85] demonstrated a modest reduction in the incidence of diarrhea among 245 American travelers who received LGG for 1 to 3 weeks, compared with control patients who received placebo (incidence of diarrhea, 3.9% per day for persons who received LGG vs. 7.4% per day for control patients; *P* = 0.05).

**Antibiotic-associated diarrhea.** Diarrhea is the most common gastrointestinal side effect of antibiotic therapy often associated with *C. difficile* infections in adults and children. Studies that have involved strains of *S. boulardii* [86], *Lactobacillus* species [74], and *Bifidobacterium* [87] species have reported beneficial effects in the treatment and prevention of antibiotic-associated diarrhea. Gorbach et al. [88] reported successful treatment of *C. difficile* relapsing diarrhea, without side effects, in 5 adults and 4 children who received LGG [89]. A small study that involved 16 young adults who received erythromycin for 7 days showed a substantial positive effect among those who received LGG-enriched yogurt, compared with those who received placebo yogurt. The duration of diarrhea was only 2 days among patients in the study group versus 8 days among patients in the placebo group [90].

Recently, a beneficial effect of LGG in the prevention of diarrhea induced by antibiotics was demonstrated among Finnish [91] and American [92] children with respiratory infection. Stool frequency decreased and stool consistency increased during antibiotic therapy among subjects who received LGG, compared with subjects in the placebo groups. However, other studies have also demonstrated no benefit when *L. acidophilus* and *L. bulgaricus* were given [93]. Differences in antibiotics given, variation in probiotics organisms tested, lack of a control or placebo group, and small numbers of patients have reduced our ability to interpret some of these clinical studies.

**Candidal vaginitis.** *Candida* species is the most common cause of vaginal infection, with frequent recurrence and chronic infection, so probiotics may be useful for treatment [36, 37]. Studies conducted by Hilton et al. [94] showed a significant reduction in vaginal colonization with *Candida* species after the oral administration of *L. acidophilus*, and clinical improvement with a 7-day course of vaginal suppositories of LGG [95] was shown in women with recurrent vaginitis. However, other reports have not demonstrated that probiotic treatment benefits women with vaginitis [96], so more controlled trials are needed to evaluate this clinical indication. *H₂O₂*-producing lactobacilli (LB+) are isolated from the vaginal mucosa of most healthy women but are present in <23% of women with bacterial vaginosis (BV) [97]. LB+ are toxic to *Gardenella vaginalis* at high concentrations, but their absence may allow an overgrowth of catalase-negative organisms present in women with BV. Because BV is a risk factor for sexually transmitted diseases (STDs), probiotic agents that contain *Lactobacillus* species may have a potential protective role against STDs [98]. Also, LB+ may inhibit growth of *Neisseria gonorrhoeae* by several mechanisms, including acidification of the environment, *H₂O₂* secretion, and production of protein inhibitors [99].
FUTURE DIRECTIONS

The following are some of the future possibilities for these biological products in the field of infectious diseases.

Mucosal vaccines and immunomodulation. The use of LAB as live vectors for oral immunization appears to be an exciting approach, on the basis of their safety, ability to persist within the indigenous flora, adjuvant properties, and low intrinsic immunogenicity. Medaglini et al. [100] have recently developed a genetic system for the expression of heterologous antigens from human papillomavirus and HIV type 1 (HIV-1) in the surface of the human commensal Streptococcus gordonii and L. casei. Local and systemic immune responses were detected in BALB/c mice and Cynomolgus monkeys after vaginal colonization with the aforementioned recombinant strains. Both macrophage activation and IL-12/γ-IFN pathway stimulation are promising areas of research with regard to resistance to intracellular pathogens by enhancement of mucosal and systemic immunity [56, 57]. More experimental and clinical studies are needed to clarify the role of probiotics as immunomodulators, not only in infectious diseases of the GI tract, but also for inflammatory and allergic conditions.

Prevention of transmission of AIDS and STDs. The role of the vaginal microflora in determining the efficiency of HIV and other STD transmission is still not well understood. Lactobacillus plays a critical role in the regulation of the vaginal microflora. It has been suggested that the production of H$_2$O$_2$, rather than a particular species of Lactobacillus, is essential in the regulation of the vaginal flora. This toxic molecule is the most potent local microbicidal present in the human vagina. The findings of experiments have suggested that LB+ given at high concentrations is viricidal for HIV-1 [98, 99]. The presence or absence of vaginal lactobacilli on culture was inversely associated with BV and gonorrhea among HIV-negative female sex workers in Kenya [101]. There was also a trend for an inverse association between vaginal lactobacilli and HIV seroconversion [101–103], and there is an association between BV and increased HIV-1 infection among women who are younger than 40 years of age [103]. These studies suggest that LB+ may play an important role in protecting women against some pathogens in the vagina. If the Th-1 cytokine pathway is stimulated, other mechanisms should be considered in the prevention or control of opportunistic intracellular infections, especially in the GI tract.

Infection control programs and eradication of multidrug-resistant microorganisms. The alarming increase of inappropriate antibiotic use and bacterial resistance, along with renewed interest in ecological methods to prevent infections, makes probiotics a very interesting field for research. No studies have been conducted to establish this potential role. However, a case report describes a 68-year-old woman from Japan with a decubitus colonized by methicillin-resistant Staphylococcus aureus who was successfully treated with a lactobacillus preparation [104]. Studies of this potential use may have profound impact in coming years.

Antibacterial effects. In vitro studies suggest multiple specific activities of different probiotic agents against several pathogens, including Listeria monocytogenes [34], Salmonella typhimurium [33], E. coli [31, 32], and H. pylori [35], among others. Therefore, probiotic agents may provide prototypic antimicrobial substances that will be useful for pharmaceutical companies in the development of new antibiotics.

CONCLUSIONS

Probiotic agents are living microorganisms belonging to the normal flora, with low or no pathogenicity and a positive effect on the health and well-being of the host. Probiotic therapy uses bacterial interference and immunomodulation in the control of several infectious, inflammatory, and immunologic conditions. An impressive list of health effects is attributed to probiotic agents, but scientific methods to select and evaluate potential microbial strains with probiotic characteristics are limited. Careful selection of agents and dose standardization of bacterial strains for commercial and scientific use are required. Studies have shown potential medical benefits of use of probiotics for the treatment and prevention of a variety of infections that involve mucosal surfaces, including pediatric gastroenteritis and vaginitis. The future of probiotics will depend in part on further elucidation of basic mechanisms, allowing scientists and clinicians to maximize their health benefits. However, the risk of transferring antibiotic resistance from probiotics to virulent microorganisms requires more evaluation. High-risk populations, such as immunosuppressed individuals and elderly persons, need special precautions, and critical evaluation of the safety of probiotic strains is required. New challenges in infectious diseases may expand the role of probiotic agents in the prevention of STDs, transmission of HIV-1, and the control of multidrug-resistant organisms. The stimulatory effect of probiotics on mucosal and systemic immunity and their effect as immunomodulators suggest that probiotics may also be useful in the development of vaccines. This is supported by studies that have demonstrated adjuvant activity and enhanced immune responses from orally administered LGG. With further research, this traditional medical therapy may still prove to be one of our most effective tools against new and emerging pathogens that continue to defy modern medicine in the 21st century.
References


