REVIEW ARTICLE

Use of probiotics in various diseases: evidence and promises

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KEY WORDS

immunonutrition, *Lactobacillus*, microbiota, probiotics, *Saccharomyces*

ABSTRACT

The utilization of live microorganisms as therapeutics has been gaining increasing attention over the last years with the addition of scientific knowledge on their traditional uses. Probiotics are defined as "live micro-organisms which when administered in adequate amounts, confer a health benefit on the host". The normal intestinal microbiota prevents the colonization of pathogenic bacteria and has important immune functions. It has been hypothesized that the sudden change in the intestinal microbiota that parallels the modern life practices of humans might have contributed to the rise in the incidence of particular diseases. Bacteria and yeasts may be used as probiotics either in the form of a single strain or combination of microorganisms or mixed with prebiotics. Probiotics have been used for various disease states from gastrointestinal diseases to infections and even to diabetes and atopic diseases. Drawing firm conclusions about the clinical efficacy of probiotics is hard because of the heterogeneity of patient populations, probiotic strains, dosages, and commercial preparations. However, probiotics represent a very exciting and promising area of research due to the ever-increasing antibiotic resistance rates and the ability of some probiotics to modify the course of diseases.

Introduction The utilization of live microorganisms as therapeutics has a long history, which dates back even before the recognition of their existence. Acetifying and fermenting milk and other food products to preserve it are traditional methods that have been used for centuries. However, the medical reflection of these methods has only recently been discovered. At the beginning of the 20th century, Elie Metchnikoff claimed that consumption of live bacteria as yogurt improves gastrointestinal system function and prolongs life.[1] Fuller[2] defined a probiotic as "a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance". A more recent definition by the Food and Agriculture Organization of the United Nations and the World Health Organization describes probiotics as "live micro-organisms which when administered in adequate amounts, confer a health benefit on the host".[3] Recognition of the distant and systemic effects of probiotics has led to the proposal of the name "immunobiotics".[4]

Intestinal microbiota are necessary for the maturation of the immune system, normal development of intestines as a natural barrier for foreign materials and bacteria, and the synthesis of vitamins K and B_{12}.[5] The functionality of the intestinal microbiota is demonstrated by the predisposition of animals which are given antibiotics or are grown in sterile conditions to infection.[6] Infection in these animals can be prevented by the introduction of fecal suspension into the intestines. Moreover, the growth of pathogenic bacteria can be inhibited in vitro by the inoculation of bacteria isolated from intestinal flora.[6] The term "colonization resistance" indicates the resistance of the normal intestinal microbiota against the colonization of pathogenic microorganisms. This resistance is damaged by many factors such as consumption of food including low amount of microorganisms, living in more sterile environment and even by simple antibacterials such as vinegar. The most common factor affecting colonization resistance is antibiotics. Frequently used antibiotics including ampicillin, amoxicillin, cephalosporins, and clindamycin are blamed. As the role

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of the intestinal microbiota is better understood, strategies to preserve this physiological environment are popularized. This understanding resulted in the search and use of different bacterial and yeast species as functional foods, i.e., probiotics. The properties of probiotics are herein summarized, with a particular emphasis on gastrointestinal disorders in adults.

Species used as probiotics In order to be utilized as a probiotic, a microorganism should survive in the acidic environment of the stomach and also resist bile acids, enzymes, and antibacterial peptides. Moreover, it should reach viable counts in the intestines and persist long enough to have an interaction with the host mucosa and immune system and should not confer health risks on the host. Various strains of microorganisms may differ in survival and colonization capabilities.[7]

Lactobacillus and *Bifidobacterium* spp. are commonly used in commercial probiotic preparations. Strains of *Lactobacillus* – *L. acidophilus* and *L. rhamnosus* [former *L. casei*] – are the first bacteria to have been used as probiotics. Lactobacilli are normal inhabitants of the human intestinal microbiota and they are the most commonly used and commercially available probiotics as *L. acidophilus* and *L. rhamnosus GG*. Lactobacilli have been demonstrated to have numerous potentially important benefits in terms of gut health and immunity.[8] They can stimulate immune mechanisms at the intestinal level, increase immunoglobulin secretion, enhance antigen presentation and macrophage activation, and inhibit mucosal attachment of pathogens. Besides, antimicrobial molecules are secreted against some pathogens such as *Clostridium difficile* and *Escherichia coli*.[8] *Lactobacillus* species also have good evidence against the occurrence and recurrence of antibiotic-associated diarrhea (AAD).[9]

The utilization of bifidobacteria in fermented dairy products has been a practice for a few decades; they are solely used for their probiotic properties contrary to other starter cultures.[10] Besides lactobacilli, other Gram-positive bacteria such as *Enterococcus*, *Streptoccus*, and *Bacillus* spp. are also increasingly used as probiotics.[11]

Saccharomyces boulardii is a very popular yeast probiotic that has a strong evidence for use in preventing AAD. *S. boulardii* is a similar species to *Saccharomyces cerevisiae*, which is known as the beer yeast. Some researches do not accept it as a different species taxonomically and call it *S. cerevisiae Hansen CBS 5926*.[12] It was first isolated from a fruit (litchi) and introduced into clinical practice to treat diarrhea in the 1950s.[13] *S. boulardii* has a temperature optimum of 37°C, is resistant to local factors and pH variation, and survives the passage through the gastrointestinal system.[14] It has been shown to induce brush border enzyme activities, increase immunoglobulin receptors in the intestinal mucosa, and increase chloride absorption during *C. difficile*-associated diarrhea. Moreover, it has the advantage of not being affected by antibiotics, so that *S. boulardii* does not confer a risk for the development of antibiotic resistance and for transferring resistance genes to the pathogenic bacteria, and can be used together with antibiotic therapy.[15]

Commercially available probiotic preparations may contain single or mixed strains of microorganisms or prebiotics. Timmerman et al.[16] defined a multispecies probiotic as "containing strains of different probiotic species that belong to one or preferentially more genera". They suggested that multispecies probiotics may in some conditions be more efficient than probiotics that contain a single strain.

Effects on various gastrointestinal diseases Increasing numbers of patients are diagnosed and treated for *Helicobacter pylori* because of the frequent utilization of noninvasive diagnostic tests. Triple therapy used as the first-line treatment may cause significant side effects in some patients, which precludes the accomplishment of therapy. Besides, the increasing frequency of antibiotic-resistant strains decreases the efficacy of the first-line treatment. Human lactobacilli are the predominant bacteria found in the stomach of fasting subjects, and it was demonstrated that they have an inhibitory effect on the attachment of *H. pylori* to gastric epithelial cells in vitro.[17] *H. pylori*-eradication therapy has been shown to increase the numbers of the facultative anaerobic component of the microbiota.[18] Moreover, *H. pylori*-eradication treatment may cause AAD – a condition that has been shown to be prevented and treated with probiotics. Recent studies have demonstrated that probiotics might be efficient in decreasing the side effects of *H. pylori*-eradication therapy while increasing the eradication rates and tolerability of the triple therapy.[19,20] Not only concomitant therapy, but also pretreatment with probiotics has been shown to increase the efficacy of *H. pylori* treatment. Supplementation with a yeast probiotic, *S. boulardii* was also effective by preventing AAD when started concomitantly with the eradication therapy.[21]

Inflammatory bowel diseases are thought to develop due to an altered response to the normal intestinal microbiota.[22] In-vitro studies demonstrated that the release of tumor necrosis factor-α from the inflamed tissues of the colon was reduced when inflamed tissues were cultured with probiotic bacteria.[23] Although good results of probiotic use in ulcerative colitis have been published, a very recent Cochrane review has not demonstrated any benefit.[24,25] While probiotics added to standard therapy may reduce the disease activity in patients with mild to moderately severe ulcerative colitis, no improvement in the overall remission rates have been observed in the 4 eligible randomized controlled trials. Probiotic use in Crohn's disease (CD) is rather disappointing. Although small trials demonstrated a reduction in CD activity, especially in patients

with frequent diarrhea, larger scale trials and another Cochrane review of CD also could not demonstrate a positive result in terms of the maintenance of remission in CD.[26]

Irritable bowel syndrome (IBS) is actually a spectrum of diseases and the underlying causes may be complex. IBS may be secondary to infection in approximately 15% of the patients. Taken together with the evidence of low-grade inflammation, the data suggest a role for an aberrant relationship of the intestinal microbiota and the immune system in the pathogenesis of IBS. The fluctuating nature of IBS and the strong placebo effect in IBS patients make it impossible to draw a firm conclusion about the effects of probiotics in this patient population. A few long-term studies of up to 1 year reported beneficial effects of probiotics on IBS; others have found probiotics to be ineffective.[27,28]

Probiotic organisms have been shown to decrease the luminal pH, secrete bacteriocins, and inhibit bacterial adhesion, which all make them potential candidates in the treatment of acute diarrhea. Administration of *S. boulardii* to travelers decreased the incidence of diarrhea from 40% to 29%, which was statistically significant.[29] A former Cochrane review of probiotic use in infectious diarrhea, on the other hand, demonstrated that probiotics may be a useful adjunct to rehydration therapy in treating acute, infectious diarrhea especially in adults.[30] Nearly all trials reviewed in this report had a beneficial effect in reducing diarrhea.

Growth of opportunistic pathogens, destruction of normal metabolic functions of bacterial flora, allergic or toxic effects, and impairment of motility by antibiotics are involved in the pathogenesis of AAD.[31] Approximately one-third of AAD is due to *C. difficile*, while other bacterial and viral etiologies can also be identified. In the era of increasing antibiotic resistance, evidence of probiotic use in the prevention and treatment of antibiotic- and *C. difficile*-associated diarrhea is gaining importance. *S. boulardii* has the strongest evidence for the prevention of *C. difficile*-associated diarrhea.[32]

Miscellaneous effects of probiotics on human health and disease Besides modulation of gastrointestinal diseases, probiotics are promising in terms of their various beneficial effects on human health. High-quality animal studies demonstrated that modulating gut microbiota composition can regulate gut permeability, plasma endotoxin levels, fat gain, inflammation, and glucose tolerance.[33] The idea that common medical disorders, such as dyslipidemia, hypertension, and diabetes, can be prevented or treated to some extent by natural products is stimulating. Modulation of gut microbiota was shown to improve glucose tolerance of mice by altering the gene expressions involved in inflammation and metabolism.[34] Regular consumption of both probiotic and conventional yogurt for 4 weeks had a positive effect on the lipid profile of healthy women.[35] The probiotic LG2055 demonstrated lowering effects on abdominal adiposity and body weight.[36] The antihypertensive effect of probiotic bacteria were linked to their ability to produce peptides having angiotensin-converting-enzyme inhibitory activity.[37]

Beneficial effects of probiotics were also shown for extragastrointestinal infections. The intake of the probiotic combination, *L. gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, and *B. bifidum* MF 20/5, had no effect on the incidence of common cold infections, but significantly shortened duration of episodes, reduced the severity of symptoms, and led to increased numbers of cytotoxic, suppressor, and helper-T-cell counts.[38] Daily intake of *L. reuteri* was shown to reduce sick leaves related to gastrointestinal or respiratory tract diseases by 60%.[39]

Critically ill patients encompass a specific class, who are prone to infection and do need enteral or parenteral nutritional support. The probiotic VSL#3 was shown to be effective in reducing the number of liquid stools in enterally fed critically ill patients.[40]

Modification of gut microbiota composition with probiotics might have positive effects in terms of prevention of allergic, atopic, and auto-immune diseases.[41,42] Intestinal microbiota has been linked to the development of type 1 diabetes, hence factors influencing composition of the intestinal microbiota could be a target for therapeutic intervention.[43]

Safety and adverse effects Theoretically, two important threats exist with the use of probiotics: utilization of live organisms by immunocompromised patients and transfer of antibiotic-resistance genes. It has been claimed that probiotic strains may act as reservoirs to carry resistance genes and have the potential to transfer these genes to the pathogenic bacteria in the body. Lactobacilli, due to their broad environmental distribution, may act as vectors to convey resistance genes.[44] Intestinal bacteria not only exchange resistance genes among themselves but might also interact with transient bacteria to acquire and transmit antibiotic-resistance genes.[45] For instance, tetracycline, chloramphenicol, and erythromycin resistance among probiotic isolates has been demonstrated, which might be a reservoir for resistance genes.[46]

Invasive bacterial or fungal infections are concerns about the safety of probiotics. Lactobacilli, lactoccocci, bifidobacteria, and yeast have been labeled as "generally regarded as safe" by the World Health Organization.[47] Rare cases have been reported with bacteremia, endocarditis, and liver abscess, associated with *Lactobacillus* use.[48] Patients who are severely ill and have catheters or nasogastric tubes might be at increased risk for *S. boulardii* fungemia.[49] These infections respond to antibiotic therapy rapidly and do not confer a life-threatening risk.

Future prospects and conclusions Emerging multidrug-resistant pathogens are the main driving force behind the efforts to find an alternative solution as probiotics. The main goals are to decrease antibiotic consumption and fight the negative effects of antibiotic use. Moreover, it has been hypothesized that the sudden change in the intestinal microbiota that parallels the modern life practices of humans may have contributed to the rise in the incidence of autoimmune diseases.

The U.S. Food and Drug Administration accepts probiotics as dietary supplement that are not subject to regulations made for other pharmaceuticals. The European authorities are currently considering the required minimal colony-forming-unit counts of probiotics per preparation. The European Food Safety Authority has recently adopted a qualified presumption of safety approach for microorganism use in foods and feeds; however, no definitive guideline exists for commercially used probiotics.[50]

Although many trials shed light on our understanding of the mechanisms of action and the beneficial effects of probiotics, it is very hard to draw an exact conclusion from these trials and meta-analyses because of the heterogeneity of patient populations, probiotic strains, dosages, and commercial preparations. We still need well-designed, placebo-controlled, sufficiently powered studies that will reflect the actual role of probiotics.

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ARTYKUŁ POGLĄDOWY

Probiotyki: dowody i nadzieje związane ze stosowaniem ich w różnych chorobach

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SŁOWA KLUCZOWE

drobnoustroje, *Lactobacillus*, probiotyki, *Saccharomyces*, wpływ odżywiania na odporność

STRESZCZENIE

W ostatnich latach lecznicze zastosowanie probiotyków skupia coraz większą uwagę ze względu na obserwacje naukowe uzupełniające tradycyjną wiedzę. Pod pojęciem „probiotyki" rozumiemy „żywe drobnoustroje, które podane w odpowiedniej liczbie przynoszą korzyści zdrowotne organizmowi gospodarza". Prawidłowa mikroflora jelitowa zapobiega kolonizacji patogennymi bakteriami i odgrywa istotną rolę immunologiczną. Przypuszcza się, że nagła zmiana mikroflory jelitowej, która towarzyszy współczesnemu stylowi życia, może odgrywać rolę w zwiększeniu zachorowalności na niektóre choroby. Bakterie i drożdżaki mogą być stosowane albo jako pojedyncze szczepy, albo jako kombinacja drobnoustrojów, albo w połączeniu z prebiotykami. Probiotyki stosuje się w wielu stanach chorobowych: od chorób przewodu pokarmowego do zakażeń, a nawet w cukrzycy lub chorobach atopowych. Trudno jest wnioskować o klinicznej skuteczności probiotyków z uwagi na podawanie ich w różnorodnych grupach chorych i w różnych dawkach oraz stosowanie odmiennych szczepów probiotyków i zróżnicowanie preparatów. Nie ulega jednak wątpliwości, że probiotyki są pasjonującym i obiecującym obszarem badań ze względu na zwiększającą się oporność ludzi na antybiotyki oraz wpływ probiotyków na przebieg chorób.

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