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Antibacterial, antiviral, antifungal, and immunomodulatory properties of probiotics

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ABSTRACT

Probiotics are microorganisms that provide health benefits in sufficient amounts and are used in commercial dairy products such as yogurt and cheese and non-dairy products such as fruit juice and bread. Various gram-positive and gram-negative bacteria are in the category of probiotics, the most famous of which are *Lactobacillus* and *Bifidobacterium*. The most well-known application of probiotics is the removal of intestinal pathogens and stabilization of the mucosal barriers of the gastrointestinal tract. In addition, probiotics have the effects of anti-obesity, anti-diabetic, anti-cancer, cholesterol regulation, and improvement of neuropsychiatric diseases such as anxiety, depression, Parkinson's, and multiple sclerosis. Probiotics exert their antibiofilm and antibacterial properties through the production of antimicrobial compounds that inhibit the attachment to surfaces and the growth of pathogenic bacteria and prevent diseases such as tooth decay and urinary tract infection. The use of antibiotics against pathogenic bacteria leads to the possibility of antibiotic resistance, but probiotics are not like this, and even they can eliminate resistant bacteria such as methicillin-resistant Streptococcus aureus (MRSA). By producing lactic acid, fatty acids, nitric acid, H2O2, and bacteriocin and stimulating the immune system, probiotics have antiviral and antifungal activity, which in fungi, as a result, reduces toxin production. Due to the modulatory effect that probiotics have on inflammatory cytokines, different types of T lymphocytes, and other immune mediators, they can be used to improve autoimmune, inflammatory, and allergic diseases.

Keywords : Probiotic, Antibacterial, Antiviral, antifungal, Immune Mediator

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1. Introduction

According to the definition given by Lilly and Stillwell in 1965, probiotics are substances secreted by organisms to affect the growth of other organisms. Later, Marteau et al. (2002) called probiotics compounds of microbial cells that have beneficial effects on health (1). The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) define probiotics as live microorganisms that, when administered in sufficient amounts, provide health benefits to the host (2). In a more comprehensive definition by Ritchie and Romanuk, a probiotic is a product of live microorganisms that alters the host's microflora to stabilize mucosal barriers, influence the host's immune responses, and eliminate pathogens (3).

In addition to the benefits mentioned above, probiotics have anti-obesity effects through the production of short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate. Also, probiotics exert their anti-diabetic effect by neutralizing insulin resistance (4). In previous studies, it has been reported that the use of probiotic capsules (such as lactobacilli) vaginally inhibits the recurrence of urinary tract infections (UTIs) in women (5). Several cases of the improving effects of probiotics on the symptoms of neuropsychiatric diseases such as autism, depression, anxiety, Parkinson's, anorexia nervosa, Alzheimer's, schizophrenia, and multiple sclerosis have been introduced; however, their mechanism of action has not yet been accurately identified (6). Probiotics have an antidepressant effect by reducing the hyperactivity of the hypothalamus-pituitary-adrenal axis and reducing the cortisol level, as well as by regulating the level of tryptophan, which is the precursor of the 5-HT (serotonin) neurotransmitter (7). In anorexia nervosa disorder, probiotics may act as an adjunctive treatment to regulate the host's microbiota and reduce inflammation and gastrointestinal symptoms (8). Anti-cancer and angiogenic activity, blood pressure reduction, cholesterol regulation, role in lactose metabolism, and vitamin B production are other advantages of using probiotics (9).

Probiotic commercial products can be prepared in the form of dairy products such as cheese, yogurt, ice cream, and powdered milk, in the form of beverages such as fruit juice, and also in the form of bread (10). The minimum required concentration of the desired strain used in probiotic products should be 106 CFU/ml, and a total of 108 to 109 probiotic microorganisms should be used daily to be effective (11). A wide range of gram-positive and gram-negative bacteria are used as probiotics, including *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Saccharomyces*, *Streptococcus*, *Bifidobacterium*, and *Escherichia*, of which *Lactobacillus* and *Bifidobacterium* are more common and well-known (12). In this study, the antibacterial, antiviral, antifungal, and immunomodulatory aspects of probiotics will be discussed.

2. Antibacterial effects

Several studies have been conducted on the antibacterial effects of probiotics, including the evaluation of the inhibitory effect of *Lactobacillus* sp. on *Streptococcus mutans*. Dental caries is one of the complications caused by *S. mutans* due to biofilm formation and acid production. *Lactobacillus* sp. can play a role in

preventing tooth decay by inhibiting growth, biofilm formation, and the expression of genes related to biofilm formation and acid tolerance in *S. mutans* (13). In another study related to dental disease, *Lactobacillus* sp. showed antibacterial effects against two oral pathogenic bacteria, *Aggregatibacter actinomycetemcomitans* and *Actinomyces naeslundii*. Among the lactobacilli, *L. salivarius* and *L. fermentum* have an inhibitory effect on the attachment and growth of pathogenic bacteria by binding to the oral mucosa and forming a biofilm (14). When the pathogenic bacteria form the biofilm structure, due to the multi-layered nature of the biofilm, the pathogen becomes resistant to antibiotics and the immune system. *Lactobacillus casei* exopolysaccharide has the property of inhibiting the formation of biofilm and dispersing the biofilm of bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *E. coli* O157:H7 (15).

The extraction and secretome of *Lactobacillus acidophilus* and *L. casei* have antibacterial and antibiofilm effects against *Escherichia coli* associated with urinary tract infection (16).
Lactobacillus promotes its antibacterial properties by producing organic acids, antimicrobial peptides (AMPs), and bacteriocins, which have an inhibitory effect on the growth of pathogenic bacteria. Cell-free supernatant of *Lactobacillus paracasei* has more antibacterial effect against gram-positive bacteria than gram-negative bacteria, which is due to the inherent resistance of gram-negatives to bacteriocin and acid produced by lactobacilli (17). In digestive diseases related to *Helicobacter pylori*, it has been observed that the administration of probiotics, including *Lactobacillus*, with bismuth for more than two weeks causes the elimination of *H. pylori* and its complications. The inhibitory effect of probiotics occurs by preventing the attachment of H. pylori to the stomach wall, reducing urease activity, and destroying the cell wall of *H. pylori* (18).

In another study, in addition to the antibacterial effect, *Lacticaseibacillus* spp. probiotics had the ability to inhibit MRSA biofilm formation by 82.81 to 87.24% (20). Under *in vitro* conditions, *Lactobacillus fermentum* prevents the binding of MRSA to Caco-2 cells. Therefore, this probiotic has the potential to prevent colonization and also remove MRSA from the epithelial surface of the gastrointestinal tract (21).
Probiotic bacteria such as bifidobacteria can be found in human milk, which plays a role in gastrointestinal colonization and the development of the infant's immunity. According to the previous study, bifidobacteria species have antibacterial activity against *E. coli, Shigella dysenteriae, Salmonella typhi,* and *Listeria monocytogenes* (22). *Saccharomyces cerevisiae* reduces the pathogenicity of *Vibrio cholerae* by binding and inhibiting the toxin of *Vibrio cholerae*, as well as preventing electrolyte leakage from the intestine by inhibiting cAMP. Also, S. cerevisiae acts against the toxin and receptor related to *Clostridium difficile* through the protease enzyme and prevents the occurrence of *C. difficile* infection. Antibacterial effects of *S. cerevisiae* against *Bacillus anthracis, Shigella, E. coli, Helicobacter pylori,* and *Salmonella* have also been reported (23).

Some probiotics, such as *Bacillus coagulans*, have beneficial effects on the human intestinal microflora. *B. coagulans* improves intestinal microflora disorders by increasing probiotic bacteria such as *Bifidobacterium*, *Prevotella*, and *Firmicutes* and decreasing *Bacteroides* and *Shigella* (causes of intestinal inflammation) (24).

3. **Antiviral effects**

As well as antibacterial effects, several studies have investigated the antiviral effects of probiotics against various pathogenic viruses. For example, lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, *Lactobacillus amylovorus*, and *Enterococcus hirae* can act against enteroviruses E7 and E19 (25). It has been reported that the combination of *L. acidophilus* with *Glycyrrhiza glabra* (a type of plant) has antiviral effects against herpes simplex virus-1 (HSV-1) and vesicular stomatitis virus (VSV) (26).

The prevalence and pathogenicity of rotavirus as a cause of diarrhea and dehydration in children have made it the second most common cause of death in children under five years of age. Rotavirus causes diarrhea by lysing enterocytes and producing the enterotoxin NSP4, which plays a role in interfering with intracellular electrolyte homeostasis. A previous study revealed that metabolites of *L. casei* and *Bifidobacterium adolescentis* reduce the production of enterotoxin NSP4, and these probiotic metabolites can be considered antiviral agents (27).

In addition, probiotics such as *lactobacillus* and *bifidobacterium* have antiviral activity against rotavirus through the production of lactic acid, nitric oxide, H_2O_2, bacteriocin, and inducing the production of antimicrobial peptides, mucin, and IgA in the host and activating innate immune responses (28).

Another viral disease that is more common in children under the age of 5 is hand, foot, and mouth disease (HFMD), in which coxsackievirus and enterovirus 71 (EV71) are the main causes of this disease. In in vitro conditions (cell lines), Lactobacillus reuteri has an antiviral effect against the two mentioned pathogens, while this feature has not been reported for *L. casei* (29). Also, among bifidobacteria, three species of *B. adolescentis*, *Bifidobacterium longum*, and *Bifidobacterium pseudocatenulatum* can be active against coxsackievirus (30). As discussed in the following sections, probiotics can have beneficial therapeutic effects by regulating the immune system. *Lactobacillus rhamnosus* prevents the multiplication and maturation of the influenza A virus by stimulating the production of interleukin-1β and type I interferon in macrophages (31).

The combination of *B. longum* with the microalgae *Chlorella sorokiniana* increases the expression of interferon-α in HT-29 cells (human colon tumor cells) during pre-infection and also interferon-β after infection with rotavirus, which has been effective in reducing the amount of virus (32). Another antiviral mechanism of probiotics is engulfing or binding to the virus, which prevents it from attaching to the target cells in the host. Also, the protein produced or the biofilm formed by some probiotics by covering the receptor interferes with the binding and pathogenicity of the virus (33).

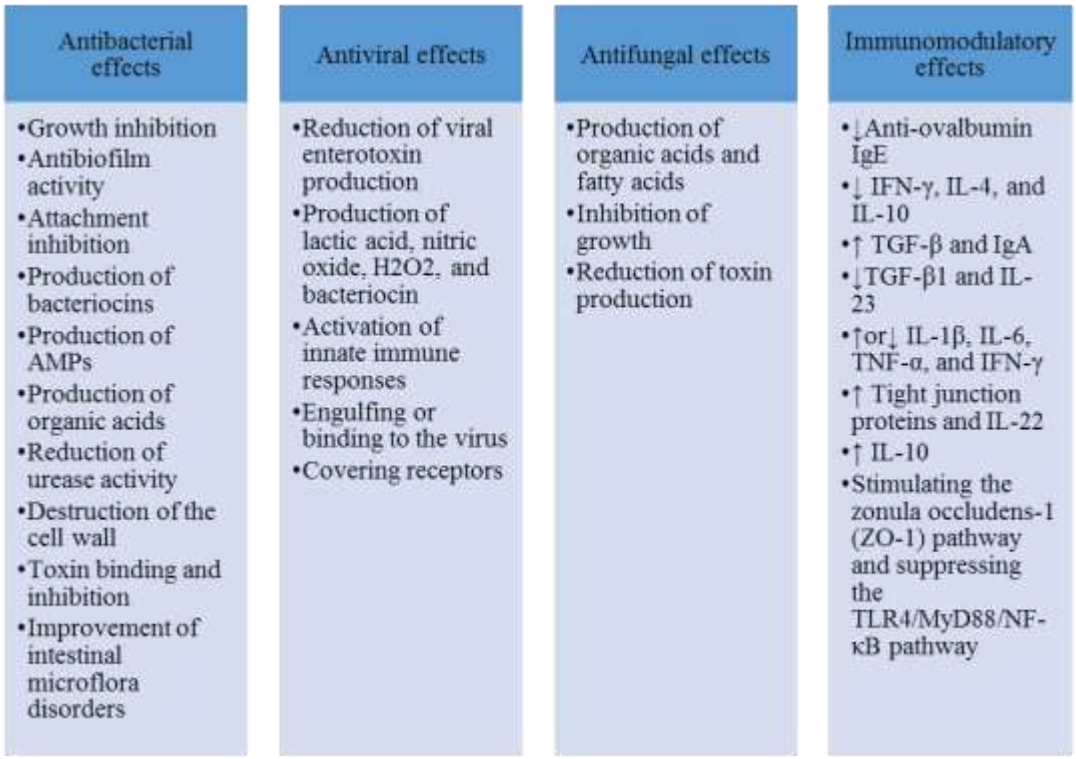
4. Antifungal effects

Metabolites such as organic acids and fatty acids from some lactobacillus species have antifungal properties against the filamentous fungi *aspergillus flavus* and *aspergillus fumigatus* (34). For example, organic acids, including lactic acid, acetic acid, phenyllactic acid, and hydroxyl-phenyllactic acid from *l. Plantarum*, have an inhibitory effect on the growth of different species of aspergillus and ochratoxin a (ota) (35, 36). Also, saccharomyces cerevisiae, which is a yeast, prevents the growth and production of ota in different fungal species, except in situ conditions, where the production of ota increases on the contrary due to the presence of aspergillus niger (37). In addition to inhibiting the growth of a. Flavus and the spores of this fungus, *l. Plantarum* can have a destructive effect on aflatoxin b1, a carcinogenic and hepatotoxic toxin (38).

Similarly, *l. Plantarum* and its metabolites reduce the production of deoxynivalenol (don) mycotoxin by creating an inhibitory effect on the growth of *fusarium* sp. Also, this probiotic can detoxify mycotoxin by binding or transforming don into another metabolite. Deoxynivalenol (don), or vomitoxin, is a mycotoxin produced by *fusarium* sp. That contaminates food, including grains, and causes diarrhea, vomiting, and gastroenteritis in humans (39, 40). The inhibitory effect of bacillus coagulans on fusarium growth and don production has also been reported (41). A study has reported that cells and cell-free supernatants of *l. Acidophilus* and *l. Plantarum* have antifungal activity against candida species isolated from the mouths of aids patients (42).

4. Immunomodulatory effects

A previous study has shown that oral administration of *l. Acidophilus* strain l92 killed in mice reduces anti-ovalbumin ige. Also, this strain reduces the level of cytokines related to th1 and th2, such as interferon-γ (ifn-γ), il-4, and il-10, and increases transforming growth factor β (tgf-β) (treg activator) and iga. In another study, *l. Acidophilus* suppressed the expression of tgf-β1 (a differentiation of th17) and il-23 to prevent excessive activity of th17 in colitis and ibd. Therefore, according to the immunomodulatory feature of *l. Acidophilus*, this strain can be effective in the treatment of diseases such as allergic rhinitis, colitis, and autoimmune and inflammatory diseases (43–45). Il-1β increases intestinal tight junction permeability by activating the nuclear factor-κb (nf-κb) pathway. *L. Acidophilus* and *bifidobacterium infantis* can protect the intestinal barrier by inhibiting this pathway (46). Also, co-cultures of *l. Acidophilus* and *bacillus subtilis* have an increasing effect on the level of tight junction proteins and il-22, which is an anti-inflammatory cytokine (47). *Lactobacillus reuteri* biofilm exerts its immunomodulatory properties by suppressing tumor necrosis factor (tnf) production (48). A previous study reported that extracellular polysaccharide from *l. Plantarum* has antioxidant properties and increases il-1β, il-6, tnf-α, and ifn-γ cytokines (49). *Infantis* alone has immunomodulatory effects such as reducing th2 and th17 and increasing treg, which can be effective in the treatment of guillain-barré syndrome (50).



Also, reducing pro-inflammatory cytokines (tnf-α and il-6) is another capability of *b. Infantis* (51). Anti-allergic and modulating effects have been observed in the case of *bifidobacterium breve*, which occurs by inhibiting the activation and maturation of dendritic cells and t cells such as th2 and stimulating the activity of treg (52–54). *Bifidobacterium lactis* exerts its anti-inflammatory effects by suppressing the production of tnf-α and il-6 and increasing the level of il-10 (55, 56). In the animal model of immunosuppressed mice, where the intestinal mucosa is damaged and inflamed by cyclophosphamide, it has been observed that *bacillus coagulans* improves and restores the intestinal barrier by stimulating the zonula occludens-1 (zo-1) pathway and suppressing the tlr4/myd88/nf-κb pathway (24). Therefore, due to their immunomodulatory properties, probiotic bacteria have the potential to be used in the treatment of autoimmune, inflammatory, and allergic diseases.

Conclusion

Although the mechanism of action of probiotics is far from being fully understood, their stabilizing effects on the digestive system are well known. In addition, there have been reports of the anti-obesity, anti-diabetic, anti-cancer, cholesterol-regulating, and improving neuropsychiatric effects of probiotics. Probiotics exert their inhibitory effect on pathogen bacteria through the production of organic acids, antimicrobial peptides, and bacteriocin, which may be able to obtain more favorable results only by extracting these compounds and concentrating them, which requires other studies. Another mechanism is that probiotic microorganisms prevent pathogens from binding to their receptors and causing disease. The antiviral activity of probiotics is also based on the production of metabolites and inhibition of binding, in addition to the fact that they prevent the activity of viruses by stimulating the

production of cytokines. The antifungal effects of probiotics occur by inhibiting the growth and production of toxins by the pathogen. Also, probiotic microorganisms can improve autoimmune, inflammatory, and allergic diseases by modulating immune mediators.

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