Review Article

Probiotics: an update on mechanisms of action and clinical applications

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Abstract

Probiotics are live microbial feed supplement and can provide health benefit to the host if administered in sufficient amounts. The most predominant species that have been used as probiotic include Lactobacilli and bifidobacteria. Proper administration of probiotics could be efficient in the treatment of various disorders. However; their mechanism of action is poorly understood. The effects of probiotics may be classified in following modes: reinforcement of the intestinal mucosal barrier against pathogens, competition with pathogens for adherence to the mucosa and epithelium, competitive exclusion of pathogenic microorganisms, production of antimicrobial substances, modulation of the immune system and interference with quorum sensing signaling. Exploration of the clinical features of probiotic strains, their modes of action and investigation based on probiotic therapy may be beneficial in treatment of various diseases.

Key words: Probiotics, Lactobacillus, Bifidobacterium, UTI

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Introduction

According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), probiotics are live microorganisms that can confer a health benefit to the host if administered in adequate amounts. The name probiotic comes from the Greek 'pro bios' which means 'for life'. At the beginning of the 20th century, Probiotic was first conceptualized by the Russian Nobel Prize winner and father of modern immunology, Elie Metchnikoff. In 1965, the first introduction of probiotic term was reported by Lilly and Still wellin to describe 'substances secreted by one microorganism which stimulate the growth of another'. In 1974 Parker proposed that probiotics are organisms and substances which contribute to intestinal microbial balance. In 1989, Fuller introduced the modified definition of probiotic. According to the Fuller definition, probiotics are a live microbial feed supplement which beneficially affects the host by improving its microbial balance. According to definition of Salminen et al, in 1998, probiotics are foods which contain live bacteria which can be beneficial to health, whereas Marteau et al in 2002 defined probiotics as microbial cell or components of microbial cells that have a beneficial effect on the health and well-being.

There are several characteristics for assessment of a good probiotic product. Many investigators believe that the definition of an ideal probiotic is based on the following options: resistant to gastric acid digestion and to bile salts, high ability to multiply in the gut,
strong adhesive capability with the digestive tract of the host, lacking side effect; do not have pathogenic and toxic effect on host cells, durable enough, ability to reduce the pathogenic microorganisms²⁹.

The most predominant species that have been used as probiotic bacteria are summarized in Table 1. However, probiotic bacteria can also be derived from the intestinal microbiota of healthy humans, nonhuman strains used in the fermentation of dairy products or species from genera such as Streptococcus, Bacillus and Enterococcus. Due to some of genera such as *Streptococcus*, *Bacillus*, and *Enterococcus* containing many pathogenic species there is some concerns about safety of such probiotics. Yeasts especially Saccharomyces have also been used as probiotics for many years¹⁰⁻¹³.

Table 1. Commercial probiotic microorganisms

<table>
<thead>
<tr>
<th>Lactobacillus species</th>
<th>Bifidobacterium species</th>
<th>Other bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>B. bifidum</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>L. casei (rhamnosus)</td>
<td>B. longum</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>L. johnsonii</td>
<td>B. breve</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>L. bulgaricus</td>
<td>B. infantis</td>
<td>Enterococcus faecalis</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>B. lactis</td>
<td></td>
</tr>
<tr>
<td>L. reuteri</td>
<td>B. adolescentis</td>
<td></td>
</tr>
<tr>
<td>L. lactis</td>
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</tbody>
</table>

**Discussion**

A prebiotic is non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one species or a limited number of species in the colon. Oligosaccharides such as lactulose, galactooligosaccharides, inulin and fructooligosaccharides, are some of the prebiotics that are able to promote the growth and activities of probiotics. Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism¹⁴.

**Mechanisms of action of probiotics**

Probiotic bacteria have various effects on the host cells and are advocated for the prevention and treatment of a wide range of disorders. The main mode of probiotic actions include 1) enhancement of the epithelial barrier 2) increased adhesion to intestinal mucosa and simultaneous inhibition of pathogen adhesion 3) competitive exclusion of pathogenic microorganisms 4) production of anti-microbial substances 5) modulation of the immune system. Recent data exhibit the effect of probiotics in interference with quorum sensing.¹⁴⁻¹⁶

**Enhancement of the epithelial barrier**

Intestinal barrier in healthy individual consists of the mucous layer, antimicrobial peptides, secretory IgA and epithelial cells that form tight junctions¹⁵. Disruption of the intestinal barrier function may result in enteric infections, celiac disease, some autoimmune diseases such as type1 diabetes and inflammatory bowel disease (IBD). Consumption of probiotic bacteria can contribute to increasing intestinal barrier function¹⁶⁻¹⁹.

Intestinal barrier integrity may be increased by enhancing the expression of genes involved in tight junction signaling²⁰. Some of the probiotics are able to induce signaling pathway involved in tight junction. For instance, *Lactobacilli* modulate the regulation of E-cadherin and β-catenin, in a T84 cell. Moreover, incubation of intestinal cells with *lactobacilli* modulates tight junction protein phosphorylation²¹.

Probiotics may also repair the barrier function after damage. *Escherichia coli* Nissle 1917 (EcN1917) not only counteract the disruptive effects of enteropathogenic *E. coli* (EPEC) but also restores mucosal integrity in Caco-2 cells. This effect is
achieved by increasing expression and repartition of tight junction proteins of the zonula-occludens (ZO-2) and altering protein kinase C signaling. In a TLR-independent pathway, Probiotics can protect the integrity of the mucosal gut barrier against the destructive action of enteropathogenic Escherichia coli. Alteration in levels of pro-inflammatory cytokines can lead to intestinal permeability in intestinal disease such as IBD. Consumption of probiotic bacteria prevents cytokine-induced epithelial damage. Soluble peptides secreted by Lactobacillus rhamnosus GG (LGG), p40 and p75 can prevent TNF-a-mediated cell apoptosis by activating the anti-apoptotic factor Akt and protein kinase B (PKB/Akt) and inhibiting the pro-apoptotic p38/mitogen-activated protein kinase (MAPK). Induction of mucous secretion by probiotic bacteria reinforces barrier function and the exclusion of pathogens. Some of Lactobacillus species increases the expression of MUC2, MUC3 and MUC5AC in HT29 cells.

Probiotic strains can also release antimicrobial proteins (AMPs) such as α defensins, β defensins, cathelicidins, C-type lectins and ribonucleases from epithelial cells. These small proteins can stabilize intestinal barrier function. Defensins display antimicrobial activity against a wide range of bacteria fungi and viruses. Cathelicidins are involved in host defense against pathogens.

Increased adhesion to intestinal mucosa
Probiotic bacteria are able to adhere to epithelial cells, thereby, can block adherence of pathogens. The anti-adhesive effect might be the result of competition between probiotic strains and pathogens for the same receptor or the induction of mucin production by probiotics.

Several studies exhibited that different lactobacilli proteins promote mucous adhesions and bacterial surface adhesions mediate attachment to the mucous layer. MUB (mucus-binding protein) produced by Lactobacillus reuteri and MapA (mucus adhesion-promoting protein) by L. reuteri and L. fermentum has been reported to mediate the binding to mucous. Probiotic bacteria competitively inhibit adhesion of pathogenic bacteria. Lactobacillus GG and Lactobacillus plantarum 299V competitively inhibit the attachment of enterohemorrhagic E. coli 0157H7 to HT-29 cells.

Competitive exclusion of pathogenic microorganisms
Based on several reported researches, probiotic bacteria are able to exclude or reduce the growth of pathogens by one of following ways: creation of a hostile microenvironment, physical blocking of available bacterial receptor sites, production and secretion of antimicrobial substances and selective metabolites and competition for essential nutrients.

Production of antimicrobial substances
Antimicrobial substances produced by probiotics can lead to inhibition of pathogen replication. These components are almost always low-molecular-weight (LMW) compounds (< 1,000 Da). The most important of these LMW compounds are short chain fatty acids. Organic acids especially acetic acid and lactic acid inhibit the growth of Gram-negative bacteria. Also low-molecular-weight bacteriocins (LMWB) and high-molecular-weight bacteriocins produced by lactobacilli can inhibit pathogen replication. Some of lactic acid bacteria (LAB) produce bacteriocins. Lacticin B is produced by L. acidophilus and plantaricin by L. plantarum andnisin. These bacteriocins have a narrow activity spectrum against related species such as other lactobacilli and taxonomically related Gram-positive bacteria or broad activity spectrum against across genera such as Gram-positive and Gram-negative bacteria as well as yeasts and molds. Some of the most probable mechanisms of bacteriocins are formation of pore in cell membrane and inhibition of cell wall synthesis.

Studies done on probiotic bacteria exhibited that some of them can produce antibacterial compounds. Lactobacillus reuteri can produce reuterin antibiotic that is a broad spectrum antibiotic which not only is active against Gram-positive and Gram-negative bacteria but also against yeast, fungi, protozoa and viruses. Intestinal bifidobacteria and lactobacilli also produce fatty acids such as conjugated linoleic acid (CLA) which is a potent anti-carcinogenic agent. CLA-
Probiotics and the immune system

Probiotic bacteria can effect on numerous cell types involved in the innate and adaptive immune responses such as epithelial cells, dendritic cells, monocytes/macrophages, B cells, T cells, regulatory T cells and NK cells and thereby exert their immunomodulatory effect.\(^{55}\)

Effects of Probiotic Bacteria on Dendritic Cells

Dendritic cells (DCs) are antigen-presenting cells that act as messengers between the innate and the adaptive immune systems. Specialized functions of DC in intestine lead to induction of oral tolerance to dietary antigens by development regulatory T cells and IgA-producing B cells through production of cytokines such as IL-10 and TGFβ.\(^{56-59}\) Probiotic bacteria can down-regulate Th1 response and inhibit the production of pro-inflammatory cytokines, IL-12, TNF-α, and IFN-γ by DC.\(^{60}\)

Effects of Probiotic Bacteria on B Lymphocytes

Administration of \(L.\ rhamnosus\ GG\) to children with acute gastroenteritis increased a nonspecific humoral immune response by an enhancement in IgG, IgA, and IgM secretion from circulating lymphocytes.\(^{62}\) In vaccination trial, the effects of probiotic bacteria on B lymphocytes have been proven. Studies indicate that combination of \(L.\ rhamnosus\ GG\) and Salmonella vaccination can increase \(Salmonella\)-specific IgA levels.\(^{63}\)

Effects of Probiotic Bacteria on Natural Killer (NK) Cells

More recently, in an animal study it have been proven that \(L.\ casei\) ssp. casei with prebiotic such as dextran enhanced the NK cell activities in spleen mononuclear cells of BALB/c mice and its oral administration increased production of IL-12 in human blood mononuclear cells.\(^{64}\) Secretion of IL-22 by NK cells has anti-inflammatory effects on epithelial cells in vitro.\(^{65}\) In addition, activity of NK cells will increase with consumption of a low fat milk drink with \(B.\ lactis\ HN019\).\(^{66}\)

Effects of Probiotic Bacteria on T cells

T cell response is mainly controlled by interactions between DC and T cells. Assessment of effect monocyte-derived dendritic cell cultured with \(L.\ rhamnosus\) exhibited that T-cell proliferation and T-cell cytokine production, particularly IL-2, IL-4, and IL-10 had decreased dramatically.\(^{67}\)

Interference with signaling factor of quorum sensing

Bacteria communicate with each other and their surrounding environment through chemical signaling molecules that are called auto-inducers. This phenomenon is known as quorum sensing (QS) that can measure the population density, nutrient concentration and other ecological characteristics. In addition to, QS can control the gene expression of the entire community in response to changes in cell number.\(^{68,69}\)

Probiotic bacteria such as lactobacillus, bifidobacterium and \(B.\) cereus strains can produce auto-inducers that can control virulence gene expression in numerous microorganisms. Lactobacillus acidophilus La-5 secretes a compound that reduces the production of auto-inducer by \(E.\ coli\) O157 and through it, leads to significant reduction in the transcription of genes involved in colonization.\(^{70}\) Recently, many studies have reported similar results for \(B.\) cereus and \(B.\ toyoi\).\(^{71,72}\)

Applications of probiotics

In the past century the beneficial roles of nonpathogenic bacteria in the establishment of health were described. Recently it is proved that there are clinical benefits to use specific nonpathogenic organisms and also there is strong evidence for their efficacy in some clinical scenarios. Considering beneficial roles of probiotics, they are now widely used in many countries by consumers and in clinical practice.
Diarrheal diseases
Diarrhea is defined as the passage of 3 or more unformed stools for at least 2 consecutive days. Probiotics are able to protect the host against bacterial toxins and in this way impede diarrhea. *L. casei rhamnosus* (Lcr35) is found to inhibit colonization of large variety of pathogens in Caco-2 cell line and therefore prevent traveler’s diarrhea.73

*Bifidobacterium lactis* HN019 reduced the severity of diarrhea associated with both rotavirus and *E. coli* in pigs. This effect is attributed to the increase of immune-mediated protection.74 A meta-analysis done on probiotics exhibited that Saccharomyces boulardii and the bacterium Lactobacillus acidophilus in combination with L. bulgaricus, L. rhamnosus strain GG are effective in prevention of antibiotic-associated diarrhea. Another meta-analysis study done on probiotic bacteria showed that Lactobacillus species, Enterococcus species, and S boulardi are effective in the treatment of infective diarrhea in both adults and children (00). The preventive effect of *Lactobacillus GG* on antibiotic-associated diarrhea (AAD) has been assessed in children receiving different antimicrobial treatments.75

Saccharomyces boulardii is effective in prevention and decrease of recurrent *C. difficile* infection (CDI). The effect of *S. boulardii* on *C. difficile* toxin in rats and human colonic mucosa is probably due to proteolytic digestion of toxins by secretion of protease. Although, they are used as a preventive and therapeutic measure but their role in treatment and prevention of CDI remains controversial. The best studied probiotic agents in CDI are Saccharomyces boulardii and Lactobacillus. Several studies shown that mixtures of probiotics can be useful in treatment and prevention of ADD and CDI.76, 77

Urinary tract infections
There is a close correlation between the loss of the normal microbiota of genital tract, especially *Lactobacillus* species, and an increased incidence of UTIs. The role of probiotics in prevention of UTI is obvious.78 *Lactobacillus plantarum* and *Lactobacillus rhamnosus* inhibit the adherence of *E. coli* to the gastrointestinal (GI) tract wall by inducing the production of mucin and leading to inhibition of the adhesion of pathogens.79 Recent research has shown that two probiotics strains, *Lactobacillus acidophilus* PXN35 and *Lactobacillus plantarum* PXN47 have good anti-bacterial effects in inhibiting *E. coli* growth.80 In study performed by Kontiokari et al. in 2003, they showed that consumption of fermented milk products containing probiotic bacteria more than three times a week was associated with a decreased risk of recurrent UTIs (RUTIs).81 When *Lactobacillus fermentum* orally administer with low doses of ampicillin, was effective in the treatment of *E. coli* urinary tract infection in a mouse model.82

Helicobacter pylori infections
During recent years, several studies in order to assess probiotics effect in the treatment of *H. pylori* infections have been done.83 In vitro studies demonstrated that strains of lactobacilli, bifidobacteria and *B. subtilis* inhibit the growth or the adhesion of *H. pylori* to mucus cells.84-86 In mice model, Lactobacillus salivarius eliminated the colonization of *H. pylori*.87

Vaginal dismicrobism
The vaginal microbiota of healthy women consists of a wide variety of anaerobic and aerobic bacterial genera and species dominated by the genus Lactobacillus. Lactobacillus especially during pregnancy helps to maintain the natural healthy balance of the vaginal microbiota. Nad thereby decreases the rate of bacterial vaginosis (BV) and aerobic vaginitis (AV). In a study conducted by Hilton, oral administration of *L. johnsonii* effectively decreased candida vaginitis.88 The production of hydrogen peroxide and lactic acid by *lactobacilli* contribute to reduction of pH and may inhibit growth of *Gardnerella* and other bacteria.89, 90

Food allergy
Small peptides and amino acids produced by some bacteria are able to induce allergic reactions. Several investigators have shown that imbalance between *bifidobacteria* and *clostridia* can lead to allergies.84 Enzymes derived from *L. casei* GG degrade Small peptides and amino acids, in result it will produce molecules with inhibitory effects on lymphocyte proliferation.91 Mechanisms of anti-allergic probiotic bacteria include increasing intestinal barrier integrity,
modulation of the immune system through the production of inflammatory cytokines or enhancement of specific IgA responses and degradation of food antigens by means of productive enzymes of probiotics.

**Lactose intolerance**

Lactose intolerance (LI), also known as lactose malabsorption is the most common type of carbohydrate malabsorption. Due to low levels of lactase enzyme activity in patients, they are not able to digest lactose into glucose and galactose. Intensive intestinal distress with characteristic bloating, flatulence and abdominal pain could appear 30-minutes to 2 hours after consumption of food products containing lactose disaccharide. Lactases produced by L. acidophilis and Lactobacillus delbrueckii ssp. bulgaricus by β-galactoside galactohydrolase (β-gal) and LactococciStactics ssp. lactis and Lactococcus lactis ssp crenzoris by β-D-phosphogalactoside galactohydrolase (β--P-gal) are able to hydrolyze and ease absorption of lactose. The production of hydrogen in breath is an indicator of bacterial metabolism of lactose in the colon 92.

**Hypercholesterolemia**

Several studies showed that the reduction of serum cholesterol can be created by oral administration of L. johnsonii and L. reuteri in pigs. Probiotic bacteria can decrease blood cholesterol level by cholesterol absorption, de-conjugation of bile acids through bacterial hydrolysis, attachment of cholesterol to bacterial cell walls and inhibition of hepatic cholesterol synthesis 93, 94.

**Anticancer effects**

There are claims for anti-cancer activity of probiotics. *Lactobacillus*, *Bifidobacteria* and *E. coli* Nissle 1917 strains have anti-mutagenic activities due to ability to metabolize and inactivate compounds of mutagen 95. The anticancer effects of probiotics probably are due to several mechanisms such as inhibition of procarcinogen transformation to active carcinogens, inactivation of mutagenic compounds, production of anti-mutagenic compounds, suppression of the growth of pro-carcinogenic bacteria, reduction of the absorption of mutagens from the intestine and reinforcement of immune system function 96.

**Conclusion**

According to the clinically proven benefits of probiotics it is clear that probiotics can be used for the prevention of various diseases and even their management. Therefore, it is possible to conceptualize the use of probiotics due to their low cost, decrease in antibiotic resistance, modulation of immune system and inhibition of pathogens providing good alternative over the antibiotics.

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