Food Microbiology & Food Preservation
for
Vidyasagar University

e-Content

Chapter 6
Probiotics

Module No. 2
Health Benefits of Probiotics, including Mechanisms of Action

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1. Probiotics

The word "probiotic" comes from Greek, and it means "for life". Most probably, it was Ferdinand Vergin who invented the term "probiotic" in 1954, in his article entitled "Anti-und Probiotika" comparing the harmful effects of antibiotics and other antibacterial agents on the intestinal microbiota with the beneficial effects ("probiotika") of some useful bacteria.

According to the FAO and WHO report 2021, probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host.

2. Probiotics Used in Human Nutrition

- Probiotic products may contain one or more selected microbial strains.

- Human probiotic microorganisms belong mostly to the following geni: *Lactobacillus, Bifidobacterium*, and *Lactococus, Streptococcus, Enterococcus*. Moreover, strains of Gram-positive bacteria belonging to the genus Bacillus and some yeast strains belonging to the genus *Saccharomyces* are commonly used in probiotic products.

- Probiotics are subject to regulations contained in the general food law, according to which they should be safe for human and animal health. In the USA, microorganisms used for consumption purposes should have the GRAS (Generally Regarded as Safe) status, regulated by the FDA (Food and Drug Administration). In Europe, EFSA introduced the term of QPS (Qualified Presumption of Safety). The QPS concept involves some additional criteria of the safety assessment of bacterial supplements, including the history of safe usage and absence of the risk of acquired resistance to antibiotics.

- They are available as capsules, gels, pastes, tablets, packets, liquids, or powders, and are contained in various fermented foods, most commonly yogurt or dairy drinks.

Type *Lactobacillus*	Type *Bifidobacterium*	Other Lactic Acid Bacteria	Other Microorganisms
L. acidophilus [(a),*] *L. amylovorus* [(b),*] *L. casei* [(a),(b),*] *L. gasseri* [(a),*] *L. helveticus* [(a),*] *L. johnsonii* [(b),*] *L. pentosus* [(b),*] *L. plantarum* [(b),*] *L. reuteri* [(a),*] *L. rhamnosus* [(a),(b),*]	*B. adolescentis* [(a)] *B. animalis* [(a),*] *B. bifidum* [(a)] *B. breve* [(b)] *B. infantis* [(a)] *B. longum* [(a),*]	*Enterococcus faecium* [(a)] *Lactococcus lactis* [(b),*] *Streptococcus thermophilus* [(a),*]	*Bacillus clausii* [(a),*] *Escherichia coli Nissle 1917* [(a)] *Saccharomyces cerevisiae (boulardi)* [(a),*]

[(a)] Mostly as pharmaceutical products; [(b)] mostly as food additives; * QPS (Qualified Presumption of Safety) microorganisms.

3. Health Benefits of Probiotics

- In the face of widespread diseases and ageing societies, the use of knowledge on microbiocenosis (the group of interacting microbial organisms living in a specific habitat) of the gastrointestinal tract and on the beneficial effect of probiotic bacteria is becoming increasingly important.

- The consumption of pre-processed food (fast food), often containing excessive amounts of fat and insufficient amounts of vegetables, is another factor of harmful modification of human intestinal microbiota.

- There is currently no doubt about the fact that the system of intestinal microorganisms and its desirable modification with probiotic formulas and products may protect people against enteral problems, and influence the overall improvement of health.

- Probiotics may be helpful in the treatment of inflammatory enteral conditions, including ulcerative colitis, Crohn's disease, and non-specific ileitis.

- Numerous studies assessed the use of probiotics in the treatment of lactose intolerance, irritable bowel syndrome, and the prevention of colorectal cancer and peptic ulcers.

- A positive effect on the urogenital system [prevention and treatment of Urinary Tract Infections (UTIs) and bacterial vaginitis (BV)] constitutes an excellent example of the benefits associated with the use of probiotics.

- There were attempts to apply probiotics to pregnant women and neonates in order to prevent allergic diseases such as atopic dermatitis.

- There is evidence that the consumption of probiotics-containing dairy products results in the reduction of blood cholesterol, which may be helpful in the prevention of obesity, diabetes, cardiovascular diseases, and cerebral stroke.

- There are many reports on the application of probiotics in the treatment of diarrhoea. The application of *Saccharomyces boulardii* yeast to patients with acute, watery diarrhoea resulted in the cure and reduced frequency of that type of complaints in two subsequent months. The efficacy of probiotic strains in the therapy of nosocomial, non-nosocomial, and viral diarrhoeas has also been documented. It turns out that probiotics may increase the amount of IgA antibodies, which leads to the arrest of a viral infection.

- Antibiotic-associated diarrhoea (AAD) is a common complication of most antibiotics and *Clostridium difficile* disease (CDD), which also is incited by antibiotics, and is a leading cause of nosocomial outbreaks of diarrhoea and colitis. Using meta-analyses, three types of probiotics (*Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG, and probiotic mixtures) significantly reduced the development of antibiotic-associated diarrhoea. Only *S. boulardii* was effective for CDD.

4. Mechanism of Action of Probiotics

Molecular and genetic studies allowed the determination of the basics of the beneficial effect of probiotics, involving four mechanisms:

(a) Antagonism through the production of antimicrobial substances;

(b) Competition with pathogens for adhesion to the epithelium and for nutrients;

(c) Immunomodulation of the host;

(d) Inhibition of bacterial toxin production

4.1. Antagonism and competition

- Antagonism and competition are directly associated with their effect on other microorganisms.

- Those mechanisms are important in prophylaxis and treatment of infections, and in the maintenance of balance of the host's intestinal microbiota.

- The ability of probiotic strains to co-aggregate, as one of their mechanisms of action, may lead to the formation of a protective barrier preventing pathogenic bacteria from the colonisation of the epithelium.
- Probiotic bacteria may be able to adhere to epithelial cells, thus blocking pathogens. That mechanism exerts an important effect on the host's health condition.

- Moreover, the adhesion of probiotic microorganisms to epithelial cells may trigger a signalling cascade, leading to immunological modulation.

- Alternatively, the release of some soluble components may cause a direct or indirect (through epithelial cells) activation of immunological cells. This effect plays an important role in the prevention and treatment of contagious diseases, as well as in chronic inflammation of the alimentary tract or of a part thereof. There are also suggestions of a possible role of probiotics in the elimination of cancer cells.

- Low-molecular-weight substances produced by probiotic microorganisms (e.g., hydroperoxide and short-chain fatty acids) inhibit the replication of pathogens.

For example, *Lactobacillus* genus bacteria may be able to produce bacteriocins, including low-molecular-weight substances (LMWB - antibacterial peptides), as well as high-molecular-weight ones (class III bacteriocins), and some antibiotics.

- Probiotic bacteria (e.g., *Lactobacillus* & *Bifidobacterium*) may produce the so-called de-conjugated bile acids (derivatives of bile acids), demonstrating stronger antibacterial effect than the bile salts produced by their host.

- The nutrient essential for nearly all bacteria, except for lactic acid bacteria, is iron. It turns out that *Lactobacillus* bacteria do not need iron in their natural environment, which may be their crucial advantage over other microorganisms. *Lactobacillus delbrueckii* affects the function of other microbes by binding iron hydroxide to its cellular surface, thus making it unavailable to other microbes.

4.2. Immunomodulation

The immunomodulatory effect of the intestinal microbiota, including probiotic bacteria, is based on three:

(a) Induction and maintenance of the state of immunological tolerance to environmental antigens (nutritional and inhalatory);
(b) Induction and control of immunological reactions against pathogens of bacterial and viral origin;
(c) Inhibition of auto-aggressive and allergic reactions.

- Probiotic-induced immunological stimulation is also manifested by the increased production of immunoglobulins (Igs), enhanced activity of macrophages and lymphocytes, and stimulation of γ-interferon (IFN) production.

- Probiotics may influence the congenital and acquired immunological system through metabolites, components of the cellular wall, and DNA, recognised by specialised cells of the host (e.g., those equipped with receptors).

- The principal host cells that are important in the context of the immune response are intestinal epithelial cells and intestinal immune cells. Components of the cellular wall of LAB stimulate the activity of macrophages. Those, in turn, are able to destroy microbes rapidly by the increased production of free oxygen radicals and lysosomal enzymes.

- Probiotic bacteria are also able to stimulate the production of cytokines by immunocompetent cells of the gastrointestinal tract.

- On the other hand, the immunological activity of yeast is associated with the presence of glucans in their cellular wall. Those compounds stimulate the response of the reticuloendothelial system (RES).

4.3. Inhibition

- Inhibition of the production of bacterial toxins—is based on actions leading to toxin inactivation and help with the removal of toxins from the body.

- Help in detoxification from the body can take place by adsorption (some strains can bind toxins to their cell wall and reduce the intestinal absorption of toxins), but can also result from the metabolism of mycotoxins (e.g., aflatoxin) by microorganisms. However, not all probiotics exhibit detoxifying properties, as it is a strain-related characteristic. The effectiveness of some probiotics in combating diarrhoea is probably associated with their ability to protect the host from toxins.

Short-type Questions

i) Probiotics are

a) pathogens, b) live microbes, c) protein, d) none of the above.

ii) Which of the following is used for human probiotics?

a) Klebsiella spp., b) Shigella spp., c) Enterococcus spp., d) Salmonella spp.

iii) *Saccharomyces boulardii* is a bacterial probiotic. (True/False)

iv) Probiotics have the potential to prevent peptic ulcer. (True/False)

v) Class III bacteriocins are

a) high mw proteins, b) high mw proteins, c) carbohydrates, d) none of the above.

vi) Probiotics enhance the phagocytic activity of macrophages as one of the mechanisms of immunomodulatory effects. (True/False)

vii) What is the full form of QPS microorganisms?

viii) Strains of Gram-negative bacteria mostly belong to Bacillus are used as probiotics. (True/False)

ix) Which component of yeast probiotics stimulates the activity of RES?

a) nucleic acid, b) glucans, c) steroids, d) none of the above.

x) Which of the following nutrient essential for pathogens is sequestrated by probiotic organisms?

a) Manganese, b) Cobalt, c) Nickel, d) Iron

Answer Keys to the Short-type Questions

i) b, ii) c, iii) F, iv) T, v) a, vi) T, vii) Qualified Presumption of Safety, viii) F, ix) b, x) d

Suggested Readings

i) Jay JM, Loessner MJ, Golden DA. Modern Food Microbiology. 7th Ed. Springer. 2005.

ii) Ray B. Fundamental Food Microbiology. 3rd Ed. CRC Press, Washington D.C. 2005.

iii) Montville TJ, Matthews KR, Kniel KE. Food Microbiology, An Introduction. 3rd Ed. ASM Press, Washington D.C. 2012.