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Probiotics and food safety: an evidence-based review

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ABSTRACT

Probiotics are commonly defined as live microorganisms (yeast or bacteria), when getting ingested in adequate amounts, they exhibit the beneficial effects on the host. During the past two decades, probiotic microorganisms as health-promoting agents have been increasingly added to various types of food products, especially in fermented food and also drugs. Due to the importance of food safety aspects of the human diet and with regards to some adverse effects of probiotics for human, we decided to carry out a review on probiotics and their adverse effects by research in literature. Previous studies indicated that several aspects, including safety, functional and technological characteristics, have to be considered in the selection of probiotic microorganisms. Safety aspects include origin (gastrointestinal tract of healthy human), non-pathogenicity and antibiotic resistance. Some probiotic microorganisms such as enterococci have been considered as an opportunistic pathogen for humans and cause disease, possess agents for antibiotic resistance and potential virulence factors. The bacteria used as a probiotic in food should be completely safe. Probiotic bacteria should be chosen from the healthy human micro-flora and should not have any antibiotic resistance that would prevent treatment of a rare probiotic infection. This review focused on key issues concerning the safety aspects of probiotics added to particular food products for improvement of general health and also discussed the criteria for probiotic selection in details.

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1. Introduction

In recent years, great attention is being paid to the concept of food safety because unsafe food propels a cyclical process of disease formation and malnutrition, especially in infants, young children, the elderly, and the sick (1).

Recent tendency to use health-friendly bacteria called probiotics in the food industry and commercial products such as dietary supplements containing live bacteria is increasing on an ongoing basis due to their beneficial effects on the immune system and generally on the human health. These bacteria are added intentionally to food as technological additives (such as starter cultures) or as functional additives because of their benefits to human health (2,3).

Probiotics are microorganisms (bacteria and yeasts) when getting ingested in sufficient amounts alive, show positive effects on human health (4-6). Over the past two decades, probiotic microorganisms as health promoting agents have been used increasingly in various types of foods, especially fermented products. Probiotics are also available as food supplements or pharmaceutical products. The results of some studies have shown that the best effect is when these bacteria are added to the food (7,8). Such products have been named in various terms such as probiotic, synbiotic and functional foods (2). A major advance in functional foods is related to the diet including probiotics and prebiotics that promote the

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health of the intestinal microbial flora. There is a growing scientific evidence to support the concept that maintaining healthy gut microbiota can provide protection from digestive disorders such as gastrointestinal infections, irritable bowel syndrome (IBS) and even cancer (9). In addition to the use in human foods, probiotics are also used in animal foods to strengthen and stabilize animal growth and products (8).

Gibson and Roberfroid defined probiotics as a viable microbial dietary supplement, which improves the balance of gut microbiota in the host (10). The human digestive system is host to more than 500 known species of germs; this microflora of the human intestine is metabolically active and has many beneficial effects on the host. The human gut microflora can be altered in three ways of taking antibiotics, prebiotics and probiotics (11,12). In order to make probiotics effective for humans, the count of living microorganisms should be greater than 6 log cfu/g to provide a suitable daily dosage of 10^6-10^9 living bacteria (13).

The common probiotic bacteria used in food and drug products mainly include specific species of *Bifidobacterium* and lactic acid bacteria, especially *Lactobacillus* and *Streptococcus* (3). Probiotic products can also include a combination of species. *Escherichia coli*, *Enterococcus faecalis* and *Saccharomyces boulardii* are less commonly used (14).

Lactic acid bacteria (LAB) have been used traditionally as natural biopreservatives of food and feed preventing the growth of spoilage microorganisms. LAB have a generally recognized as safe status and researches have shown that they may improve human and animal health as probiotics. LAB are widely applied for the production of fermented foods such as dairy, meat products, and vegetables. LAB produce some antagonistic compounds such as lactic acid, organic acids, fatty acids, hydrogen peroxide, and bacteriocins, which are able to control pathogenic bacteria and undesirable spoilage microflora (15).

Lactobacilli are commonly found in the oral cavity (10^3-10^4 cfu/g), ileum (10^3-10^7 cfu/g) and colon (10^4-10^8 cfu/g); they are dominant microorganisms in vagina (16). Among Lactobacilli, *L. acidophilus* and *L. casei* are normal flora of the human intestine and are compatible with the digestive system. *L. plantarum* is a compatible bacterium that can live in a variety of environments, such as dairy, meat, and many fermentative vegetables (17).

Information on the safety of probiotic products can be extracted through scientific or experiential documentation of their long and safe use history and consumer satisfaction. Today, researchers are constantly identifying newer and more specific species of probiotics. Obviously, the safety of products containing new probiotic species should be evaluated before adding to the products. The questions are whether probiotics strains increase the risk of opportunistic infections, whether the currently used probiotics increase the likelihood of opportunistic infections among people with immune deficiency, and whether probiotics are safe for infants and children. Therefore, it is very important to assess the risk and criteria needed to formulate guidelines and instructions for determining the safety of probiotic species, especially new ones and the need for extensive research in this field.

2. Health Effects of Probiotics

The use of live microorganisms in food, especially LAB, has a long history to maintain and improve human health. In the past, a Roman historian recommended the use of fermented milk products to treat gastroenteritis. Elie Metchnikoff, in 1900, suggested that the consumption of yogurt containing lactobacillus would reduce toxigenic bacteria in the intestine, thereby increasing the host's lifespan. It has now been proven that when the concentration of Lactobacilli increases in the intestinal mucosa, the count of gram-negative anaerobic bacteria, Enterobacteriaceae and sulfite-reducing clostridia will be reduced (18).

The mechanism of beneficial effects of probiotics on human digestive health is still not fully understood. Generally, the probiotics through adhesion and establishment in GI-tract inhibit the growth of pathogenic bacteria, improve the gut microbial balance and enhance the function of the gastrointestinal mucous barrier. The probiotics control the transmission of food antigens and stimulate the systemic and mucosal immune system of the host. They are capable of removing carcinogens (11,19).

The efficacy of probiotics is influenced by the food matrix used to deliver. An ideal probiotic should survive during the passage of the digestive tract, to be permanently located in the intestine, and to exert beneficial effects on host health by enhancing immune responses, synthesis of compounds such as short chain fatty acids, lactic acid, and bacteriocin, and other mechanisms (20).

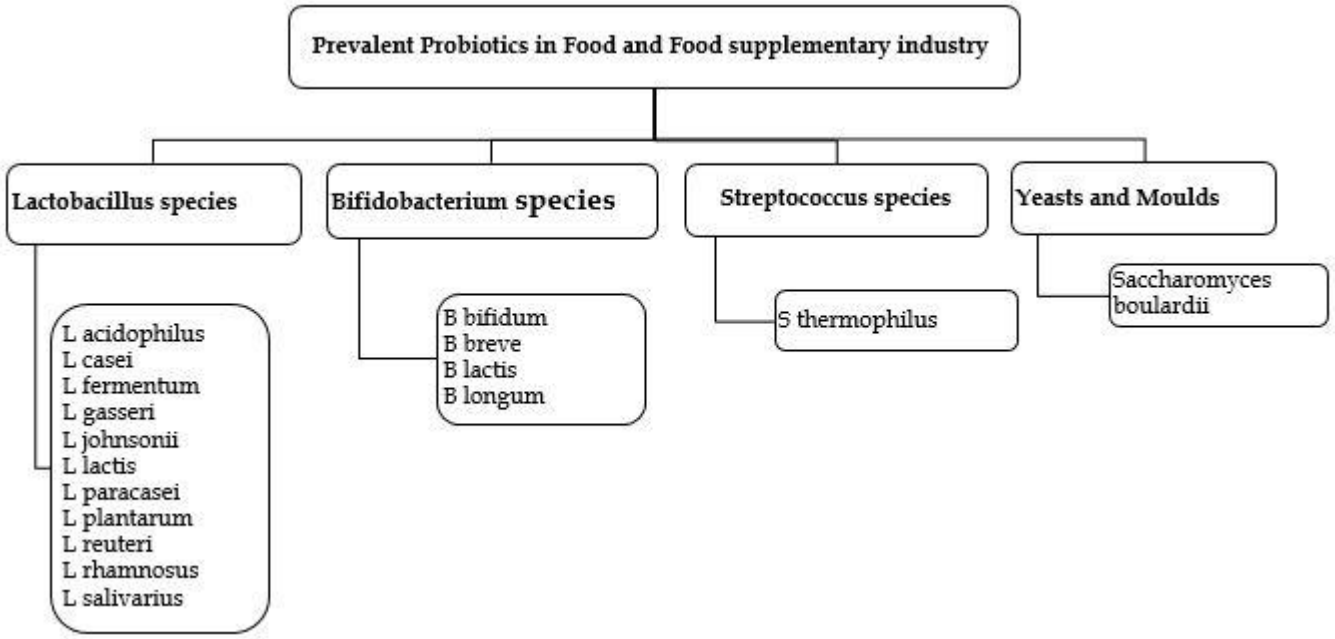

Figure 1. Common Probiotic Microorganisms that are used in yogurt, fermented milk products and food-supplementary

The potential of immune system modification by probiotics has proposed a new therapeutic strategy for coping with inflammatory and infectious conditions and hope for the use of probiotics in the treatment of infectious diseases and chronic diseases, such as inflammatory bowel syndrome. Some of the effects of probiotics on health of human GI-tract and the prevention and treatment of gastrointestinal diseases include helping to treat diarrheal disease such as infectious diarrhea, diarrhea caused by antibiotics, diarrhea caused by *Clostridium difficile*, inflammatory bowel diseases such as ulcerative colitis, Irritable bowel syndrome, lactose intolerance, constipation, allergies and *Helicobacter pylori* infection (3,21). There are many proposed mechanisms to justify the ability of probiotics to protect the host against gastrointestinal disorders. All processes in which bacteria inhibit the colonization and the establishment of other bacterial species in the body called "colonization resistance". Different species of Bifidobacteria are known to be resistant to the colonization of pathogenic bacteria in the large intestine (20).

3. The Risk of Probiotic's Infections

Some probiotic microorganisms, such as enterococci, not only help maintain the gut microflora, stimulate the immune system and increase the nutritional and organoleptic value of food, but also are opportunistic pathogens leading to human disease and have antibiotic resistant agents and virulence factors. Due to this dual nature of *Enterococci* and also the increased Enterococcal infections, their usage as probiotics is still questionable. The high prevalence of Enterococcal infections is reported mainly from *E. faecalis* or *E. faecium*. The safe species of *Enterococci* used as probiotics should not have any of the virulence factors and cannot acquire an antibiotic-resistant gene (22). It is necessary to consider the safety evaluation in relation to the nature of the microorganisms used, the method of application, exposure levels, the health status of the consumer and the physiological functions (5).

Although, *Bifidobacteria* and *Lactobacilli* have a long history of safe and prolonged use (23), infection with some of these species has been observed in rare cases. Since *E. coli* and *Enterococci* are known to be a class of pathogens, the use of these species as probiotics and the safety assessment of these strains should be carefully investigated (2).

Many resources of *Lactobacilli* and *Bifidobacteria* are available, including the probiotic products, fermented foods (such as yogurt, cheese, cabbage, olives and other fermented vegetables) as well as host micro-flora (16). Infections caused by *Lactobacilli* and *Bifidobacteria* are rare and are estimated 0.05% -0.4% of infectious and bacterial endocarditis. Over the past 30 years, 180 cases of *Lactobacillus bacteremia* have been reported, 69 of which were *Lactobacillus endocarditis*

during the same period (16). Some clinical studies have shown that there is a relationship between *Lactobacillus* and endocarditis, meningitis and pneumonia (24).

In Finland, where the registration of all bacterial isolates are mandatory, a number of infections, including *Lactobacillus* species, were reported to the National Public Health Institute (Helsinki, Finland), which remained at a low level of 10-20 cases a year. Increased consumption of probiotic *Lactobacilli* and *Bifidobacteria* in consumers did not lead to an increase in such opportunistic infections. The average incidence is 0.2% for 1995-1999 without apparent trend. This constant level has occurred versus a significant increase in the consumption of probiotic products. Regarding the incidence rate of infection with *L. rhamnosus*, (25, 26). The most common cases of *Lactobacillus* infection have occurred in patients with specific conditions. Most of these patients die in the course of one year as the infection progresses. Lactobacillus bacteremia can often lead to serious illness (27). At present, the risk of infection with probiotic Lactobacilli and Bifidobacteria is reported to be one case per million, and the risk of death is almost impossible (16).

Immunocompromised patients are more vulnerable to opportunistic infections. However, there is no conclusive evidence that probiotic *Lactobacilli* or *Bifidobacteria* increase the risk of opportunistic infections. In addition, some clinical studies aimed at assessing the safety of probiotics in small groups have shown that when certain probiotics are safely administered to immunocompromised patients (e.g., HIV patients), immature infants and elderly patients and those with Crohn's disease, there would be no specific adverse effects. The results of this study support the safety of taking probiotics and the poor pathogenicity of opportunistic microorganisms (5,28, 29).

In some cases, the invasive function of GI-tract and other organs (especially when population of *Lactobacilli* or *Bifidobacteria* is high), along with the chronic immunosuppressive status and antibiotic therapy, cause an increased risk. Because few statistical studies are available in this field, so there are currently no medical prescriptions for hospital patients caused by consumption of probiotics or other products that contain viable *Lactobacilli* or *Bifidobacteria*. Based on available evidence, there is no concern for probiotic *Lactobacilli* or *Bifidobacteria* in food. However, there are instructions for the preparation of probiotic yeasts (16). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have recently provided guidelines for "evaluation of probiotics in food to ensure their safety and efficacy" (30).

Recently, several cases of fungal disease related to probiotic *Saccharomyces boulardii* have been reported, which has interrupted the patient's treatment. An examination of these cases showed that the infection was caused by contamination during the production (31).

In some case studies, information has been reported on the infection caused by some probiotic species. For example, the *L. rhamnosus GG* strain isolated from a 74-year-old elderly woman with a liver abscess accompanied by a lung infection with a history of hypertension and non-insulin dependent diabetes. She consumed about 0.5 liters of dairy products containing *L. rhamnosus GG* per day that caused to exacerbate her symptoms over the past four months (32).

Table 1. Infection potential of prevalent probiotic microorganisms

Organism	Infection Potential
Lactobacillus	Mainly non-pathogens, some opportunistic infections (usually in immunocompromised patients).
Lactococcus	Mainly non-pathogens
Leuconostoc	Mainly non-pathogens, some isolated cases of infection.
Streptococcus	Oral *Streptococci* mainly non-pathogens (including *Streptococcus thermophilus*); some may cause opportunistic infections.
Enterococcus	Some strains are opportunistic pathogens with hemolytic activity and antibiotic resistance
Bifidobacterium	Mainly non-pathogens, some isolated cases of human infection.
Saccharomyces	Mainly non-pathogens, some isolated cases of human infection.

In another study, the probiotic strain caused endocarditis in a 67-year-old man with mitral valve prolapse who referred to a doctor for dental caries. The man reported a consumption of probiotic capsule containing a combination of *L. rhamnosus*, *L. acidophilus,* and *S. faecalis*. Because the capsule was too large to swallow, he drained the contents of the capsule in his mouth and then swallowed with milk. A few days after tooth surgery, the patient's endocarditis was progressed and *L. rhamnosus* was isolated from his blood cultures, which was from the contents of the probiotic capsule (24).

Such findings suggest that, although probiotic products have been used without risk for many years,

severe infections may occur occasionally, especially in patients with immune deficiency.

4. Safety Assessment of Probiotic Species

The purpose of this study was to evaluate the safety of products containing probiotic strains. Observations suggest that these products are increasingly helping consumers with immunocompromised system or vulnerable people, such as the elderly, children and people with immune deficiencies. Such an attitude raises concerns about safety of probiotics. The probiotic strains should be completely non-pathogens and should not be harmful to the host. It is not easy to obtain evidence to prove this claim (2).

The use of food additives requires a careful assessment in terms of efficacy and safety that is currently being conducted in Europe. Experiential studies on this issue are also ongoing at the Scientific Committee for Animal Nutrition (SCAN) (33). Today, these studies have focused on the use of probiotics for humans (14).

Several studies have been carried out on the health effects of probiotics on a variety of diseases, while little information is available on the safety and the gastrointestinal tolerance to long-term consumption of probiotic-containing products and disorders caused by certain probiotics, such as opportunistic *Lactobacilli* pathogens. Occasionally, it is possible that these products will be sold without legal control. Some cases of bacteremia and adverse effects of probiotics have been reported in populations at risk. There has not been enough research hitherto on the effect of receiving high doses of probiotics for a long time, especially in newborns (5,34).

A large number of *Lactobacilli* and *Bifidobacteria* are present naturally in the human gut (35). Lactic Acid Bacteria are often harmless and are known as generally recognized as safe (GRAS) in the United States (36). However, as previously mentioned, some *Lactobacillus* species are associated with opportunistic infections (37). Safety aspect is not always a concern for all members of a species or genus (38).

There are several methods in the Probiotic Safety Assessment studies: 1. Study on the intrinsic properties of probiotic strains 2. Study on the pharmacokinetics of probiotic strains (mechanism of drug effect) 3. Study on the interactions between probiotics and host (9). Animal models can have the most useful performance in assessing the safety of the new probiotic in the immunocompromised host. Gnotobiotic mice with immune deficiency are now used to assess the safety of probiotic bacteria (16). The results of these studies should be reflected in human studies.

The need for further research on probiotics can be divided into four broad categories. In the first step, there is a need for a better understanding of host and microbial agents that play an important role in lactobacillus infections. Secondly, the efficacy of probiotics should be proven with the aim of treating or preventing diseases and infections. Third, there is a need for a better understanding of the mechanism of action of probiotics when the probiotic efficacy is proven. Finally, based on previous criteria, there is a need for research into the development of improved probiotics, in particular, the targeted use of new probiotic species as therapists or therapeutic supplements, including the development of probiotics as a vaccine (16).

Dimensions of probiotic safety include the following characteristics (9):

1. Probiotic strains for human consumption should be preferable of human origin.
2. They should be isolated from the healthy human gastrointestinal tract.
3. They should not have a history of pathogenicity.
4. They should not have any relation to diseases such as infective endocarditis or gastrointestinal disorders.
5. They should not destroy bile salts by hydroxylation or decongestion in the small intestine.
6. They should not have transferable antibiotic resistance genes.

The identification is an important step in assessing the safety of the new probiotics (39). In the clinical setting, the identification is done for two main reasons: a diagnostic purposes and epidemiological purposes. The identification should be investigated by molecular studies due to physiological characteristics (16).

There are no legal standards around the world to assess the safety of food supplements containing probiotics. Different approaches are available in the United States, the European Union, and EU countries. The EU rules on food additives are restrict and limited. Therefore, microorganisms or a mixture of bacteria or yeast must be officially approved as a probiotic additive before use (8,40). This assessment seeks for a pre-sale approval and a precise risk assessment of strain and its effects on human health. Safety aspects are considered to be in contact with the microorganism, depending on the final consumer, the target species, the environment and safety of the workers during production or application (2).

5. The Risk of Gene Transfer

Some bacteria are able to absorb the surrounding DNA and integrate with their genome DNA (2). Because probiotics naturally form colonies in the intestine, there is a risk of gene transfer, especially resistant genes that can threaten human health due to produce antimicrobial resistance (41). These genes can be transferred to other strains via integration or transformation. The resistance gene transfer is a natural process, which will be dangerous only when resisted to a drug used in a therapeutic condition (39). Therefore, the safety of bacterial products should be considered with respect to the antibiotic resistance, the ability to absorb the foreign DNA and the duration of toxicity or pathogenicity. If pathogens are present in the environment the absorption of destructive genes by probiotic species may occur (2).

Given the diversity and complexity of gut micro-flora and the in vivo transfer rate, we consider the probiotics as a mediator for transferring resistance genes to pathogens. To prevent resistance, consumers should not use antibiotics when taking probiotics. Avoid taking probiotics is recommended, preferably during treatment with antibiotics (43).

6. Antibiotic Resistance and Virulance Factors

According to the indiscriminate use of antibiotics in human and veterinary medicines, the antibiotic resistance has become an increasing feature of microorganisms, thereby developing serious problems in the treatment of microbial infections (44,45). Antibiotic resistance in bacteria may be inherent or acquired trait. The inherent resistance is a natural property and can be recognized as a feature of the species, while the acquired resistance is derived from any of the genetic mutations or foreign DNA acquisition from other bacteria. Some species of *Lactobacillus* shows a wide range of antibiotic resistance naturally, but in most cases, antibiotic resistance is non-transferable. *Lactobacillus* species with non-transferable antibiotic resistance usually do not cause safety concerns. Several species of *Lactobacillus* include *L. rhamnosus* and *L. casei* are inherently resistant to vancomycin (46). It is accepted that the antibiotic resistance is not in itself dangerous unless the probiotics are incurable in rare cases of infection or unless it can be transferred to pathogens potentially resistant to therapies. The vancomycin resistance gene in *Lactobacillus* species appear to be on the chromosome and cannot easily be transferred to other genera (47). If the antibiotic resistance is related to the plasmid, the probability of transferring resistance to the pathogenic genera and species will be important. Since vancomycin is one of the most recent antibiotics in the treatment of multidrug-resistant pathogens, the transferable *Enterococcal* resistance to glycopeptide antibiotics such as vancomycin and teicoplanin should be considered (36).

Most *Bifidobacteria* are inherently resistant to nalidixic acid, neomycin, polymyxin B, kanamycin, gentamicin, streptomycin, and metronidazole (48). Many vancomycin-resistant *Lactobacillus* species have a long history of safe use as probiotics, and there is no evidence that vancomycin-resistant *Lactobacillus* can transfer resistance to other bacteria (47). A study reported the presence of Antibiotic resistance in the *Weissella* (60%), *Pediococcus* (44%), and *Lactobacillus* (33%), but not in *Lactococci* and *Leuconostocs*. *E. faecalis* strains showed acquired resistance to second generation quinolones (ciprofloxacin and/or norfloxacin), rifampicin, nitrofurantoin, glycopeptides (vancomycin and teicoplanin) and/or erythromycin (49).

particular safety concerns based on virulence factors and antibiotic resistance were identified in the *Enterococcus* (86%) (*Enterococcus faecalis*, 100%; *E. faecium*, 79%). Regarding *E. faecalis*, positive gelatinase reaction and hemolytic activity was found. Also, positive gelatinase reaction was identified in E. faecium but none of them showed hemolytic activity (49).

Another study reported the sensitivity of *E. faecalis* CP58 to vancomycin, tetracycline, rifampicin and erythromycin but resistant to kanamycin and chloramphenicol (50).

7. Selection Criteria for Probiotics

Before a probiotic can be useful in the interest of human health, it must have several criteria: it should have the desired technological properties so that it can be produced and added to the food products without losing its survival and function, or creating an unpleasant taste or tissue; it must survive when passing through the gastrointestinal tract and must reach live site and should be able to function in the intestinal environment (51). Several aspects should be considered in the selection of appropriate probiotic (9):

1- Safe strains, species, and genera of probiotics

2- Viability and bioactivity during the process and storage

3- Gastrointestinal survival and resistance to gastric acid and bile acids

4- Stimulating the selection of beneficial bacteria and suppressing harmful bacteria (through the

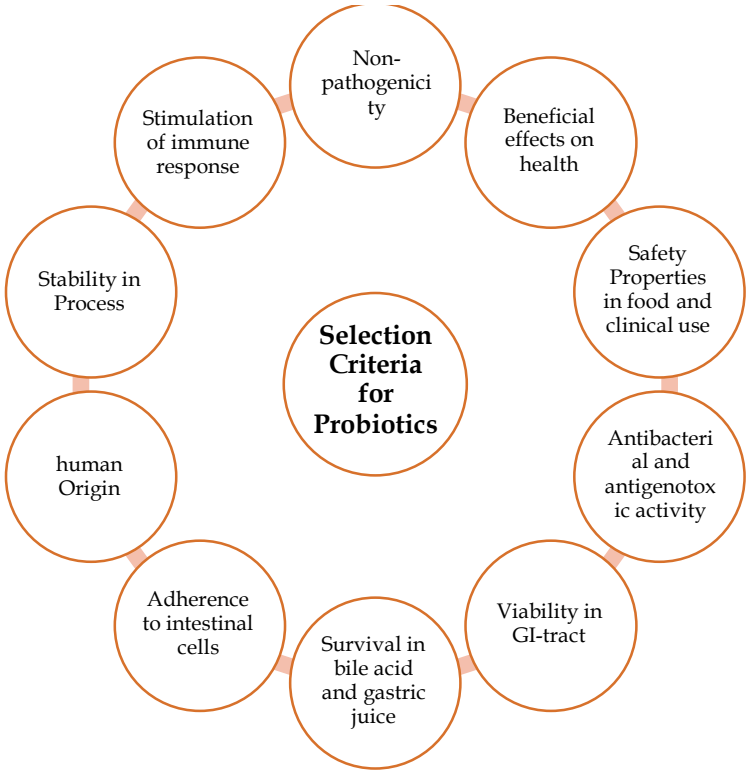

Figure 2. The main selection criteria for probiotic microorganisms according to safety, functional and technological properties

production of antimicrobial compounds and competitive elimination)

5- Antagonistic activity against pathogens such as *Helicobacter pylori, Salmonella, Listeria monocytogenes* and *Clostridium difficile*.

6- Adhesion to the intestinal epithelium

7- Anti-mutagenic and anti-carcinogenic properties

8- Modification and improvement of the immune system

Knowledge of probiotic survival in the gastrointestinal tract, establishment and migration properties, and the active components derived from probiotics are important for evaluating the positive and negative effects of probiotic intake. The survival of different probiotic strains varies in different parts of the gastrointestinal tract; some strains are rapidly eliminated in the intestine, while others can cross whole intestines (52). In the development of new probiotics, the species should ideally be selected from fecal flora of a number of healthy volunteers who have not consumed the products for one month or from probiotic LABs that have a long history of safe use in food products (16). Summary of properties for the selection of probiotic microorganisms in terms of safety, functional and technological properties is shown in Figure 1.

8. Conclusion

The use of live bacteria called probiotics in food and dietary supplements requires accurate safety assessment. The bacteria used in probiotic products should be completely safe. Various dimensions, including safety, functional and technological characteristics, should be evaluated in the process of selecting probiotic microorganisms. Concerning the safety aspects, the origin of microorganisms (healthy human gastrointestinal tract), non-pathogenic nature and antibiotic resistance is very important. The species should be selected from human micro-flora, without pathogenic characteristics such as virulence factors, and without transferable antibiotic resistance gene or lacking resistant to antibiotics, as well as should not prevent the treatment of rare probiotic infections. Although, many research tools based on animal models or Lab techniques are available, probiotic safety should also be confirmed in human studies.

Conflict of Interest

The authors declare no conflict of interest.

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