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Review Article

PROBIOTIC: AS EFFECTIVE TREATMENT OF DISEASES

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ABSTRACT
Probiotic are defined as live organisms, which confer benefits to the host. Their efficiency was demonstrated for the treatment of gastrointestinal disorders, respiratory infections, and allergic symptoms, but their use is mostly limited to bacterial and viral diseases. During the last decade, probiotic as means for the control of parasite infections were reported covering mainly intestinal diseases but also some nongut infections that are all of human and veterinary importance. In most cases, evidence for a beneficial effect was obtained by studies using animal models. In a few cases, cellular interactions between probiotic and pathogens or relevant host cells were also investigated using in vitro culture systems. WHO have given special guidelines for probiotic. Bifidobacteria have been studied for their efficacy in the prevention and treatment of a broad spectrum of animal and/or human gastrointestinal disorders, such as colonic transit disorders, intestinal infections, and colonic adenomas and cancer.
KEYWORDS Probiotic, Bifidobacteria, colonic transit disorders, intestinal infections

INTRODUCTION

There is evidence that the oral consumption of microorganisms produces a protective effect on the gut flora. Probiotic are defined as the viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance. The term probiotic is defined by a United Nation and World Health Expert Panel as live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotic are also defined as the viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance. The concept of probiotics. probably dates back to 1908, when Noble Prize winner Eli Metchnikoff suggested that the long life of Bulgarian peasants resulted from their consumption of fermented milk products. The term probiotic was first used in 1965, by Lilly and Stillwell for describing substances secreted by one organism which stimulate the growth of another. Marteau et al, in 2002 defined them as microbial preparations or components of microbial cells that have a beneficial effect on health and well being. Humans live in close association with vast numbers of micro-organisms. The GIT harbors a rich flora of more than 500 different bacterial species, stimulating the immune system, the use of antibiotics, immunosuppressive therapy and irradiation, amongst other means of treatment, may cause alterations in the composition and have effect on the flora. Therefore, the introduction of beneficial bacterial species into the GI tract may be a very attractive option to re-establish the microbial equilibrium and prevent disease[1,2].

Properties

an ideal probiotic preparation should have the following properties. For adequate amount of health benefits, a dose of five billion colony forming units a day ($5x10^9$ CFU/day has been recommended, for at least five days. The microorganisms used in probiotic preparations should be generally recognized as safe (GRAS), they should be resistant to bile, hydrochloric acid and pancreatic juice, have anti-carcinogenic activity and stimulate immune-system, have reduced intestinal permeability, produce lactic acid, able to survive both acidic conditions of the stomach and alkaline conditions of the duodenum. Foods for human consumption that contain mainly lactic acid bacteria include fermented milks, cheeses, fruit juices, wine, and sausages. Single and mixed cultures of live microorganisms are used in probiotics preparations. Experiments into the potential health effects of supplemental probiotics include the molecular biology and genomics of Lactobacillus in immune function, cancer, and antibiotic-associated diarrhoea, travellers' diarrhoea, paediatric diarrhoea, Inflammatory bowel syndrome and bowel syndrome. For testing of a probiotic using specific strain under study[3].

Stain Identification: Guidelines For The Evaluation Of Probiotics

In order to claim that a food has a probiotic effects, Joint FAO/WHO (Food and Agriculture Organization of the United Nations/ World Health Organization) Working Group generate guidelines for their evaluation. A scheme outlining these guidelines for the evaluation of probiotics for food use is shown in figure 1.

DNA-DNA hybridization is the reference method to specify that a strain belongs to a species: however, as it is time consuming and beyond the resources of many laboratories, requiring a large collection of reference strains, the use of DNA sequence encoding 16S rRNA is suggested as a suitable substitute. Strain typing has to be performed with a reproducible genetic method or using a unique phenotypic trait. Pulsed Field Gel Electrophoresis (PFGE) is the gold standard. Randomly Amplified Polymorphic DNA (RAPD) can also be used.

In- Vitro Tests To Screen Potential Probiotics

In-vitro tests are useful to gain knowledge of strains and the mechanism of the probiotic effect. It was noted that *in- vitro* data available for particular strains are not sufficient for describing them as probiotic. Probiotics for human use will require substantiation of efficacy with human trials. Appropriate target- specific *in- vitro* tests that correlate with *in- vivo* results are recommended. Conway et al. correlated the *in- vitro* bile salts resistance with the gastric survival *in-*

vivo. A list of the main currently used *in- vitro* tests for the study of probiotic strains is as follows- Resistance to gastric acidity, Bile acid resistance, Adherence to mucus and/ or human epithelial cells and cell lines, Antimicrobial activity against potentially pathogenic bacteria, Ability to reduce pathogen adhesion to surfaces, Bile salt hydrolase activity, Resistance to spermicides (applicable to probiotics for vaginal use).

Safety Consideration

Probiotics are viable organisms, and therefore it is feasible that they could infect the host. Historical data indicates that probiotic *lactobacilli* and *bifidobacteria* associated with food have been considered to be safe[4]. Their occurrence as normal commensals of the mammalian micro biota and their established safe use in diverse food and supplement products worldwide support this conclusion. However, probiotics may theoretically be responsible for four types of side effects. Systemic infection, deleterious metabolic activities, Excessive immune stimulation in susceptible individuals, Gene transfer.

Phase 2 Clinical Studies

Standard methods for clinical evaluations are comprised of Phase1 (safety), Phase2 (efficacy), Phase3 (effectiveness) and Phase4 (surveillance). Phase2 studies, generally in the form of randomized, double blind, placebo-controlled (DBPC) design, measure efficacy compared with placebo. The outcomes for the individual should be a statistically and biologically significant improvement in condition, symptoms, signs, well beings, or quality of life, reduced risk of disease or longer time period to the next occurrence, or faster recovery from illness. More clinical evidence of this type is needed to gain credibility among the broader medical community. These need to provide a physician with the name of the strain, its product formulation, and the specific use for which it has been shown to be effective.

Phase 3 Clinical Studies

In other words, Phase3 studies require careful planning and an evaluation of multiple endpoints before probiotics should be discarded from the health care armamentarium. The performance of more Phase3 studies on probiotic strains is required to determine fully their place, if any, in the treatment and prevention of more serious clinical conditions and whether or not this approach can replace or complement[5].

Health Claims And Labelling

Currently, in most countries, only general health claims are allowed on foods containing probiotics, specific health claims are allowed on drug-approved probiotics that have gone through Phase3 clinical studies. Such specific health claims should be permitted on the label and promotional materials. For example, a specific claim that states a probiotic reduce the incidence and severity of rotavirus diarrhea in infants would be more informative to the consumer than a general claim that states improve gut health.

Corporate contact details for consumer information ISAPP'S role for establishment of standards for probiotic products

There are a number of problems related to the quality of commercial products of probiotics. These products do not adequately meet label requirements Problems exists in the following areas- 1.The number of live microbes of each strain delivered through the end of shelf life is often not accurately reflected on the label. 2. Microbes contained in the product are not always named in accordance with scientifically valid nomenclature. 3. Claims of efficacy are not always adequately substantiated. 4. Use of the term 'probiotic'on the labels of products with no established record of a physiological (health) benefit in humans. Although a scientifically recognized consensus definition of the term 'probiotic' was established by an FAO Expert Consultation, the term is misused on products that lack a minimum of studies validating efficacy. Acceptance and enforcement world wide of a 'Standard of Identity' for use of the term 'probiotic' would go a long way toward bolstering consumer confidence when purchasing probiotic products, building a strong foundation for a growing industry and informing the legislation for claims.

Standards of identity for the term probiotic

Safe, Microbiologically defined, Impact on human health or physiology documented by at least one controlled study in humans of suitable size and statistical power to be considered valid by experts in the field. Product labels accurately indicate the genus, species and strain of all contained types of microbes according to nomenclature accepted by the microbiological community. Product labels accurately indicate per serving or per dose levels of each probiotic microbe contained in the product through the end of shelf life. Any efficacy statements made on product labels or in promotional materials or websites are truthful and not misleading and are based on scientifically valid studies.

Probiotic Selection

Probiotic strain selection shown in Figure 2

Comparison Between Probiotic, Probiotic And Symbiotic[6,7]

Term	Definition	Examples	Advantages	Possible Future developments
Probiotic	A live microbial food supplement which beneficially affect host animal by improving its intestinal microbial balance	Lactobacilli, Bifidobacteria Enterococci streptococci	Strain may have proven health values .Useful when gut flora may be compromised	New product developments based on synbiotics, that may improve probiotic survival
Prebiotic	A non –digestible food ingredient that beneficially affects the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon ,and thus improves host health	Fructo-oligosaccarides (FOS),inulin,galacto-oligosaccarides	Genus –level changes occur in gut flora .product survival not problematic.low dose required and can be incorporated into many different food delivery system	Manufactur of novel multiple-function prebiotics, that may stimulate the "beneficial flora; exert antiadhesiv properties attenuate pathogen virulence.probiotic derived from dietary fiber –type polysaccarides
Synbiotic	A mixture of pro-and prebiotics which beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract.	Fructooligosaccharides+(FOS),+bifidobacteria;Lactilol+lactobacilli	Dual effect of both entities .Probiotic survival should be improved	Design of new symbiotic through molecular engineering (based on specific probiotic enzyme)

Factor Affecting Probiotic

Physiological state

The physiological state of bacteria when prepared and remaining in a product itself are important factors for survival of the probiotic. Dryness in a food product keeps the bacteria in a relatively quiescent state during storage, while a wet product establishes potentially active metabolism. Temperature affects shelf life of the bacteria, with low temperature providing conditions for possible long term survival.

Temperature

During processing, temperatures over 45–50°C will be detrimental to probiotic survival, this means that the higher the temperature, the shorter the time period of exposure required to severely decrease the numbers of viable bacteria, ranging from hours or minutes at 45–55°C to seconds at higher temperatures. Therefore it is obvious that probiotics should be added downstream of heating/cooking/pasteurization processes in food manufacture to avoid the high temperatures.

pH

Some bacteria like Lactobacilli and bifidobacteria can tolerate lower pH levels because produce organic acid and products from carbohydrate metabolism. Indeed, numerous in vitro and in vivo studies have demonstrated that in gastric transit where the cells are exposed to low pH values and with a time of exposure relatively short, some probiotic organisms can survive. In fermented milks and yogurts with pH values between 3.7 and 4.3. lactobacilli are able to grow and survive, while Bifidobacteria tend to be less acid tolerant, with most species surviving poorly in fermented products at pH levels below 4.6.

Water activity

The higher moisture levels and water activity, the lower survival of probiotics. There is a substantial interaction between water activity and temperature with respect to their impact on the survival of quiescent probiotics. As the storage temperature is increased, the detrimental impact of moisture is magnified.

Oxygen

Maintaining probiotic viability in moderate water activity foods (0.4–0.7) is a great challenge and solutions such as microencapsulation or incorporation of probiotics into fat phases of products can provide improved survival. Most probiotic bifidobacteria do not grow well in the presence of oxygen, although, many bifidobacteria have enzymatic mechanisms to limit the oxygen toxicity. For oxygen sensitive strains, some strategies can be used to prevent oxygen toxicity in food products. Antioxidant ingredients have been shown to improve probiotic survival, as well as the use of oxygen barrier or modified-atmosphere packaging. Therefor, it is advisable to minimize processes that are highly aerating, particularly when using bifidobacteria. Toxicity of ingredients. Interactions between probiotics and other ingredients could happen and those interactions can be protective, neutral, or detrimental to probiotic stability. Obviously, the inclusion of antimicrobial preservatives can inhibit probiotic survival and elevated levels of ingredients such as salt, organic acids, and nitrates can inhibit probiotics during storage, while starter cultures can sometimes inhibit the growth of probiotics during fermentation through the production of specific bacteriocins.

Growth factors, protective, and synergistic ingredients

Probiotic lactobacilli and, in particular, bifidobacteria are only weakly proteolytic and grow relatively slowly or poorly in milk. The growth of bifidobacteria can be improved by the presence of suitable companion cultures, which can aid in protein hydrolysis and through the production of growth factors. Some growth substrates such as carbon sources, nitrogen sources, and growth factors or antioxidants, minerals and vitamins can be added to improve growth. Finally, the food matrix itself can be protective like in the cheese, where the anaerobic environment, high fat content and buffering capacity of the matrix helps to protect the probiotic cells both in the product and during intestinal transit.

Freeze–thawing

The damages made to cell membranes freezing probiotics is detrimental to survival, and also can make the cells more vulnerable to environmental stresses. To prevent or at least mitigate cell injury, protectants are usually added to cultures to be frozen or dried. Once frozen, probiotics can survive well over long shelf lives in products such as frozen yogurts and ice-cream. Using alternative methods of freezing, such as slow-cooling rates or pre-freezing stress, can significantly improve cell survival. Repeated freeze–thawing cycles are highly detrimental to cell survival and should be avoided.

Shear forces

Probiotic lactobacilli and bifidobacteria are Gram-positive bacteria with thick cell walls that are able to tolerate the shear forces generated in most standard food production processes such as high-speed blending or homogenization that may result in cell disruption and losses in viability.

Mechanism Of Action Of Probiotic

Several mechanisms have been postulated regarding action of Probiotics. Partial lactose digestion and stimulation of the intestinal mucosal lactase activity has been postulated as a possible mechanism against some types of diarrhoea. *Lactobacilli* used in the fermented milk industry have active beta-galactosidase to decrease the lactose concentration in dairy products, which may affect the severity of osmotic diarrhea due to organisms as rotavirus. Lactic acid bacteria produce several metabolites like fatty free acids, hydrogen peroxide, bacteriocins etc. which prevent the growth of food borne pathogens in dairy products Probiotics can also use enzymatic mechanisms to modify toxin receptors and block toxin mediated pathology. Probiotic agents also prevent colonization of pathogens The other suggested mechanisms for the effect on intestinal microflora are lowering the intestinal pH,release of gut protective metabolites, regulation of intestinal motility and mucus production. Gastrointestinal mucosa is the primary interface between the external environment and the immune system. Whenever intestinal microflora reduces, antigen transport is increased indicating that the normal gut micro flora maintains gut defences. The non-pathogenic probiotic bacteria interact with the gut epithelial cells and the immune cells to start the immune signals. These bacteria must interact with M cells in the Peyers patches, with gut epithelial cells, and with associated immune cells. Probiotic bacteria have been shown to modulate immunoglobulin production. Secretory IgA plays an important role in mucosal immunity, contributing to the barrier against pathogenic bacteria and viruses. The increase in the number of IgA producing cells was the most remarkable property induced by probiotic organisms and also

by fermented milk yogurt. T independent IgA induction has also been demonstrated[8]. The increase in profiles of certain cytokines (TNF- α IFN- γ, IL-10) has also been observed due to stimulation with probiotic bacteria[9]. The release of cytokines is induced to up or down regulate the immune responses and maintain intestinal homeostasis. Interactions between probiotic micro-organisms and GALT(Gut associated lymphoid tissue), mechanisms of immunomodulation and anti-inflammatory properties are not yet fully understood.

Potential Effect Of Probiotic

Diarrhoea

Some probiotic have been shown in preliminary research to possibly treat various forms of gastroenteritis. They might reduce both the duration of illness and the frequency of stools. Fermented milk products also reduce the duration of symptoms[10].

Antibiotic-associated diarrhoea

Antibiotic associated diarrhoea (AAD) results from an imbalance in the colonic microbiota caused by antibiotic therapy. Microbiota alteration changes carbohydrate metabolism with decreased short term fatty acid absorption and an osmotic diarrhoea as a result. Another consequence of antibiotic therapy leading to diarrhoea is overgrowth of potentially pathogenic organisms such as Clostridium difficult. Probiotic treatment might reduce the incidence and severity of AAD as indicated in several metanalysis[11]. For example, treatment with probiotic formulations including Lactobacillus rhamnosus may reduce the risk of antibiotic-associated diarrhoea, improve stool consistency during antibiotic therapy, and enhance the immune response after vaccination. However, further documentation of these findings through randomized double-blind, placebo-controlled trials are required to confirm specific effects and attain regulatory approval, which currently does not exist. Potential efficacy of probiotic AAD prevention is dependent on the probiotic strain(s) used and on the dosage[12] Up to a 50% reduction of AAD occurrence has been found in preliminary studies. No side-effect have been reported in any of these studies. Caution should, however, be exercised when administering probiotic supplements to immunocompromised individuals or patients who have a compromised intestinal barrier.

Lactose intolerance

As lactic acid bacteria actively convert lactose into lactic acid, ingestion of certain active strains may help lactose intolerant individuals tolerate more lactose than they would have otherwise[13].

Colon cancer

In laboratory investigations, some strains of LAB Lactobacillus delbruekiti suspension have demonstrated anti-mutagenic effects thought to be due to their ability to bind with hetrocyclic amines which are carcinogenic substances formed in cooked meat Animal studies have demonstrated that some LAB have evidence for acting against colon cancer in rodents, though human data are inconclusive. Some human trials hypothesize that the strains tested may exert anti-carcinogen effects by decreasing the activity of an enzyme called glucuronidase.

Cholesterol

Animal studies have demonstrated the efficacy some strains of LAB to be able to lower serum cholesterol levels, presumably by breaking down bile in the gut thus inhibiting its reabsorption (which enters the blood as cholesterol). A meta-analysis that included five double blind trials examining the short term (2-8weeks) effects of a yogurt with probiotic strains on serum cholesterol levels found a minor change of 8.5 mg/dL (0.22 mmol/L) (~4% decrease) in total cholesterol concentration, and a decrease of 7.7 mg/dL (0.2 mmol/L) (~5% decrease) in serum LDL concentration. A slightly longer study evaluating the effect of a yogurt with probiotic strains on twenty-nine subjects over six months found no statistically significant differences in total serum cholesterol or LDL values. However, the study did note a significant increase in serum HDL from, 50 mg/dL (1.28 mmol/L) to 62 mg/dL (1.6 mmol/L) following treatment. This corresponds to a possible improvement of LDL/HDL ratio[14]. Studies specifically on hyper-lipidemic subjects are still needed.

Blood pressure

Although not a confirmed effect, some studies have indicated that consumption of milk fermented with various strains of LAB may result in modest reductions in blood pressure, an effect possibly related to the ACE-inhibitor like peptidase produced during fermentation.

Immune function and infections

Some strains of LAB may affect pathogen by means of competitive inhibition (i.e., by competing for growth) and there is evidence to suggest that they may improve immune function by increasing the number of IgA-producing plasma cells, increasing or improving phagocytosis as well as increasing the proportion of T-lymphocytes and Natural Killer cells[15].

Helicobacter pylori

Some strains of LAB may affect infections (which may cause peptic ulcer) in adults when used in combination with standard medical treatments, but there is no standard in medic al practice or regulatory approval for such treatment[16].

Inflammation

Some strains of LAB may modulate imflammation and hypersensitivity responses, an observation thought to be at least in part due to the regulation of cytokines function. Clinical studies suggest that they can prevent reoccurrences of inflammation bowel disease in adults, as well as improve milk allergies. They are not effective for treating eczema, a persistent skin inflammation. How probiotics may influence the immune system remains unclear, but a potential mechanism under research concerns the response of T – lymphocytes to pro-inflammatory stimuli.

Bacterial growth under stress

In a study done to see the effects of stress on intestinal flora, rats that were fed probiotics had little occurrence of harmful bacteria latched onto their intestines compared to rats that were fed sterile water.

Irritable bowel syndrome and colitis

In one study, a commercial strain of Bifidobacterium infantis improved some symptoms of irritable bowel syndrome in women. A separate small study showed that a strain of lactobacillus plantarum may also be effective in reducing IBS symptoms. A study focused on Bifidobacterium animalis showed a reduction in discomfort and bloating in individuals with constipation-predominant IBS, as well as helping to normalize stool frequency in said individuals. For maintenance of remission of ulcerative colitis, Mutaflor

(*E.coli* Nissle 1917) randomized clinical studies showed equivalence of Mutaflor and mesalazine (5-ASAs).

Application of probiotic[16]

Examples of various stains that have been positively affect health.

Indication	Genus , species, strain
Inflammatory bowel condition	Eight strain combination of 3bifidobacterium strains,4 lactobacillus strains and s.thermophilus
Antibiotic associated diarrhea	S cerevisiae,L rhamnosus, L casei, L acidophilus
Gut transit time	Bifidus regularise
General wellness	L reuteri,L casei
Lactose intolerance	L bulgarius,S thermophilus
Immune support	B lactis, L casei
Irritable bowel syndrome symptoms	B infantis

L= Lactobacillus strain, B = bifidobacteium strain, S=Streptococcus thermophilus strain

CONCLUSION

The concept that probiotics could control the development of eukaryotic pathogens is Emerging. Therapeutic approaches with probiotic could help to reduce the risks of infestation by specific parasites or complement classical anti-parasite treatments. Probiotic therapy has already made its way in the treatment of number of conditions-Infectious, inflammatory, neoplastic and allergic. There is a long list of potentials of giving probiotics in a number of these conditions. But before bringing probiotic into routine usage, proper evaluation of these products is essential. Several important criteria and standards regarding quality and reliability have to be met. Thus future well designed placebo controlled studies with validated results are required for ascertaining the true health benefits of these products. The important point is careful selection of the probiotic agent, its dose standardization and a thorough knowledge of its beneficial effects over and above the toxic effects, so that this traditional therapy proves to be an effective tool for medical therapy.

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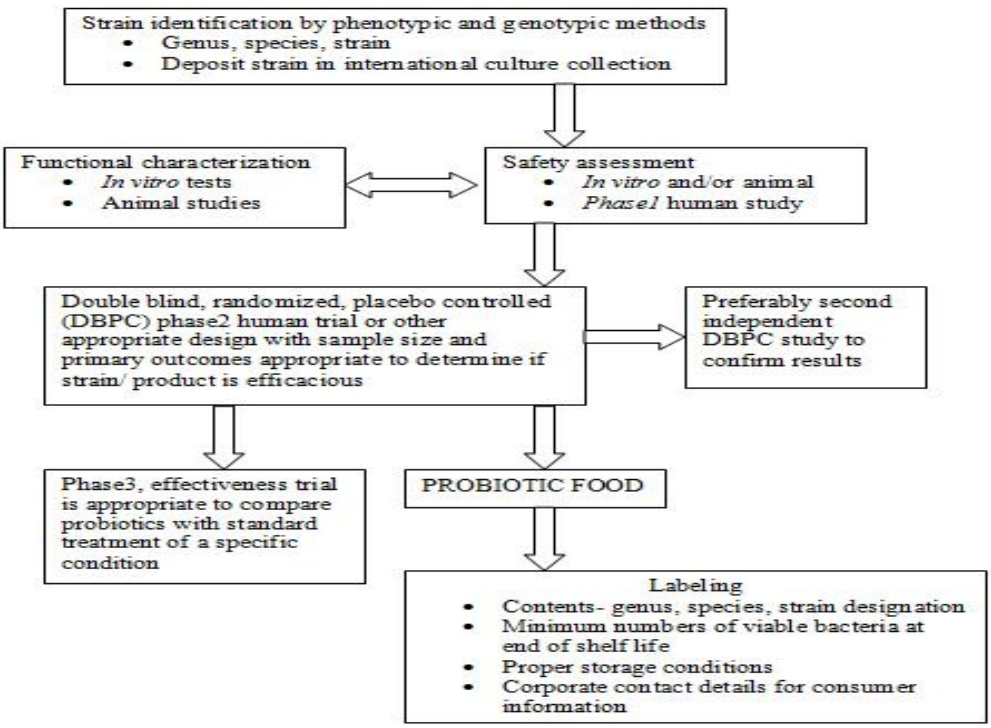

Fig. 1. Guidelines for the evaluation of probiotics

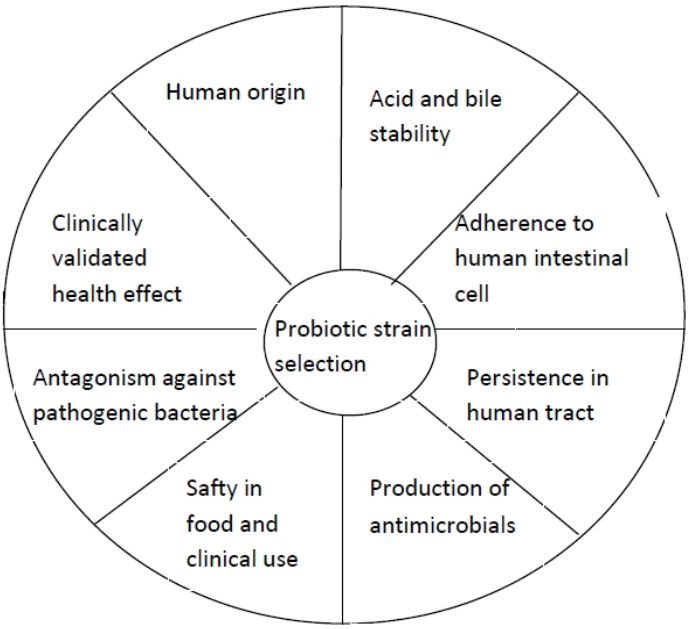

Fig. 2. Probiotic strain selection