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Review Article

Desired Characteristics and Mechanism of Action of Probiotics for Human Health Management

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Abstract:

Human body is a living colony of more than 100 trillion bacteria that constitute approximate 5 pounds of weight. Good bacteria empowers your body to fight against health-threatening microbes, provide immunity and help in digestion while bad bacteria cause heart, respiratory and digestive problems including bone health. The use of probiotics help to maintain a balance between good and bad bacteria in gut flora and facilitate in the natural management of your health. A large number of probiotics and prebiotics are available in global market and the probiotic industry is facing new challenges due to increased regulatory mechanism for their acceptance as food, public knowledge and awareness of the value of functional food consumption, particularly after Covid-19, and increased international competition in the food and dairy market. Vast scientific literature on choice and characterization of novel species and strains of probiotics and prebiotics bacteria and yeasts is available in the academic world. The World Health Organization's (WHO) has prescribed basic selection criteria, such as host-associated stress resistance, epithelial adhesion capability, and antibacterial activity that are generally adopted during scientific investigations. These criteria are used to make sure that the probiotic candidate can work properly and endure the demanding conditions of the human digestive system. The use and applications of probiotics in children's and older people's food including in the treatment of common ailments has gained the importance in recent years not only in western countries but in Asian and African developing countries and their market is expanding every year. It cannot be expected that these unique microbial strains will provide the biological and health advantages associated with probiotics.

Key words: functional food; stress resistance; antibacterial activity; probiotics; prebiotics; human digestive system

Introduction

Microbiota such as bacteria, fungi, archaea, protists, planktons, amoebae are ubiquitous and have been exploited for their potential for beneficial effects for human, animals and plants (Venkatesh et al., 2024). The human microbiome is essential in modulating health and disease. The overall human microbiota is believed to comprise roughly 10^{13} to 10^{14} microorganisms, maintaining a 1:1 ratio with human cells. The gut constitutes the most densely populated area in the human body, housing almost tenfold the number of cells found in the entire organism and surpassing the genetic content of the human genome by more than one hundred times (Sender, et al., 2016, Thursby, et al., 2017 and Wu, et al., 2018). In the beginning of the 20th century, probiotics were assumed to enhance host intestinal micro-flora which produce inhibiting compound against pathogenic bacteria (Anukam and Reid, 2008). According to Harlé et al. (2020), they are mostly utilised in the food and dairy industries as starting cultures for the preparation of fermented dairy, vegetable, cereal, and meat products. Since their first isolation from milk, lactic acid bacteria (LAB) have been found in a variety of foods and fermented goods, including meat, milk, vegetables, drinks, and baked goods (Reis et al., 2016). According to Goswami et al. (2017), LAB may be found in fermented foods and have been isolated from sewage, manure, water, soil, and other natural environments. In addition to flavouring and texturising compounds, LAB is found in the human gastrointestinal system and in certain oral flora, which can lead to dental caries (Ramalingam et al., 2013; Fguiri et al., 2017; Marlina et al., 2020).

The term "gut microbiota" refers to the microorganisms that inhabit solely the gastrointestinal system. The primary gut microbiota is non-pathogenic and is essential for providing health benefits to the host (Grimoud, et al., 2010). The many segments of the gastrointestinal tract display unique physiological traits, chemical compositions, and environmental conditions. As a result, the varieties and amounts of microbiomes vary throughout different places. The transition from the stomach to the small intestine and thereafter to the large intestine demonstrates a notable rise in bacterial populations. These microbes have been extensively studied and may possess similar significance to bacteria, as they presumably affect both the host's and importantly, the gut microbes' functioning (Bakhshinejad and Ghiasvand, 2017 and Barr, et al., 2017). Since Hippocrates stated in 400 B.C. that "Death sits in the bowls," there has been a persistent recognition of the connection between intestinal health and general human well-being. The profound influence of gut microbiota on human health and disease has been investigated in several research globally (Abo Nahas, et al., 2022).

The concept of lactic acid bacteria as a distinct group of microorganisms was first introduced in the early 20th century, with significant contributions from Louis Pasteur. These probiotic bacteria have multiple useful effects on health, such as lowering pH of the gut, producing essential vitamins and digestive enzymes, and generating antibacterial substances like bacteriocins, , organic acids, diacetyl, acetaldehyde, the lactoperoxidase system, hydrogen peroxide, and lactones. They also support the restoration of a healthy gut microbiome and can alleviate issues caused by diarrhea, antibiotic treatments, and radiation therapy. Moreover, they help reduce cholesterol levels in the blood, enhance immune responses, inhibit bacterial infections, detoxify carcinogens, enhance calcium assimilation, and decrease enzyme activity of fecal (Zubillaga et al., 2001; Ouwehand et al., 1999; Holzapfel and Schilling, 2002; Hossain et al., 2017; De Melo et al., 2018). Probiotic microorganisms need to be non-pathogenic, unassociated with diarrhoeagenic bacteria, unable to transport antibiotic resistance genes, and capable of maintaining genetic stability (Molina et al., 2015). To be identified as functional food components, they must exhibit specific characteristics: stability in acidic and bile environments (Tankoano et al., 2019; Bisht and Garg, 2021), barrier to digestive enzymes, ability to adhere to intestinal surfaces, antagonistic effects against human pathogens (Kang et al., 2019; Arya et al., 2018; Komalben et al., 2022), anti-carcinogenic and anti-mutagenic activities (Lee et al., 2010), cholesterol-lowering effects (Shewale et al., 2014), immune system stimulation without causing inflammation, enhanced bowel motility, management of mucosal integrity (Marlina et al., 2020), production of vitamins and enzymes and improved bioavailability of food compounds (Cruz et al., 2012; Ouwehand et al., 1999). The technological properties of bacteria are crucial in the production of probiotics (Kang et al., 2019; Saarela et al., 2000; Bisht and Garg, 2021). Probiotic supplements may contain large doses of probiotic microbes may or may not be present in gut flora as colonizer. They are shown to promote healthy gut environment (Bisht and Garg, Garg et al, 2024) and facilitate the restoration of balanced intestinal flora, especially after the disturbances created by the usage of antibiotic therapy.

Lactic acid bacteria (LAB)

In 1857 a significant contribution in this field was made by Louis Pasteur who for the first the time developed the methods of isolation pure bacterial culture and *Bacterium lactis* was reported by Lister in 1873. Beijerinck (1901) described *Lactobacillus* as Gram positive organism. According to Orla-Jensen (1919), LAB are a naturally occurring group of rod- or cocci-shaped, Gram-positive, non-spore-forming , non-motile organisms that lack catalases and ferment carbohydrates to create lactic acid (Gautam et al., 2014, Garg et al., 2024), are anaerobic bacteria, devoid of cytochrome, fastidious, acid-tolerant and strictly fermentative (Ding *et al.,* 2017). The classical approach to bacterial taxonomy was totally based on morphological and physiological characters. LAB degrade hexoses to produce lactate (homo-fermentative) or lactate and add- on products for example acetate, ethanol, CO_2, format or succinate (hetero-fermentative) (Ho and Sze, 2018).

Lactic acid bacteria (LAB) include both rod-shaped organisms, such as Lactobacilli and Carnobacteria, as well as cocci like Streptococci. Carnobacterium, in particular, produces bacteriocins that are effective against *Listeria monocytogenes*. Various species of LAB, including *Streptococcus*, *Leuconostoc*,, *Lactobacillus*, *Pediococcus*, *Enterococcus*, *Aerococcus*, *Vagococcus*, and *Carnobacterium*, have adapted to thrive in diverse environmental conditions and are capable of growing, developing, and sporulating under these conditions (Ni et al., 2015). These bacteria are commonly located in the gastrointestinal tracts of humans and other animals, along with dairy, soil and seafood products, and on plant surfaces (Ringø and Gatesoupe, 1998; Patel et al., 2015). Although LAB are not the dominant microorganisms in the typical intestinal microbiota, efforts have been made artificially increase to their presence (Verschuere et al., 2000; Tosungnoen et al., 2014; Bisht and Garg, 2019, 2021). Depend on their carbohydrate metabolism, LAB are classified into two groupsLactic acid is the main product of the Embden-Meyerhof (glycolytic) route, which is used by the homo-fermentative group. In contrast, hetero-fermentative LAB, such as *Leuconostoc* and *Weisella*, utilize the phosphoketolase pathway to produce equal amounts of lactate, CO2, ethanol, and acetate from glucose (Perez et al., 2014; Ho and Sze, 2018). The homo-fermentative group includes *Lactococcus*, *Enterococcus*, *Pediococcus*, and *Streptococcus*, while the hetero-fermentative group comprises *Leuconostoc* and *Weisella* (Vasiljevik and Shah, 2008).Supplementation with selected probiotic strains could help to reduce diarrhea and constipation. Various stains of *Lactobacillus reuteri*, *Bifidobacterium lactis* and *Saccharomyces boulardii* have been shown to be most beneficial and they support weight loss, may reduce depression and act as antimicrobial (Garg et al., 2024)

Lactic acid bacteria (LAB) are a varied group of bacteria found in numerous environments, such as dairy products (both unfermented and fermented), vegetables, meats, and the gastrointestinal and urogenital tracts of humans and animals, as well as in soil and water (Liu et al., 2014). These bacteria are primarily known for their ability to generate lactic acid as a key byproduct during anaerobic metabolism. In addition to lactic acid, they produce series of metabolites which enhance the nutritional, sensory, and technological charactersties of fermented foods. Thanks to their versatile metabolism, LAB are widely used in three main areas: (i) in fermentation processes as starter cultures, (ii) as probiotics that provide health benefits, and (iii) in the synthesis of valuable compounds, such as nutraceuticals (Naeem et al., 2012; Emerenini et al., 2013). Fermented foods are produced with the assistance of lactic acid bacteria (LAB), that are Gram-positive, catalase-negative microorganisms. These bacteria, typically non-spore-forming, can appear as either cocci or rods under a microscope. In industrial fermentation, LAB are used to enhance the flavor and quality of food and feed products (Kaban, G et al., 2008; Hati, S et al., 2013). Lactic acid, along with other organic acids, acts as a natural preservative in fermented foods, and it has been shown to be effective in meat decontamination (Ray, B et al., 1992). The antibacterial effect of lactic acid is primarily due to its un-dissociated form, which can penetrate the cytoplasmic membrane, lower the intracellular pH, and disrupt the proton motive force across the membrane.

A notable feature of LAB is their ability to synthesize porphyrin groups, like heme (Alessandro et al., 2010), which plays an essential role in their physiology. In laboratory conditions lacking hematin or similar substances, LAB do not possess a "true" catalase or cytochromes. Instead of an electron transport chain, they rely on fermentation and substrate-level phosphorylation for energy production. While some LAB may exhibit catalase activity through non-heme "pseudocatalase," the absence of cytochromes can be a more reliable marker for identifying them than the traditional catalase test. Lactobacilli, a key group of LAB, help regulate harmful microflora in the gut, preventing the growth of infectious bacteria by producing antimicrobial metabolites. As natural biological preservatives, they are often found in fermented foods. Dairy products containing LAB are particularly resistant to harmful bacteria. Several studies have explored the antimicrobial properties of probiotics, demonstrating their effectiveness against both Gram-negative and Gram-positive bacteria, including *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* (Oyetyo VO et al., 2004).

Probiotics

The term "probiotic" has been defined in various ways over time. It was first introduced by Lilly and Stillwell (1965) to illustrated “substances produced by one microorganism that promote the growth of another.” Parker (1974) later expanded this definition, describing probiotics as "organisms and substances that contribute to the balance of intestinal microbes." Marteau et al. (2001) defined probiotics as "microbial cell preparations or components that have a beneficial effect on health and well-being." However, the most comprehensive definition views probiotics as microbes or groups of microbes that reside in the gut and provide internal nourishment to the host (Gibson and Roberfroid, 1995; Hamasalim, 2016). Eli Metchnikoff, often regarded as the "father" of modern probiotics and a Nobel laureate, recognized the beneficial effects of certain bacteria. The current definition, proposed by Havenaar and Huisin Veld (1912), describes probiotics as viable single or mixed bacterial cultures that positively impact the host by improving the properties of the indigenous microbial community. Over the past two decades, there have been significant advancements in the identification and characterization of specific probiotic strains, revealing their considerable health benefits.

Over 100 to 1000 different microbial species may be found in each person's gastrointestinal system, with bacteria accounting for fifty percent of the moist weight of colonic material. Bacterial colonization of the gut starts at birth and evolves over a lifetime, influenced by diet, genome, lifestyle, and antibiotic use (Lloyd-Price., 2016). A decrease in the Bacteroidetes/Firmicutes ratio and a notable drop in bifidobacteria are two age-related alterations in the gut microbiota composition that occur in people over 60 and are correlated with a deterioration in immunological function. The intestinal microbiota's advantageous function, known as "colonisation resistance" or the "barrier effect," protects the body against dangerous infections and maintains the population of natural gut bacteria. Manipulating gut microflora to increase "beneficial bacteria" can positively impact immune function,0 metabolism, digestion and brain to gut communication. Changes in microbial diversity can lead to disorders and diseases, often resistant to conventional medicine. The use of nano-encapsulated multimodal supplements as a remedy has been investigated though they may be costly and impractical for everyday use. Therefore, probiotics offer a minimalist, low-cost means to enhance host health by providing shelter against enteric pathogens (Qadir, 2015).

Probiotics are recognized for their ability to improve gut barrier function, compete with harmful microorganisms, and promote colonization. They help modify and regulate the host's immune system, activate specific genes, and influence the release of gastrointestinal hormones, as well as brain activity through the gut-brain axis. Additionally, probiotics are essential for promoting intestinal angiogenesis through the signalling of the vascular endothelial growth factor receptor (VEGFR), which helps manage inflammation in the intestinal mucosal tissue, especially in conditions like inflammatory bowel disease (IBD) (Chen X et al., 2013). In the early 20th century, it was discovered that beneficial bacteria, particularly lactic acid bacteria (LAB), have a positive effect on digestion and immune function in the body (Anukam and Reid, 2008; Dowarah et al., 2018). Most probiotic microorganisms are Gram-positive, including species such as *Lactobacillus* and *Bifidobacterium*, which are widely used to treat gastrointestinal disorders (Marco et al., 2006; Tankoano et al., 2019; Colombo et al., 2020). However, some Gram-negative bacteria are also utilized as probiotics. Research has shown that probiotic bacteria play vital roles in modulating immune, respiratory, and digestive functions (Floch et al., 2011). These probiotics are typically consumed as live cultures, containing bacteria like lactococci, lactobacilli, or bifidobacteria, which are isolated from native environments (Bongaerts and Severijnen, 2016).

Since ancient times, lactic acid fermentation has been recognised to improve human health (Bisht and Garg, 2023). The Bible also mentions sour milk several times. Many recipes for fermented milk were made by Ancient Romans and Greeks. A specific type of sour milk was made from cow, goat milk or buffalo, called “leben raib”, consumed in ancient EgyptPeople have been consuming yogurt for a long time, with fermented milk drinks being known to Indians and Turks as far back as 800-300 B.C. In the early 20th century, a Russian scientist and immunologist at the Pasteur Institute in Paris developed a keen interest in lactic acid fermentation, earning the Nobel Prize in medicine in 1907. Probiotics' many qualities have long been recognised as important health boosters. Probiotic strains' viability and culture conditions during processing and storage, as well as their susceptibility to low pH levels, stomach fluid, bile, and pancreatic enzymes, have been the main topics of recent studies (Vantsawa et al., 2017; Somashekaraiah et al., 2019). Table 1 presents a selective list of various bacterial species actively used as probiotics.

Though weight loss may be attributed to various factors, researchers have suggested probiotic supplements help in reducing obesity and facilitate in control of body mass index. Probiotics may help in management of depression but still it is suggested that they must not be used as “antidepressant medications” (Bisht and Garg 2021; Garg et al., 2024). It is also not very clear that they help equally in all humans. Trials are still going on with large number of people among various types of group of people to find out the scientific truth by WHO and Medical Organizations of various countries. The probiotics are available in different forms including capsules, pills and liquids and their prescription and dosage as recommended by medical doctors should be strictly adhered and should be taken prior or after meals as per the suggestions of the manufacturer, however, they are more effective when taken empty stomach. Most healthy people do not need any supplement of probiotics as probiotics may cause overgrowth of certain bacteria and may create imbalance in gut microflora. Probiotics may be safe for infants, children and elderly people when prescribed and monitored by healthcare provider.

Although the health benefits of probiotics are widely recognized, recent studies have focused primarily on examining the viability of probiotic strains, as well as the conditions of their processing and their storage. These studies have also investigated the strains' sensitivity to various factors such as low pH levels, bile, pancreatic, gastric fluid and intestinal fluids, and intestinal or respiratory mucus. Additionally, research has explored probiotics' interactions with other microorganisms, including pathogens, and their adherence to cell cultures or isolated cells.

S.No	Probiotic bacterial genera	Species involved	Health claims	References
1.	*Lactobacillus*	*L. plantarum, L. paracasei, L. acidophilus, L. casei, L. rhamnosus, L. crispatus, L. gasseri, L. reuteri, L. bulgaricus*	Treatment of acute gastroenteritis in children, Reduction of risk for Rhinovirus infections in infants, Treatments for traveller's diarrhea, reduction of irritable bowel syndrome symptoms	(Dixit *et al.*, 2016), (MacFarland, 2007), *(Ciccarelli, et al., 2013), (Luoto, et al., 2013), (Otiz, et al., 2014)*
2	*Bacillus*	*B. coagulans, B. subtilis, B. laterosporus*	Treatment of diarrhoea and aiding in *H. pylori* eradication	(Nguyen *et al.*, 2016, *(Tompkins, et al., 2010)*
3	*Lactococcus*	*L. lactis, L. reuteri, L. rhamnosus, L. casei, L. acidophilus, L. curvatus, L. plantarum*	Treatment of antibiotic-associated diarrhea, antimicrobial and probiotic properties	(Eid *et al.*, 2016), (Johnston, et al., 2011), (Fernandez, et al., 2-13)
4	*Enterococcus*	*E. faecium*	Antibiotic and antioxidant activity, adherence to colonic tissue and anti-inflammatory activity, Treatment of antibiotic-associated diarrhoea	(Onyenweaku *et al.*, 2016), (Pieniz, et al., 2013), (Raz, et al., 2007), (Zelaya, et al., 2014)
5	*Pediococcus*	*P. acidilactici, P. pentosaceus*	Bacteriocin production, elimination of *H. pylori* infections	(Sornplang and Piyadeatsoontorn. 2016), (Mehta, et al., 2013), (Kaur, et al., 2014)
6	*Streptococcus*	*S. sanguis, S. oralis, S. mitis, S. thermophilus, S. salivarius*	Reduction of irritable bowel syndrome symptoms, antibiotic resistance of yogurt starter culture	(Arora *et al.*, 2013), (Wu, et al., 2013), (Qin, et al., 2013)
7	*Bifidobacterium*	*B. longum, B. catenulatum, B. breve, B. animalis, B. bifidum*	Treatment of functional constipation in adults, Reduction of hospital stay of children with acute diarrhea, treatment of gastrointestinal diseases	(Westermann *et al.*, 2013), (Chmielewska, et al., 2010), (Phavichitr, et al., 2013), (Yu, et al., 2013)
8	*Bacteroides*	*B. uniformis*	Treatment of C.difficile associated diarrhea, Treatment if rare conditions of Immune disorder	(Kobyliak *et al.*, 2016), (Tufail, et al., 2024)
9	*Akkermansia*	*A. muciniphila*	Helps in the treatment of immunity related disorders and obesity disorders	(Kobyliak *et al.*, 2016), (Zhao, et al., 2024)
10	*Saccharomyces*	*S. boulardii*	Treatment of travellers' diarrhea, treatment of acute gastroenteritis in children	(Chen *et al.*, 2013), (MacFarland, 2007), *(Ciccarelli, et al., 2013)*

Table 1: Microorganisms used as probiotics.

Characteristics of Desired Probiotic Strains

The intestinal flora constitutes approximately 95% of the total microbial cells in a healthy human microbiome. Occasionally, the Diseases like inflammatory bowel disease (also known as Crohn's disease and ulcerative colitis) can be influenced by the bacterial flora that lives in the gastrointestinal system. Therefore, the beneficial traits of probiotics—such as bile tolerance, acid resistance, adherence to host epithelial surfaces, and antagonistic activity against common pathogenic microbes or those suspected to promote inflammation—must be thoroughly evaluated before they are recommended as probiotics. According to the WHO, FAO, and EFSA (the European Food Safety Authority), probiotic strains need to fulfil safety and functioning requirements (Markowiak and Sliżewska, 2017; Chelakkot et al., 2018).

The most important guidline for selection of probiotics is their capacity to survive the passage through gastrointestinal tract remaining in viable state with sufficient numbers of physiologically active cells that can exert probiotic effect. Colonization ability, aggregation, antimicrobial potential, immunomodulatory action with cardiovascular and cancerous protective properties besides their human safety are considered as major selection criteria for use of probiotics as functional foods. Probiotic characteristics are independent of the genus or species of a microorganism (Hill et al., 2014). Important safety factors for choosing probiotic strains are their origin, absence of relationship with pathogenic cultures, and antibiotic resistance profile. In the gastrointestinal system, survival and exhibition of their immune modulatory effect are categorized as the functional aspects of probiotics (Chiang and Pan, 2011; Berardi *et al*., 2013) Probiotic strains must be able of surviving and preserving their beneficial properties all through the entire process, from production and storage to distribution and eventual consumption by humans.

(Lee, 2009). Formation of short chain fatty acids by probiotics is considered as an important criteria to categorize them under the category of "happy bacteria". The desired probiotics should possess well proven and well characterized antidepression, anticarcinogenic, antianxiety, antidiabetic, antiobesity, cholesterol-lowering activities and immunostimulatory,. They should withstand the stressful conditions of human digestive system. WHO has recommended that probiotics must possess plasmid associated antibiotic resistance, epithelium adhesion activity and antimicrobial activity against pathogens. In post covid-19 era, the consumers have become increasingly aware of the quality of food supplements that may be necessary and essential for the management of balance between their health, energy requirement, nutrition and environment. Over the past few years, commercial manufacturing and commercialization of functional foods and energy supplements particularly probiotics containing yogurts, milk and various preparations have lucrative and expanding markets not only in western countries but also in Asian, African and other developing nations. European Union funded programs - The Lactic Acid Bacteria Industrial Platform (LABIP) Facilitate the spread of scientific findings, foster the development of consensus, and encourage direct interaction between various stakeholders like research institutions / Universities and the manufacturers. Our group has been working on isolation and characterization of LAB from human and animal colostrum, various natural habitats and the human feces from the children to adults living under different environmental conditions (Siddique and Garg, 2022; Arya et al., 2018; Komalben et al., 2022). The key factor in the collection and processing of sample is that the researcher should have least time gap as some of LAB are highly sensitive for exposure to oxygen especially when we are isolating the gut microbes for feces.

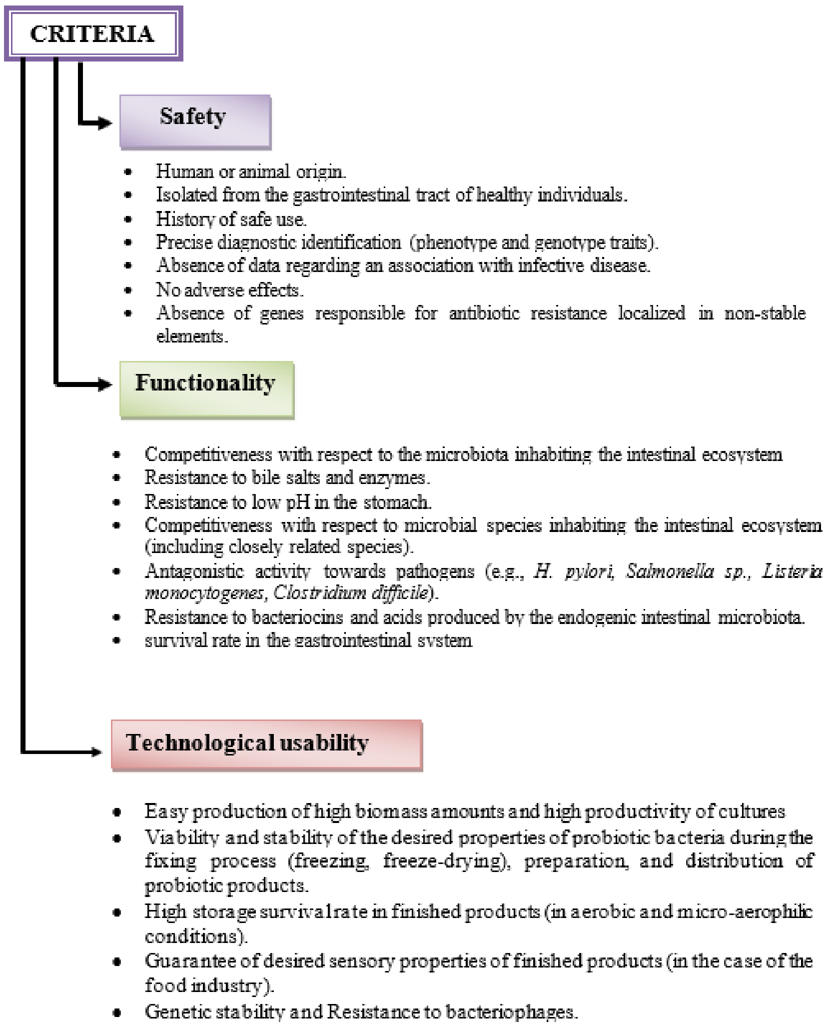

Figure 1: Desired Characters of Probiotics (EFSA, 2005 and FAO, 2002)

General Aspects

Origin

The origin of probiotics totally depends on its application and origination from a targeted animal microflora. Probiotic strains may be isolated from several sources, including human origins such as the large intestine, small intestine, or breast milk; animal origins; and dietary sources such raw milk or fermented foods (Dash 2009; Elmer *et al*., 2007, Komalben et al., 2022; Siddique and Garg, 2022). The probiotic strains derived from human microflora exhibit greater adherence to the human gut wall compared to others and are considered safe (Shewale *et al.*, 2014; Vantsawa *et al.*, 2017). The natural microbiota of human milk serves as the optimal source for isolating new probiotic bacteria. The strain must be accurately isolated and identified prior to utilisation (Dash 2009; Elmer *et al*., 2007; Arora *et al.*, 2013; Ibrar *et al*, 2017; Carlson *et al*., 2018; Bogucka *et al*, 2019; Bisht and Garg,2019).

Genus, Species and Strain Identification

Probiotics are specific to their strains, necessitating identification at the genus, species, and strain levels, as outlined in the WHO/FAO guidelines from 2006. Various genera of bacteria and yeasts have been suggested as probiotic cultures, with Lactobacillus and Bifidobacterium species being the most frequently utilised (Devi et al., 2021) (Table 2). In addition to Streptococcus, the genera Pediococcus, Enterococcus, as well as the yeast-like genera Saccharomyces, Aspergillus, and Torulopsis, are also utilised (Nemcova 1997). The main criteria for identification include cell morphology, metabolite determination, enzyme activity, carbohydrate fermentation, and the development of molecular tools for identifying probiotics through the analysis of nucleic acids and other macromolecules (Shewale *et al.*, 2014; Tankoano *et al.*, 2017). Although identification technologies may change over time and may vary depending on the microorganism, several molecular approaches are already widely used since phenotypic methods alone are not enough for accurate identification. As long as precise reference sequences are used, 16S ribosomal DNA sequencing is an established and reliable technique for species identification. The obtained sequence can first be compared to a large reference library that includes almost all known bacterial species, but final validation is best done using carefully selected databases such as PATRIC (Wattam et al., 2017).

The DNA-DNA hybridisation method is still used since it takes a lot of time and effort. Fatty acid methyl ester (FAME) and the DNA sequence encoding 16SrRNA are also utilised for identification purposes. The World Health Organisation advises that all strains be submitted to a culture collection that is internationally recognised (Zhang *et al.*, 2011; Perin and Nero, 2014; Serrano -Nino *et al.*, 2016; Fahrurrozi *et al.*, 2019).

Biosafety

It is essential that the chosen strains are non-pathogenic. In general, Lactic acid bacteria demonstrate a strong safety record (Fig 2) (Tongwa et al., 2019). The strains of microorganisms must be classified as Generally Recognised as Safe (GRAS) and adhere to the Qualified Presumptions of Safety (QPS) as evaluated by the European Food Safety Authority (EFSA). Prior to the selection of additional probiotics, it is mandatory to implement toxicological studies. Organisms and spores derived from soil are asserted to possess probiotic properties (Harlé *et al*.,2020).

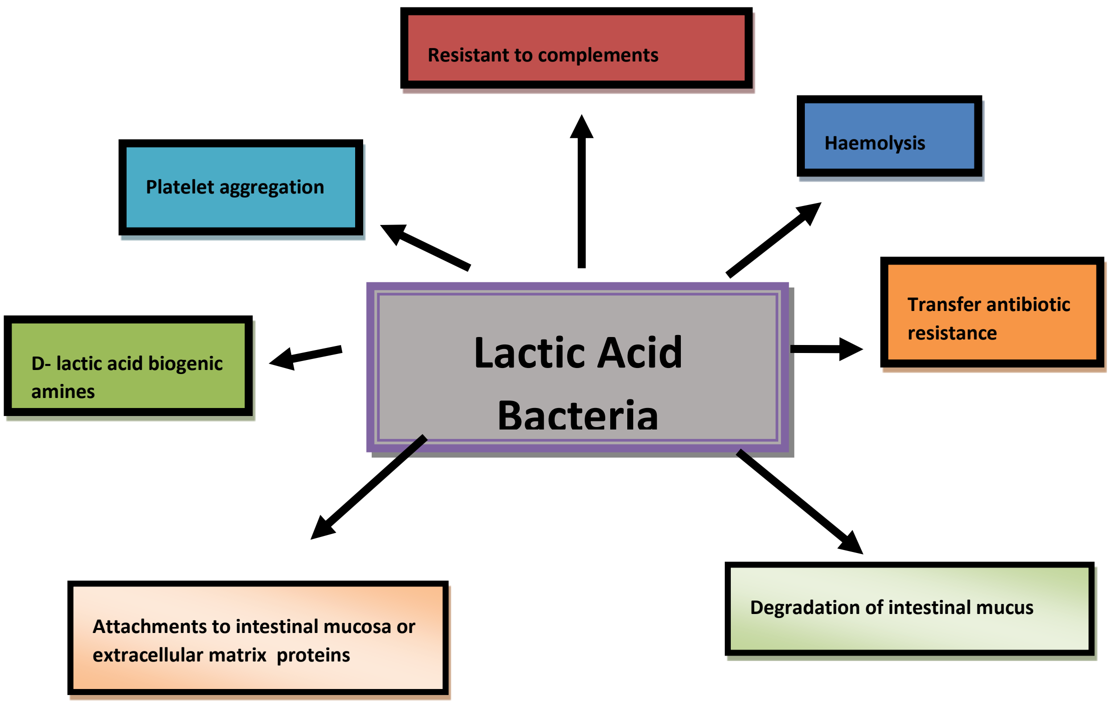

Figure 2: Potential risk factors to determine the safety of lactic acid bacteria.

Understanding the mechanism of action of probiotics

The primary pathway through which probiotics operate involve strengthening the epithelial barrier, enhancing adhesion to the intestinal mucosa (thereby inhibiting pathogen adhesion), competitively excluding infectious microorganisms, producing antimicrobial compounds like bacteriocins, and regulating the immune system (Fig 3) (Kumari *et al.*, 2008; Gomand *et al.*, 2019; Chugh and Kamal-Eldin, 2020).

Enhancement of the Epithelial Barri

The luminal material and the intestinal epithelium are constantly in touch. It is an essential defence mechanism that aids in preserving the integrity of the epithelium and providing protection from the outside world. The intestinal barrier is defended by the mucous layer, secretory IgA, antimicrobial peptides, and epithelial junction adhesion complexes (Ohland and Macnaughton, 2010; Gomand et al., 2018).

S.No	Food and feed application	*Lactobacillus species*
	Dairy products	
1.	Fermented milk	*Lb. acidophilus; Lb. rhamnosus; Lb. reuteri; Lb. casei; Lb. plantarum; Lb. johnsonii; Lb. crispatus; Lb. paracasei; Lb. gasseri*
2.	Frozen yogurt	*Lb. delbrueckii subsp. bulgaricus; Lb. acidophilus*
3.	Ice cream	*Lb. acidophilus; Lb. johnsonii La1*
4.	Cheddar cheeses	*Lb. paracasei*
5.	Argentine fresh cheese	*Lb. acidophilus; Lb. casei*
	Non dairy products	

6.	Dry sausages	*Lb. species*
7.	Chocolate	*Lb. acidophilus Rosell 52*
8.	Juice drinks	*Lb. plantarum 299v*
9.	Dry sausages	*Lb. curvatus LBPE*

Table 2: Diversity of probiotic *Lactobacilli* in dairy and non dairy products

Antigens have the ability to penetrate sub-mucosa and can also trigger inflammatory responses, including inflammatory bowel disease (Sartor, 2006). The presence of non-pathogenic bacteria could potentially disrupt the epithelial barrier. The intestinal barrier's integrity depends on the expression of genes related to tight junction signalling (Anderson et al., 2010). In a T84 cell barrier model, lactobacilli help control many genes that encode adherence junction proteins, including catenin and E-cadherin (Brito et al., 2012). Several investigation have shown that prolonged exposure of intestinal cells to lactobacilli influences the levels of protein kinase C (PKC) isoforms and the phosphorylation of adherence junction proteins (Hummel et al., 2012).

Probiotics have the potential to commence the restoration of barrier function following injury. Escherichia coli Nissle 1917 (EcN1917) plays a vital role in preventing the breakdown of the mucosal barrier caused by enteropathogenic E. coli, while also contributing to the restoration of mucosal integrity in T84 and Caco-Two cells, Lactobacillus casei DN-114001 (Parassol et al., 2005) and VSL3 (a mixture of pre- and probiotics), demonstrate the ability to maintain intestinal barrier function through comparable mechanisms. A recent research found that VSL3 increases tight junction protein expression in both in vitro and in vivo situations, protecting the epithelial barrier via activating the p38 and extracellular signal-regulated kinase signalling pathways (Dai et al., 2013).

The protection against cytokine triggered cell apoptosis involves the isolation and purification of two peptides produced by Lactobacillus rhamnosus GG (LGG), namely p40 and p75. These peptides activate the anti-apoptotic protein kinase B (PKB/Akt) through a phosphatidylinositol-3 kinase-dependent pathway, while also restricting the pro-apoptotic p38 (Yan *et al*., 2017; Guerin *et al.*, 2017).

Different low-molecular-mass peptides released from LGG promote the synthesis of heat shock proteins and stimulate mitogen-activated protein kinase (MAPKs) (Tao et al., 2006). In epithelial mucus, the primary macromolecular components are mucin glycoproteins (mucins), which have strongly correlated with both health and disease. The secretion of mucous enhances barrier functions and aids in the exclusion of pathogens through the use of probiotics. Recent studies indicate that various Lactobacillus species enhance mucin expression in human intestinal cell lines. The protective effect of Lactobacillus is entirely reliant on its adhesion to the cell monolayer in vitro, whereas this effect is not observed in vivo (De Melo et al., 2018). The cell extract of Lactobacillus acidophilus A4 and VSL3 from certain Lactobacillus species enhance MUC2 expression in HT29 cells (Kim *et al.,* 2016). Only few in vivo investigations have been performed for continuously 14 days where mice given VSL3 daily which do not show changed mucous layer thickness or mucin expression (Gaudier *et al.,* 2005). Similarly, for 7 days rats given VSL3 daily have a 60-fold increase in MUC2expression and a concomitant enhancement of mucin production (Caballero *et al.*, 2007).

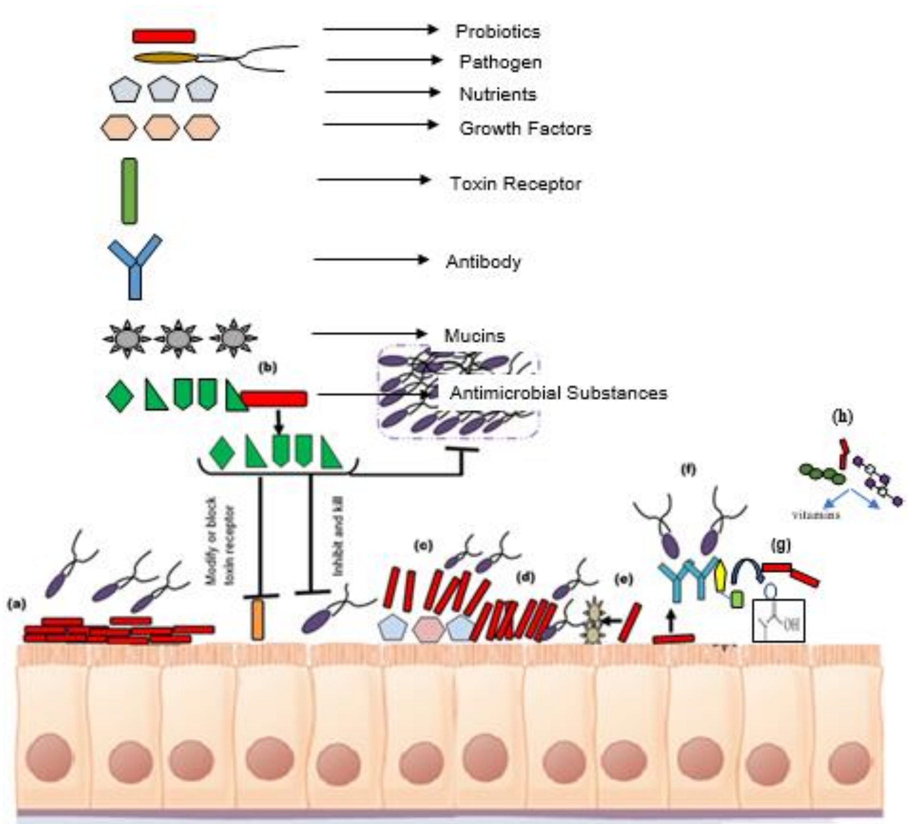

Figure 3: Major Mechanisms of action of Probiotics a) Competitive exclusion of pathogenic microorganisms. b) Production of antimicrobial substances. c) Competition for nutrients & growth factors. d) Increase adhesion to intestinal mucosa. e) Enhanced epithelial barrier function. f) Enhanced Ig A secretion (Immune stimulation). g) bioconversion of, for example, sugars into fermentation products with inhibitory properties. h) production of growth substrates, for example, EPS or vitamins, for other bacteria.

Increased Adhesion to Intestinal Mucosa

For probiotics to interact effectively with the host, adhesion to the intestinal mucosa is a key requirement (Beachey, 1981; Schiffrin et al., 1997; Juntunen et al., 2001; De Melo et al., 2018). Immune system modulation by probiotics involves two essential factors: their ability to stick to the intestinal mucosa (Perdigon et al., 2002) and their ability to combat pathogens (Hirano et al., 2014). As a result, adhesion has become a crucial criterion in selecting new probiotic strains (Ouwehand, 1998; Collado et al., 2005) and is linked to the specific health benefits associated with probiotics (Castagliuolo et al., 2005).

Lactic acid bacteria (LABs) exhibit a spectrum of surface determinants that play a role in their interaction with intestinal epithelial cells (IECs) and mucus. Main component of mucus is a complex mixture of glycoproteins, specifically mucin, which is secreted by intestinal epithelial cells and plays a important role in preventing the adhesion of pathogenic bacteria (González-Rodríguez et al., 2012; Collado et al., 2005). Alongside mucous, various other substances are present, including immunoglobulins, salts, lipids, and free proteins (Neutra and Forstner, 1987). Lactobacillus reuteri serves as the most extensively researched instance of bacterial adhesions that target mucus, specifically MUB (mucus-binding proteins) (Buck et al., 2005; Hynönen et al., 2002). MapA (mucous adhesion-promoting protein) has been shown to facilitate the association of L. reuteri and L. fermentum to mucus (Ouwehand et al., 2002). Reports indicate that L. plantarum induces MUC2 and MUC3 mucins, which suppress the adherence of enteropathogenic E. coli.Research indicates that the combination of VSL3 and probiotics enhances the production of cell surface mucins, which in turn influences mucin gene expression (Caballero et al., 2007). The suggested dosage of Lactobacilli in cfu/day is presented in table 2.2. Probiotics prevent pathogen binding by inducing qualitative changes in intestinal mucins (Kim and Ho, 2010). The association of protease-resistant strains of LB and BG2FO4 L. acidophilus with the bacterial surface has been documented in various studies (Chauvière et al., 1992; Coconnier et al., 1992; Greene and Klaenhammer, 1994). Defensins are small peptides or proteins secreted by certain probiotic strains, exhibit activity against bacteria, fungi, and viruses. Additionally, they play a role in stabilising gut barrier function (Furrie et al., 2005). The LAB adhesion process encompasses a variety of mechanisms, including lipo-teichoic acids, hydrophobic interactions, electrostatic interactions, steric forces, passive forces and specific structures like external appendages that are covered by lectins.

Competitive Exclusion of Pathogenic Microorganisms

In 1969, Greenberg firstly introduced the concept of 'competitive exclusion' to describe the circumstance in which one bacterial species competes more effectively for receptor sites in the intestinal tract compared to different species (Bisht and Garg, 2021). A single bacterial species employs various mechanisms to inhibit or decrease the growth of another species, such as creating an unfavorable micro-ecology, blocking available bacterial receptor sites, producing and releasing antimicrobial substances and selective metabolites, and competitively depleting essential nutrients (Rolfe, 1991). The communication between surface proteins and mucins plays a role in the adhesive properties of bacteria, which may prevent the colonization of harmful pathogens, contributing to the antagonistic effects observed in some probiotic strains against gastrointestinal pathogens (Servin, 2004; Bisht and Garg, 2019). Lactobacilli and bifidobacteria have been shown to exhibit a wide spectra of antagonistic activities against pathogens like *E. coli*, *Helicobacter pylori*, *Salmonella*, *Listeria monocytogenes*, and *Rotavirus* (Tsai et al., 2008; Chenoll et al., 2011; Nakamura et al., 2012; Muñoz et al., 2011; Bisht and Garg, 2022). Notable examples of environmental modification through antimicrobial substances include lactic and acetic acids (Tankoano et al., 2019; Harlé et al., 2020). Certain entero-pathogens share carbohydrate-binding specificities with lactobacilli and bifidobacteria (Nesser et al., 2000; Fujiwara et al., 2001), allowing these probiotic strains to rival with pathogens for receptor sites (Mukai et al., 2002). The role of probiotic bacteria in competitive removal of pathogens has been clearly demonstrated in studies using human mucosal samples (in vitro) (Ouwehand et al., 1999; Hirano et al., 2014), as well as mucosal samples from chickens (Hirn et al., 1992) and pigs (in vivo) (Genovese et al., 2000). A strain of *L. rhamnosus* with strong adherence capabilities has been shown to stop the internalization of entero-hemorrhagic *E. coli* (EHEC) in a human intestinal cell line, as noted by Hirano et al. (2014).

Production of Antimicrobial Substances

Probiotics are involved in the production of low molecular weight compounds (less than 1,000 Da), such as organic acids, as well as the synthesis of antibacterial agents like bacteriocins (greater than 1,000 Da). The main antimicrobial compounds manufactured by probiotics, which help inhibit pathogens, include organic acids like acetic acid and lactic acid, which have shown inhibitory effects against Gram-negative bacteria (Alakomi et al., 2000; De Keersmaecker et al., 2006; Makras et al., 2006; Emeka et al., 2020; Bisht and Garg, 2022). These organic acids' undissociated form enters the bacterial cell and separates in the cytoplasm. As a result, the intracellular pH drops and the ionised form of the organic acid builds up, which may kill the pathogen (Ouwehand, 1998; Agim et al., 2020). Small antimicrobial peptides (AMPs) and bacteriocins are two examples of the antibacterial peptides that are produced by lactic acid bacteria (LAB). Gram-positive bacteria are usually the ones that create certain bacteriocins, which have a limited range of activity, such as lactobacilli. Examples include nisin from *Lactococcus*, plantaricin from *L. plantarum*, and *lactis* lactacin B from *L. acidophilus*. These substances are effective against foodborne pathogens (Kumari et al., 2008; Nielsen et al., 2010; Bisht and Garg, 2021).

The main ways that bacteriocins work are by causing pore development in target cells and preventing the creation of cell walls (Hassan et al., 2012). For instance, nisin and lipid II, a cell wall precursor, combine to generate a compound that prevents spore-forming bacilli from producing their cell walls (Bierbaum, 2009). The production of bacteriocins helps establish beneficial microorganisms while directly inhibiting the growth of harmful pathogens in the gastrointestinal tract (O'Shea et al., 2012; Kumari et al., 2008). Intestinal bacteria also produce various beneficial fatty acids that contribute to overall health. It has been demonstrated that several strains of Lactobacilli and Bifidobacteria have strong anti-carcinogenic properties, mostly because they produce conjugated linoleic acid (CLA) (Macouzet et al., 2009; O'Shea et al., 2012).

The treatment of Bifidobacteria and Lactobacilli that produce CLA is the only way to modify the fatty acid content of the liver and adipose tissue in a mouse model (O'Shea et al., 2012).

Probiotic bacteria can generate de-conjugated bile acids, that are forms of bile salts. Compared to bile salts produced by the host organism, de-conjugated bile acids have more antibacterial action. It is widely recognised that certain strains of probiotics generate metabolites that suppress the growth of fungi and other bacteria (Coloretti et al., 2007; Lindgren and Dobrogosz, 1990). Some studies have indicated that Lactobacillus generates antifungal substances including benzoic acid, methyl hydantoin, and mevalono lactone (Prema et al., 2008; Niku-

Paavola et al., 1999). In 2001, Magnusson and Schnürer identified that Lactobacillus coryniformis is capable of producing proteinaceous compounds with antifungal properties. Dal Bello et al., 2007 documented the discovery of antifungal substances generated by L. Plantarum FST 1.7 comprises lactic acid, phenyl lactic acid, and two cyclic dipeptides: cyclo(L-Leu-L-Pro) and cyclo(L-Phe-L-Pro).

Probiotics and the Immune System

It is possible for probiotic microorganisms to alter immune responses. They engage in interactions with lymphocytes, monocytes/macrophages, epithelial cells, and dendritic cells (DCs). There are two categories of immune systems: innate and adaptive.

B and T cells, which are specialised to recognise certain antigens, are essential to the adaptive immune response. On the other hand, pathogen-associated molecular patterns (PAMPs) trigger the innate immune system (Gómez and Muñoz, 2010). The initial reaction to pathogens is initiated by pattern recognition receptors (PPRs), like toll-like receptors (TLRs), which interact with PAMPs. The interaction between host cells and probiotics predominantly occurs with IECs. Furthermore, probiotics can interact with dendritic cells, which are crucial in both innate and adaptive immune responses. Both of IECs and DCs have the capability to interact with and respond to gut microorganisms via their PPRs (Lebeer et al., 2010; Gómez and Muñoz, 2010; Bisht and Garg, 2019; Arya et al., 2018). It can be inferred that in the current landscape of awareness, probiotic supplements have gained significant popularity, offering various advantages when taken and overseen according to the guidance of healthcare professionals.

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