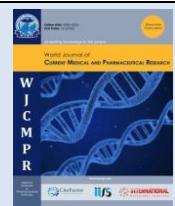




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ROLE OF PROBIOTICS IN HUMAN HEALTH AND ITS APPLICATIONS

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Abstract



This review is aimed to explore the health beneficial effects of probiotics which are live microorganisms that provide a positive health influence on humans when taken in sufficient quantity. Lactic acid bacteria, bifidobacteria, and yeast are frequently used as probiotics. Microorganisms occupy a prominent position in the industrial sector due to their unique properties, such as the limited time and space required for their growth and proliferation, as well as their easy manipulation of the genetic material. Among all the microorganisms, probiotics have grabbed the attention of researchers because of their non-pathogenic nature and immersive application in treating digestive ailments and vitamin deficiency, boosting immunity, and detoxifying harmful chemicals. Probiotics, like lactic acid bacteria, are non-pathogenic microbes that exert health benefits to the host when administered in adequate quantity. Currently, research is being conducted on the molecular events and applications of probiotics. Considering the functional properties, probiotics are being used in the dairy, beverage, and baking industries. Thus, the potential of probiotics can efficiently be utilized on a commercial scale in food processing industries. Hence, probiotics have become an alternative to several drugs including antibiotics. In addition, probiotic efficacy also depends on the delivery system as the delivery agents help the bacteria to survive in the harsh environment of the human gut. This review explored a portrait of the beneficial effects of probiotics on human health. Probiotics have become increasingly popular over the past two decades due to the continuously expanding scientific evidence indicating their beneficial effects on human health. Therefore, they have been applied in the food industry to produce functional food, which plays a significant role in human health and reduces disease risk.

Keywords: Probiotics, gut microbiota, metabolism, anti-biotics, health benefits

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Introduction

Probiotic is executed from the Greek word 'pro bios' which means for "life" as opposed to 'antibiotic' which denotes 'against life' [1]. Microorganisms, such as bacteria, fungi, archaea, protists, plankton, and amoebae, are prevalent in our day-to-day lives. The most recent estimate is that about 38 trillion microorganisms live in and on human individuals and play a crucial role in stimulating the immune system, detoxifying potential toxins, and synthesizing vitamins and amino acids essential for cellular metabolic functions. Among all the genera of microorganisms, *Lactobacillus*, *Bifidobacterium*, *Escherichia coli*, *Clostridium*, *Streptococcus*, *Peptococcus*, *Ruminococcus*, *Fusobacterium*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Bacteroides*, and *Eubacterium* are dominant in the regulation of human metabolic

homeostasis. Probiotics, in the form of supplements or food products, have emerged as the most prominent ingredient in the era of functional foods. Research has shown that the gut microbiome plays a vital role in health and disease, affecting humans' metabolism, nutrition, physiology, and immunity.

The probiotic concept has contributed towards the development of functional foods. Now scientists have developed new and innovative methods like nanoencapsulation and genetic modification, which enable probiotics to withstand harsh conditions of both processing and GI stresses in the body. Regarding the outcome of the probiotics research, a couple of guidelines about efficient strain design and development were introduced in the 1980s. As per these guidelines, therapeutic probiotics must meet all the following criteria: (a) strains must show a symbiotic, therapeutic effect; (b) they must be non-immunogenic and non-pathogenic; (c) strains should be compatible with the host system's microbial environment, and be adaptable in the host system by keeping their variability; (d) strains should protect the healthy environment of the gut microbial flora; (e) during production, formulation, and storage, strains must be stable in their metabolic activities [2].

Probiotics strain selection criteria and requirements

To meet the clinical requirements, EFSA (European Food Safety Authority), WHO, and FAO issued mandatory guidelines to probiotic researchers, stating that the strains must meet safety and functionality requirements, such as the route of strain selection, nonpathogenic, non-immunogenic nature, resistance to antibiotics, long durability in the gastrointestinal tract, and the ability to maintain their activity during production, processing, and preservation, which are crucial for patient safety [3,4].

The following critical factors are tested during the initial screening and selection of probiotics:

- Stability of phenotypes and genotypes, including plasmid stability;
- Tolerance to bile and acid, as well as survival and growth;
- The adhesion characteristics of intestinal epithelial cells;
- Antimicrobial compound production;
- Patterns of antibiotic resistance;
- Inhibition of known gut pathogens;
- Immunogenicity, spoilage organisms, or both.

lactic acid bacteria (LAB), used in yogurt, cheese, and pickles, attained a prominent position as the best probiotic supplement due to its unique properties (as mentioned by EFSA) and lack of lipopolysaccharides (LPS) and harmful extracellular proteases. During the research for efficient probiotics, researchers reported that *Lactococcus* and *Streptococcus* are predominant in the human ileum and jejunum, as well as, at lower densities, in the colon. This symbiotic relationship raised researchers' attention to the molecular mechanisms that make these strains suitable for treating intestine-related ailments. This indigenous property prompted the researchers to develop engineered therapeutic probiotics, keeping *Lactobacillus* strains as reference strains to deliver molecules directly to the mucosa .

Probiotics on epithelial barrier enhancement

The intestinal barrier serves as a significant defense system, shielding the organism from the environment while preserving epithelial integrity. The mucous layer, antimicrobial proteins, IgA, and the complex of epithelial junction adhesion are all parts of the intestinal barrier's defense. When this barrier function is compromised, bacteria and food antigens may enter the submucosa and cause inflammatory responses, leading to intestinal disorders [5]. Non-pathogenic bacteria particularly probiotic bacteria can aid in the maintenance of the intestinal barrier. *Lactobacilli* change the direction of numerous genes that encode the adherence junction proteins (E-cadherin, and β -catenin) [6]. In addition, *Lactobacillus* incubation could change adhesion junction proteins phosphorylation and modulate protein kinase C isoforms, including protein kinase C-delta (PKC δ) [7] (Fig. 2)

Mechanism of action of next generation probiotics

Probiotics can have a variety of effects on the host (Fig 4 .), including changes in the gut microbiome, modulation of the immune system, and production of bioactive compounds. The mechanisms of action of the NGPs on gut health, immune

function, and other health outcomes can vary depending on the specific strain or formulation [8,9]. However, probiotics have a wide range of beneficial effects on gut health and the immune system. They produce beneficial compounds such as short-chain fatty acids (SCFAs) and lactic acid and increase the production of antimicrobial peptides like lactobin A, curvacin A, enterocin and pediocin in the gut. These peptides play a crucial role in the defense against pathogenic microorganisms and help maintain a healthy gut microbiota, kill pathogenic bacteria and boost the immune system [10].

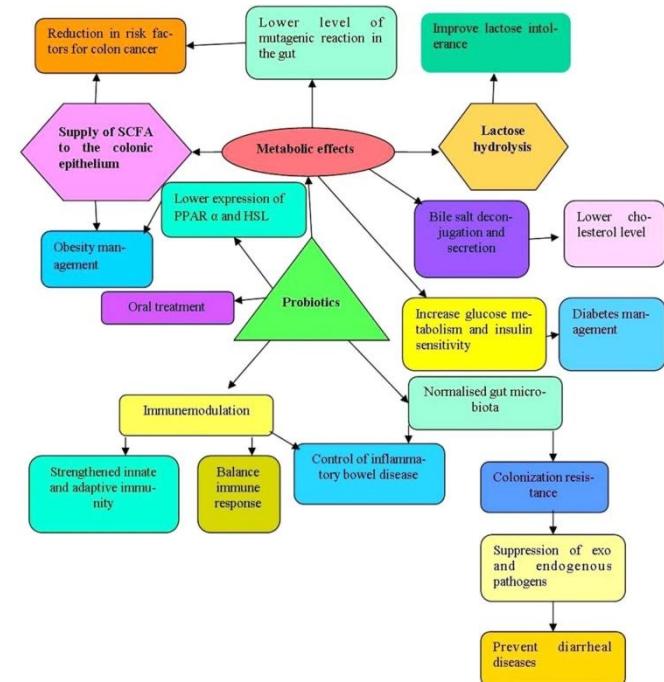


Fig. 1. Different health beneficial effects of probiotics

Probiotics colonization on gastrointestinal epithelial cells

The utilization of state-of-the-art technologies enabled the researchers to observe cells at the nanoscale level, thus easing their effort to detect and manipulate single-cell surface constituents [18]. Lactic acid bacteria are mainly Gram-positive bacteria that have thick peptidoglycan, which is a cross-linked matrix of linear carbohydrate (glycan) chains linked to one another through covalent bonds between attached peptides, allowing the covalent attachment of wall teichoic acid, surface proteins and polysaccharides [11]. The lactic acid bacteria marked no difference from other Gram-positive bacteria in the general architecture of their cell wall. Many elements exposed to the outer cell envelope, such as polysaccharides, teichoic and lipoteichoic acids and proteins, are responsible for bacterial adhesion onto intestinal cells [12] Probiotics colonization on gastrointestinal epithelial cells.

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Identification and characterization of new probiotics strains Probiotics are found in traditionally fermented food, human breast milk, human and animal gut, and the intestinal tracts of marine and freshwater fish [15]. Advances in genetic sequencing and bioinformatic analysis have enabled the identification and investigation of new probiotic strains. Dairy and dairy-related products contain probiotics such as lactic acid bacteria (LAB), bifidobacteria, and other microorganisms derived from fermented milk, which have been used for centuries [15,16].

from many ecosystems undergo several steps including isolation and identification, taxonomic classification using genotypic and phenotypic methods, and characterization and evaluation (Figure 2)

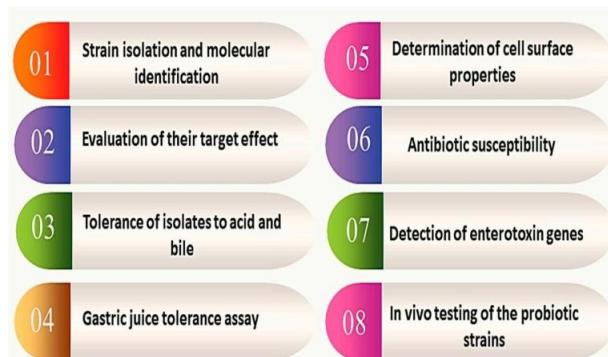


Figure 2. A diagram representing the sequential steps for the characterization of probiotics.

Health attributes of probiotics

The health benefits of probiotics are associated with preventing and reducing many diseases, i.e., allergic diseases, cancer, hypercholesterolemia, lactose intolerance, inflammatory bowel disease, diarrhea, and irritable bowel syndrome, as shown in Fig 7. shows different studies regarding the application of probiotics in different diseases. Probiotics help in the prevention and management to allergic diseases, cancer, hypercholesterolemia, irritable bowel syndrome, diarrhea, lactose intolerance, inflammatory bowel disease.

Hypocholesterolemia effect of probiotics

Probiotics can be used as an effective tool for lowering blood cholesterol levels. They can act directly or indirectly to decrease cholesterol levels in the body. The direct mechanism includes the inhibition of de novo synthesis of cholesterol by hypocholesterolemia factors like uric acid, lactose, orotic acid, and whey protein as well as the reduction in intestinal absorption of dietary cholesterol in three ways- assimilation, binding, and degradation. Cancer suppressor activity of probiotics Probiotic could be used as an adjuvant for various types of cancers based on their potential to modulate enteric flora and enhance local and systematic immunity. They prevent the initiation, progression, and metastasis of transplantable or chemically induced tumors. The effect of probiotics can be observed in suppressing both intestinal and extraintestinal cancers. The interaction of probiotics and their metabolites

(bacteriocin, peptides, and organic acids) with critical metabolic pathways such as cellular proliferation, inflammation, apoptosis, angiogenesis, and metastasis has been revealed by many researchers. Probiotics also upregulate TNF-related apoptosis-inducing ligand (TRAIL), modulate cell cycle by rapamycin (mTOR)/4EBP1 and inhibit the formation of aberrant crypt foci.

Use of Probiotics in Foods

Dairy products

Generally, probiotics can be incorporated into fermented or non-fermented dairy products, including sour and fresh milk, yogurt, kefir, and cheese. Dairy products are the main marketable probiotic foods essential in delivering probiotics to humans, providing a suitable environment for probiotic bacteria that support their growth and viability.

Plant based food

Researchers have increasingly focused on plant-based proteins like soybeans and peas in recent years, driven by their sustainability and potential as alternatives to high-quality animal protein. Soy products fermented by probiotics show potential in reducing levels of certain carbohydrates that may contribute to gas production in the intestinal system.

Fruit juices

There is a growing interest in developing probiotic products based on fruit juice. Fruit juices contain beneficial nutrients that can be an ideal medium for probiotics. Fermented beverages have highly variable characteristics based on the fermentable sugar spectrum, the probiotic strain, and nitrogen (protein).

Factors affect the viability of probiotics in food product:

Probiotics play an important role, which poses a technological challenge for selecting and delivering them efficiently while ensuring their viability. The survival of probiotics in food products during processing and storage is influenced by various factors.

Applications of probiotics

Probiotics influence the intestinal microbiota, enhance the synthesis of short-chain fatty acids (SCFA), and reduce the chance of developing diseases. The details are mentioned in Figure 3. Probiotics minimize the risk of several diseases, such as constipation, colon cancer, type 2 diabetes mellitus, and obesity, and they treat a range of intestinal disorders, including inflammatory bowel disease, among others

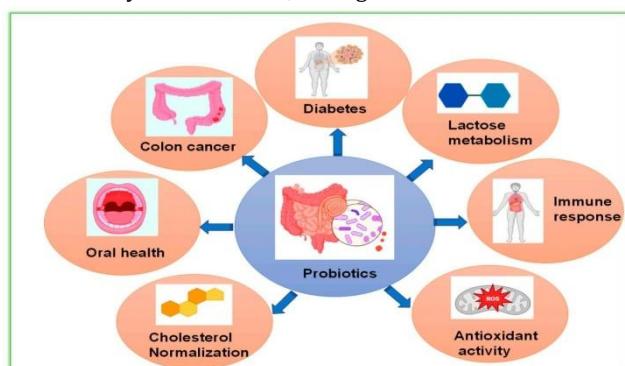


Figure 3. The probiotic clinical applications.

Quality control aspects

Probiotics are habitually not easy to use in industrial settings also. The probiotic definition has an extensive array of prospective applications for human and animal health purposes. Probiotic products are beneficial to the host's health. The widely held items intended for human utilization of milk are prepared through the fermentation process or powders or tablets. These capsules and tablets are not intended for medical use. They are only used to supplement one's fitness. Probiotics have a positive impact on the gut microbiota when consumed orally. Many reports indicated that probiotics may help with gastrointestinal microbial disorders, but clinical evidence of such products is not easy to find. The probiotic strain should be followed common quality parameters designed by the FAO and WHO such as (a) genetic identification of the claimed probiotic strains, (b) high growth in number i.e., colony-forming units (CFU) per gram of the final products of each strain, (c) stability and viability, (d) purity of the selected strain (e) and proper labelling of the probiotic products providing the valuable information about their nutritive value, the production date of that foods, and expiry date. Considering the health beneficial effects, probiotics are now being marketed worldwide.

Future Prospective

The emergence of probiotics has created robust advancement in the food industry in developing functional foods containing probiotics, especially for gut health and immunity strengthening. Human clinical trials are crucial to provide substantial evidence supporting the effectiveness of probiotics as functional foods. The significance of clinical trials in clarifying the effectiveness of probiotics is evident and underscores the need to comprehend the regulatory challenges associated with technology transfer and commercialization. The trials must be designed scientifically (typically randomized, double-blind, placebo- controlled trials) and performed according to the regulations associated with the product category.

Conclusion

This study has reviewed the health-beneficial effects of probiotics along with their mechanism of action including epithelial barrier enhancement, adhesion to the intestinal mucosa, competitive exclusion to eliminate the pathogenic organism, antimicrobial substance production, prevention of diarrhoeal diseases, prevention of inflammatory bowel disease, prevention of urogenital infection, gastric ulceration of diabetes, anti-cancer activity, increase the synthesis of shortchain fatty acids and altering the immune function to provide the host advantage. However, more human clinical trials are essential to increase the efficacy of probiotics, and thus it will help to maintain better health.

Author Contributions

All authors are contributed equally

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Declaration of Competing Interest

The Authors have no Conflicts of Interest to Declare.

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