# Exopolysaccharides from Probiotic Bacteria: Health Benefits and Therapeutic Potential

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***Abstract*** **- Exopolysaccharides (EPS) properties. These high-molecular-weight carbohydrate polymers are secreted extracellularly by various strains of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and other probiotic genera. While traditionally recognized for their role in biofilm formation and bacterial adhesion, recent advances in microbiology, immunology, and functional food sciences have highlighted their profound impact on human health. This review aims to provide a comprehensive overview of the structural diversity, biosynthesis, and functional roles of EPS derived from probiotic bacteria, with a particular focus on their therapeutic applications. EPS from probiotics exhibit diverse biological activities, including immunomodulation, antioxidative effects, antimicrobial action, cholesterol-lowering potential, and anti-cancer properties. These bioactivities are mediated through multiple mechanisms, such as modulation of immune cell signaling, inhibition of pathogenic adhesion, scavenging of reactive oxygen species, and alteration of host-microbiota interactions. Moreover, EPS contribute to gut health by enhancing mucosal barrier function and serving as prebiotic substrates for beneficial microbes. Their applications extend beyond traditional health benefits, finding relevance in the development of functional foods, drug delivery systems, and nutraceuticals. Despite their promising bioactivities, challenges such as low production yield, strain variability, and limited clinical validation remain barriers to large-scale application. This review also explores emerging research avenues including synthetic biology approaches for enhanced EPS production and targeted delivery systems. Overall, probiotic-derived EPS hold immense promise as natural, safe, and effective agents for improving human health and warrant further investigation for clinical and industrial exploitation.**

***Keywords:*** **Probiotic exopolysaccharides, Immunomodulation, Gut Microbiota, Antioxidant & Antimicrobial activity, Therapeutic application.**

## I. INTRODUCTION

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. Commonly belonging to genera such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*, these beneficial microbes are naturally present in the human gastrointestinal tract and are also consumed through fermented foods and dietary supplements. Probiotics play a pivotal role in maintaining gut microbial balance, enhancing digestive health, modulating the immune system, and protecting against pathogenic infections (4). Their relevance has expanded beyond gastrointestinal wellness, with growing evidence supporting their role in reducing inflammation, managing metabolic disorders, supporting mental health via the gut-brain axis, and contributing to skin and oral health (36, 17). The bioactive compounds secreted by probiotics, including short-chain fatty acids, bacteriocins, and exopolysaccharides (EPS), are central to their functional benefits. Among these, EPS have gained increasing attention for their structural complexity and diverse biological activities that directly influence host health. Understanding the mechanisms by which probiotics and their secreted metabolites exert therapeutic effects is crucial for developing next-generation probiotics and functional foods aimed at promoting overall well-being (5).

Exopolysaccharides (EPS) are high-molecular-weight carbohydrate polymers secreted extracellularly by a wide range of microorganisms, including probiotic bacteria. They may exist in two primary forms: capsular EPS, which remain tightly associated with the bacterial cell surface, and slime EPS, which are loosely bound or released into the surrounding environment. Structurally, EPS can be classified as homopolysaccharides—composed of a single type of monosaccharide—or heteropolysaccharides, containing repeating units of two or more different sugars. The structural complexity of EPS contributes to their wide-ranging biological properties and functionalities (7,27).

In probiotic bacteria, EPS play a fundamental role in microbial physiology and ecology. They are crucial for biofilm formation, which enhances bacterial adhesion, colonization, and persistence in the gastrointestinal tract. EPS act as protective barriers, shielding bacterial cells from desiccation, phagocytosis, antibiotics, and harsh environmental conditions such as pH fluctuations, bile salts, and oxidative stress. Moreover, EPS contribute significantly to the host–microbe interaction by modulating immune responses, either by stimulating anti-inflammatory effects or suppressing pro-inflammatory signaling pathways, depending on their structure and composition (13, 20).

From a health perspective, EPS have been associated with numerous beneficial effects including antioxidative, antimicrobial, immunomodulatory, anti-tumor, and cholesterol-lowering activities. Their ability to interact with host cells and gut microbiota also plays a role in enhancing gut barrier integrity and promoting gut homeostasis (8). In addition, EPS from probiotic bacteria can function as prebiotics by serving as substrates for beneficial gut microbes, further supporting intestinal health. Due to their safety, natural origin, and multifunctional nature, probiotic-derived EPS are increasingly being explored for applications in functional foods, nutraceuticals, and therapeutic formulations. Understanding the biosynthesis, regulation, and health effects of EPS is therefore critical for unlocking their full potential in human health (18,19). Exopolysaccharides (EPS) produced by probiotic bacteria are gaining increasing attention due to their multifunctional roles in both microbial ecology and human health. These complex carbohydrate polymers not only enhance the survivability and adaptability of the producing bacteria but also offer a wide range of health-promoting benefits to the host, making them valuable in biomedical, food, and pharmaceutical applications (23).

One of the primary roles of EPS in probiotic bacteria is to facilitate colonization and persistence within the gastrointestinal tract. EPS contribute to biofilm formation—a structured microbial community encased in a self-produced matrix—which helps probiotics adhere to intestinal mucosal surfaces, resist mechanical removal, and compete against pathogenic microbes. This stable colonization enhances the probiotic’s functional efficacy over time (32). EPS also provide protection to the bacterial cells by forming a barrier that shields them from environmental stressors such as acidic pH, bile salts, oxidative agents, and antimicrobial compounds. This enhances the viability of probiotics during gastrointestinal transit, as well as during processing and storage in functional food products. From a host perspective, EPS play a key role in modulating immune responses (11). Several studies have demonstrated that EPS can stimulate anti-inflammatory cytokines while suppressing pro-inflammatory pathways, thereby contributing to immune homeostasis. This immunomodulatory effect makes EPS-producing probiotics particularly useful in managing conditions such as inflammatory bowel disease, allergies, and infections (1,5).

Additionally, EPS exhibit strong antioxidant activity by scavenging free radicals and reducing oxidative stress, which is a key factor in aging and chronic diseases. Some EPS also possess antimicrobial properties, either by directly inhibiting pathogen growth or by disrupting biofilms formed by harmful bacteria. Furthermore, EPS from certain probiotic strains have been linked to cholesterol-lowering effects, anti-cancer activity, and enhancement of gut barrier integrity (35). Their ability to act as prebiotics—supporting the growth of beneficial gut microbes—further reinforces their role in promoting gut health. Overall, EPS produced by probiotic bacteria represent a natural, safe, and versatile class of biopolymers with significant potential for improving human health. Their multifunctional properties position them as important bioactive compounds for the development of next-generation functional foods, nutraceuticals, and therapeutic formulations (18,21).

The growing interest in functional foods and natural therapeutics has led to intensified research on the health benefits associated with probiotics and their secreted bioactive compounds. Among these, exopolysaccharides (EPS) produced by probiotic bacteria have emerged as a promising class of biomolecules due to their diverse physiological, immunological, and therapeutic effects (22). While EPS have traditionally been studied for their roles in bacterial survival and biofilm formation, recent advances have uncovered their significant contributions to human health, particularly in modulating immunity, improving gut health, combating oxidative stress, and offering potential in disease prevention and therapy (20).

This review aims to comprehensively explore the health-promoting and therapeutic potential of EPS derived from probiotic bacteria. It begins by providing a foundational understanding of probiotics and the general characteristics of EPS, followed by an in-depth discussion on the structural diversity and biosynthetic pathways of EPS. The core focus of the review is to highlight the wide-ranging biological activities of probiotic EPS—including immunomodulatory, antioxidant, antimicrobial, cholesterol-lowering, and anti-cancer properties—with supporting evidence from in vitro, in vivo, and clinical studies (18,29). Furthermore, the review addresses the emerging applications of probiotic EPS in functional food development, nutraceuticals, and biomedical formulations, emphasizing their role as natural and safe health-promoting agents. Current challenges such as low production yields, strain specificity, regulatory constraints, and the need for clinical validation are also discussed to provide a balanced perspective. By synthesizing current knowledge and identifying research gaps, this review intends to guide future studies and industrial applications focused on probiotic-derived EPS. Ultimately, it advocates for the integration of EPS-producing probiotics into evidence-based strategies for promoting human health and preventing chronic diseases (24,9).

## II. OVERVIEW OF PROBIOTIC BACTERIA PRODUCING EPS

### 2.1 Common EPS-producing probiotic genera

Several probiotic bacterial genera are well known for their ability to produce exopolysaccharides (EPS), which contribute significantly to their functional properties and health benefits. Among these, the most extensively studied genera include *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Enterococcus* (3).

#### 2.1.1 Lactobacillus

Members of the genus *Lactobacillus* are among the most widely used probiotics in the food and pharmaceutical industries. Many strains are capable of producing both capsular and free EPS, with structural variability depending on species and environmental conditions. EPS from *Lactobacillus* strains, such as *L. rhamnosus*, *L. plantarum*, *L. delbrueckii subsp. bulgaricus*, and *L. casei*, have been shown to exhibit immunomodulatory, antioxidant, and antimicrobial activities. These EPS contribute to the survival of the bacteria under acidic conditions, aid in colonization of the gastrointestinal tract, and enhance biofilm formation, which promotes persistence and probiotic efficacy (10, 11).

#### 2.1.2 Bifidobacterium

*Bifidobacterium* species, commonly found in the human gut, especially in infants, are another important group of EPS-producing probiotics. Strains such as *B. longum*, *B. bifidum*, and *B. adolescentis* have demonstrated the ability to produce heteropolysaccharides with beneficial effects on gut health and immunity. EPS from *Bifidobacterium* are known to support colonization, modulate host immune responses, and contribute to the stabilization of the gut microbiota. Their prebiotic and anti-inflammatory potential makes them valuable in managing gastrointestinal disorders and allergies (21).

#### 2.1.3 Streptococcus and Enterococcus

Certain strains of *Streptococcus thermophilus*, commonly used in yogurt and fermented dairy products, are recognized for their EPS-producing capacity, which improves product texture and contributes to host health. Similarly, some *Enterococcus* strains, such as *E. faecium*, also produce EPS with notable antimicrobial and immunomodulatory properties. Although safety concerns exist with some *Enterococcus* species, selected probiotic strains have shown promising potential in functional food formulations (27).

Together, these genera form the core group of EPS-producing probiotics that not only support bacterial survival and food quality but also offer significant health-promoting benefits, reinforcing their role in the development of next-generation probiotics and therapeutic applications (15).

### 2.2 Differences in EPS production between strains

Exopolysaccharide (EPS) production among probiotic bacteria varies widely not only between different species but also among strains within the same species. This variability is influenced by genetic, physiological, and environmental factors, resulting in differences in EPS yield, composition, molecular weight, and functional properties. At the genetic level, the presence and organization of EPS biosynthesis gene clusters differ significantly between strains (19, 6). These gene clusters encode enzymes responsible for the assembly and secretion of EPS, including glycosyltransferases, polymerases, and regulatory proteins. Variations in gene content, sequence, and expression levels contribute to distinct EPS structures and production capacities. For example, certain *Lactobacillus* strains may produce homopolysaccharides primarily composed of glucose or fructose, while others synthesize more complex heteropolysaccharides with multiple sugar residues (14,29).

Physiological differences, such as metabolic activity and growth rate, also affect EPS production. Some strains inherently have higher metabolic flux towards polysaccharide synthesis, enabling greater EPS yields. Additionally, the stage of bacterial growth influences EPS secretion, with maximal production often observed during the late exponential or stationary phases. Environmental conditions play a critical role in modulating EPS biosynthesis. Nutrient availability (e.g., carbon source type and concentration), pH, temperature, oxygen levels, and osmotic stress can upregulate or suppress EPS production (32). For instance, *Lactobacillus* strains grown on sucrose often produce higher EPS quantities compared to glucose, as sucrose acts as a substrate for specific glycosyltransferases. Similarly, stress conditions may trigger increased EPS synthesis as a protective response. Functionally, these strain-specific differences in EPS impact probiotic efficacy (23,19). Strains producing higher amounts of EPS with favorable biochemical properties tend to exhibit improved adhesion, immunomodulation, and protective effects in the host. Therefore, understanding and optimizing EPS production at the strain level is crucial for selecting potent probiotic candidates for therapeutic and industrial applications (22).

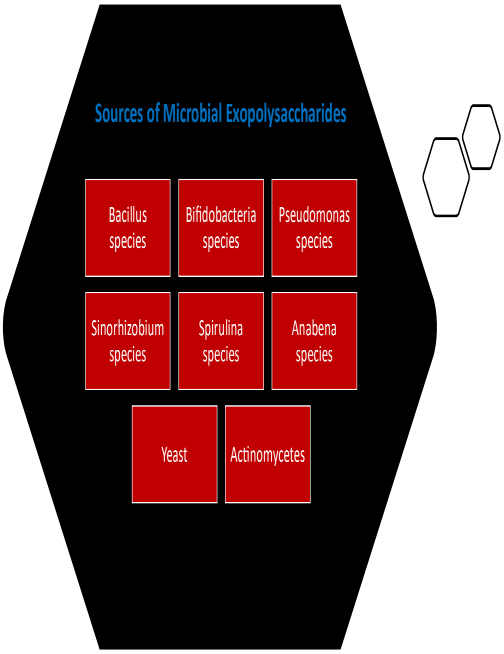


*Figure 1. Showing the types of Microbial species responsible for production of Exopolysaccharide in Industries.*

2.3 Factors affecting EPS production

The production of exopolysaccharides (EPS) by probiotic bacteria is a complex process influenced by multiple environmental and nutritional factors. Optimizing these factors is crucial for maximizing EPS yield and tailoring their structural and functional properties for specific applications. Key factors affecting EPS biosynthesis include growth conditions, carbon source, pH, temperature, and other culture parameters (12).

2.3.1 Growth Conditions

The overall growth environment, including temperature, aeration, and incubation time, significantly impacts EPS synthesis. Most probiotic bacteria produce EPS optimally within a narrow temperature range, generally between 30°C and 37°C. Extended incubation times often lead to increased EPS accumulation, typically peaking during the late exponential or early stationary phase. Oxygen availability can also influence EPS production; some strains produce higher amounts under microaerophilic or anaerobic conditions, while others require aerobic environments (1,17).

2.3.2 Carbon Source

The type and concentration of the carbon source in the growth medium profoundly affect EPS yield and composition. Sugars such as glucose, sucrose, fructose, lactose, and maltose serve as substrates for EPS biosynthesis. Sucrose is often reported to enhance EPS production, especially in strains possessing specific glycosyltransferases that utilize sucrose to build polysaccharide chains. Carbon source concentration can regulate gene expression linked to EPS biosynthesis; however, excessively high sugar levels may inhibit growth or cause osmotic stress, reducing EPS production (3).

2.3.3 pH

pH influences both bacterial metabolism and enzyme activity related to EPS synthesis. Most probiotic strains favor a slightly acidic to neutral pH (around 5.5–7.0) for optimal EPS production. Deviations from this range can inhibit bacterial growth and alter EPS structure or yield. Additionally, EPS can help bacteria tolerate acidic environments by forming protective layers (6,15).

2.3.4 Nitrogen Source and Minerals

Nitrogen availability from sources like peptone, yeast extract, or ammonium salts also affects EPS synthesis, as nitrogen is essential for microbial growth and enzyme production. Certain minerals (e.g., magnesium, manganese) act as cofactors for enzymes involved in polysaccharide assembly and can enhance EPS yield (26,8).

2.3.5 Other Factors

Osmotic stress, induced by high salt concentrations or solute levels, can stimulate EPS production as a bacterial defense mechanism. Additionally, the presence of certain metal ions or growth factors can modulate the quantity and quality of EPS. Understanding and controlling these factors are essential for the efficient production of EPS at an industrial scale and for tailoring EPS with desired functional properties for use in food, pharmaceutical, and therapeutic applications (32).

## III. STRUCTURAL AND FUNCTIONAL DIVERSITY OF EPS

### 3.1 Composition and classification

Exopolysaccharides (EPS) synthesized by probiotic bacteria are structurally diverse carbohydrate polymers composed of repeating monosaccharide units. These polysaccharides can be broadly classified into homopolysaccharides (HoPS) and heteropolysaccharides (HePS) based on their sugar composition. The structure and composition of EPS significantly influence their physicochemical properties and biological activities, including their immunomodulatory, antioxidant, and prebiotic functions (2, 14).

#### 3.1.1 Homopolysaccharides (HoPS)

Homopolysaccharides are composed of only one type of monosaccharide, which can be linked through α- or β-glycosidic bonds in linear or branched chains. The type of monosaccharide and its linkages determine the functional properties of the HoPS (16).

- Glucans: These consist solely of glucose units. They can be further categorized into:
  - α-glucans, such as dextran and mutan, commonly produced by *Leuconostoc* and *Streptococcus* species.
  - β-glucans, which are more resistant to enzymatic degradation and are noted for their immunomodulatory properties.
- Fructans: Composed of fructose units, these include:
  - Inulin-type fructans, such as levan, produced by *Lactobacillus reuteri* and other probiotic strains.
  - Fructans act as potent prebiotics by selectively stimulating the growth of beneficial gut bacteria.
  - Galactans: Less common, galactans consist of galactose monomers and may be found in specific lactic acid bacteria.

HoPS are generally synthesized extracellularly using specific enzymes (e.g., glucansucrase or fructansucrase) that polymerize sugar residues directly from sucrose substrates. They are typically high-molecular-weight polymers and can influence food texture by increasing viscosity and forming gels (17, 22).

#### 3.1.2 Heteropolysaccharides (HePS)

Heteropolysaccharides are composed of two or more types of monosaccharides, such as glucose, galactose, rhamnose, mannose, and fucose, arranged in repeating units of three to eight sugars. These polymers are usually synthesized intracellularly and then exported outside the cell.

- The biosynthesis of HePS is a more complex and tightly regulated process involving multiple enzymes and gene clusters.
- HePS are often branched, with varied linkages and side chains that contribute to their functional diversity.
- The complexity of HePS imparts unique rheological properties and broad biological activities, including immunomodulation, cholesterol-lowering effects, and antimicrobial action.

Due to their compositional diversity, HePS are considered more bioactive and structurally versatile than HoPS. Their functional properties make them particularly attractive for biomedical and nutraceutical applications (24, 30).

### 3.2 Physicochemical properties

The physicochemical properties of exopolysaccharides (EPS) produced by probiotic bacteria play a pivotal role in determining their functional applications in food, pharmaceutical, and biomedical fields. These properties are influenced by the chemical composition, molecular weight, branching patterns, and type of glycosidic linkages within the EPS structure. Understanding these attributes is essential to tailor EPS for specific uses, such as thickeners, stabilizers, bioactive agents, or delivery systems (25).

#### 3.2.1 Molecular Weight

EPS can vary widely in molecular weight, ranging from a few kilodaltons (kDa) to several million daltons (MDa). Molecular weight affects viscosity, solubility, gel formation, and bioactivity. Generally, high-molecular-weight EPS exhibit greater thickening and emulsifying properties, while lower-molecular-weight EPS are more soluble and may penetrate biological tissues more easily, enhancing their therapeutic potential (29).

#### 3.2.2 Monosaccharide Composition

The types and ratios of monosaccharides—such as glucose, galactose, rhamnose, mannose, fucose, or uronic acids—determine the charge, solubility, and functionality of EPS. The presence of uronic acids and sulfate groups can confer negative charges, enhancing water retention and metal ion binding, which may contribute to antioxidant and detoxifying activities (31).

3.2.3 Solubility and Water-Holding Capacity

Most probiotic-derived EPS are hydrophilic and highly soluble in water, which enables their use as moisture-retaining agents in food and cosmetics. Their water-holding capacity helps maintain texture, reduce syneresis, and stabilize emulsions. Some EPS can also form gels depending on the presence of divalent cations or pH conditions (13, 19).

3.2.4 Rheological Behavior

EPS can exhibit non-Newtonian (shear-thinning) flow behavior, making them valuable in food formulations where flow control and stability are needed. Their viscosity-enhancing properties depend on their concentration, molecular weight, and degree of branching. EPS can also form viscoelastic films and gels, useful in wound dressings and controlled drug release systems (31).

3.2.5 Charge and Surface Activity

EPS may be neutral or negatively charged depending on their composition. Charged EPS exhibit emulsifying and film-forming properties and can interact with other biopolymers or bioactive molecules, influencing encapsulation and delivery behavior (35).

3.2.6 Thermal and pH Stability

EPS from probiotic strains often display good thermal and pH stability, allowing them to remain functional during food processing and digestion. This resilience makes them suitable for use in a variety of industrial and biomedical settings (11).

3.3 Biosynthetic pathways and genetic regulation of EPS in probiotic strains.

The synthesis of exopolysaccharides (EPS) in probiotic bacteria is a complex, highly regulated process that involves multiple enzymes and gene clusters. These pathways are crucial not only for EPS production but also for determining the chemical composition, structure, and functional properties of the final polymer. Understanding the biosynthetic mechanisms is essential for improving EPS yield and tailoring its bioactivity through metabolic engineering or environmental manipulation (19).

3.3.1 General Biosynthetic Pathway

EPS biosynthesis in probiotic bacteria generally proceeds through the following stages:

- Precursor Formation: The process begins with the uptake and metabolism of carbohydrates from the environment (e.g., glucose, sucrose). These sugars are converted into sugar nucleotides such as UDP-glucose, UDP-galactose, GDP-mannose, or dTDP-rhamnose, which serve as activated precursors for polysaccharide synthesis (6).
- Repeating Unit Assembly: The sugar nucleotides are sequentially added to a lipid carrier molecule (undecaprenyl phosphate) embedded in the cytoplasmic membrane. This is mediated by specific glycosyltransferases, which determine the type and order of sugar residues in the repeating unit (17).
- Polymerization: The assembled repeating units are polymerized into longer chains by polymerase enzymes, which control chain length and linkage type.
- Export and Secretion: The polymerized EPS is transported across the bacterial cell membrane via a membrane-associated transport system, often involving flippase (Wzx) and polysaccharide co-polymerase (Wzy) proteins. Some EPS may remain attached as capsular polysaccharides, while others are secreted as free EPS (27).

3.3.2 Genetic Regulation of EPS Synthesis

The genes responsible for EPS biosynthesis are typically clustered together in operons or gene clusters. These clusters vary significantly among probiotic species and strains and generally include:

- Glycosyltransferases – Catalyze the transfer of sugar residues to the growing EPS chain.
- Precursor synthesis enzymes – Involved in the production of sugar nucleotides.
- Polymerases and flippases – Mediate polymerization and transport across the membrane.
- Regulatory genes – Control expression in response to environmental stimuli such as pH, carbon source, or stress conditions.

For instance, in *Lactobacillus rhamnosus* and *Lactobacillus plantarum*, EPS gene clusters can be over 20 kb long and include more than 15–20 genes with diverse functions. In *Bifidobacterium* species, EPS gene clusters also exhibit considerable heterogeneity, contributing to strain-specific EPS production and structure (14, 28, 32).

3.3.3 Regulation by Environmental and Host Factors

EPS biosynthesis is tightly regulated by both intracellular signaling and external environmental cues. Factors such as carbon availability, osmotic stress, and pH can upregulate or suppress the expression of EPS genes through signal transduction mechanisms, including two-component regulatory systems and global transcriptional regulators. Some strains also exhibit phase variation, where EPS production is switched on or off depending on growth conditions or host interaction, indicating an adaptive survival mechanism (28, 35).

## IV. BIOLOGICAL ACTIVITIES AND HEALTH BENEFITS

### 4.1. Immunomodulatory Effects

Exopolysaccharides (EPS) produced by probiotic bacteria have been shown to play a crucial role in modulating both innate and adaptive immune responses. EPS can interact with pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) on immune cells, leading to the activation of signaling pathways that stimulate macrophages, dendritic cells, and natural killer (NK) cells. This interaction promotes the release of cytokines and chemokines, enhancing the host's immune surveillance and defense mechanisms (34). In addition to immune stimulation, EPS also exhibit notable anti-inflammatory properties by downregulating the production of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β, while upregulating anti-inflammatory cytokines such as IL-10. This dual immunomodulatory effect helps maintain immune homeostasis, making EPS-producing probiotics beneficial in managing chronic inflammation, allergic responses, and autoimmune conditions. The ability of EPS to modulate immune function without triggering excessive inflammation highlights their therapeutic potential in gut health and systemic immunity (29,33).

### 4.2. Antioxidant Activity

Exopolysaccharides (EPS) produced by probiotic bacteria exhibit significant antioxidant properties, primarily through their free radical scavenging capacity and ability to protect against oxidative stress. These polysaccharides can neutralize reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, thereby reducing cellular damage caused by oxidative stress (32,30). The antioxidant potential of EPS is often attributed to their unique structural features, including the presence of hydroxyl groups, uronic acids, and branching patterns that enhance electron donation and radical stabilization. By mitigating oxidative damage, EPS help maintain cellular integrity and function, especially in the gastrointestinal tract where oxidative stress can disrupt epithelial barrier function and promote inflammation. Moreover, EPS may upregulate the expression of antioxidant enzymes such as superoxide dismutase (SOD) and catalase, further enhancing the host's defense mechanisms against oxidative insults. These properties make probiotic-derived EPS promising candidates for use in functional foods and therapeutic formulations aimed at combating oxidative stress-related disorders (17, 12).

### 4.3. Antimicrobial and Anti-biofilm Effects

Exopolysaccharides (EPS) produced by probiotic bacteria demonstrate notable antimicrobial properties, including activity against pathogenic bacteria and the ability to disrupt biofilms. EPS can inhibit the growth and colonization of harmful microbes such as *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Listeria monocytogenes* by competing for adhesion sites on mucosal surfaces, thereby preventing pathogen attachment and invasion. Additionally, EPS may exert direct bacteriostatic or bactericidal effects through mechanisms like membrane disruption or interference with nutrient uptake (31). A particularly valuable function of probiotic EPS is their ability to disrupt pre-formed biofilms or prevent biofilm formation by pathogens. Biofilms are protective matrices that shield pathogenic bacteria from antibiotics and immune responses, contributing to persistent infections. EPS from certain probiotic strains can interfere with biofilm integrity by altering quorum sensing signals or degrading the extracellular matrix, thereby enhancing pathogen susceptibility to antimicrobial agents. These properties highlight the potential of EPS as natural biotherapeutic agents for infection control and biofilm-related complications (29,19).

### 4.4. Cholesterol-Lowering Effects

Exopolysaccharides (EPS) produced by probiotic bacteria contribute to cholesterol-lowering effects through several mechanisms, notably bile salt hydrolase (BSH) activity and cholesterol assimilation. BSH enzymes deconjugate bile acids in the intestine, reducing their reabsorption and promoting their excretion (16). This process forces the liver to convert more cholesterol into bile acids to maintain the bile acid pool, thereby lowering circulating cholesterol levels. Additionally, certain probiotic strains can directly assimilate cholesterol by incorporating it into their cell membranes or binding it to EPS, reducing its absorption in the gut. EPS may also act as a physical barrier that traps cholesterol or binds bile salts, enhancing their removal. These combined actions underline the potential role of EPS-producing probiotics as natural and effective agents in managing hypercholesterolemia and improving cardiovascular health (5,7).

### 4.5. Anti-cancer Potential

Exopolysaccharides (EPS) produced by probiotic bacteria have shown promising anticancer properties, particularly through cytotoxicity against cancer cells and modulation of cancer-related signaling pathways. In various in vitro studies, EPS have demonstrated selective cytotoxic effects on tumor cell lines such as colon, breast, and liver cancers, while exhibiting minimal toxicity toward normal cells, indicating their biocompatibility and therapeutic potential (9,11). One of the key mechanisms involves the induction of apoptosis, where EPS influence pathways like the mitochondrial (intrinsic) pathway by upregulating pro-apoptotic proteins (e.g., Bax) and downregulating anti-apoptotic proteins (e.g., Bcl-2), leading to caspase activation and programmed cell death. Additionally, EPS may inhibit cancer cell proliferation by arresting the cell cycle at specific checkpoints and interfering with growth factor signaling (19,25). Some studies also suggest that EPS can reduce oxidative stress within tumor microenvironments and suppress inflammation, both of which contribute to tumor progression. These multifaceted mechanisms highlight the potential of probiotic-derived EPS as biofunctional compounds in cancer prevention and adjunctive therapy (21).

### 4.6. Gut Health and Prebiotic Function

Exopolysaccharides (EPS) produced by probiotic bacteria play a vital role in enhancing gut microbiota composition and supporting intestinal barrier function. Acting as prebiotics, EPS can selectively stimulate the growth and activity of beneficial gut microbes such as *Bifidobacterium* and *Lactobacillus*, thereby promoting a balanced and healthy microbiota. This improved microbial composition contributes to better digestion, nutrient absorption, and immune regulation (14, 23). Additionally, EPS facilitate the colonization of probiotic strains by promoting adhesion to intestinal epithelial cells, which is essential for their persistence and efficacy in the gut. EPS also contribute to strengthening the intestinal barrier by enhancing the integrity of tight junctions between epithelial cells, reducing intestinal permeability and preventing the translocation of pathogens and toxins. This barrier-supporting function is crucial for maintaining gut homeostasis and protecting against inflammatory and metabolic disorders. Thus, EPS play a dual role in shaping the gut microbiome and reinforcing mucosal defense mechanisms (8, 26).

## V. EPS AS FUNCTIONAL FOOD INGREDIENTS AND THERAPEUTICS

### 5.1 Use of EPS in fermented foods, dairy, beverages.

Exopolysaccharides (EPS) produced by probiotic bacteria are widely utilized in the food industry, particularly in fermented foods, dairy products, and beverages, due to their functional and health-promoting properties. In fermented dairy products like yogurt, kefir, and cheese, EPS improve texture, viscosity, and mouthfeel, reducing the need for synthetic thickeners or stabilizers. Their ability to bind water and form gel-like structures enhances creaminess and prevents syneresis (whey separation), thereby improving product quality and consumer appeal (25, 31). In non-dairy fermented beverages and plant-based alternatives, EPS contribute to suspension stability and homogeneity, ensuring a consistent sensory profile. Beyond their technological benefits, EPS also act as bioactive components that deliver health benefits such as improved gut health, immune modulation, and cholesterol reduction, adding functional value to foods. The inclusion of EPS-producing probiotic strains in food formulations is thus a sustainable and natural approach to developing clean-label, functional foods with enhanced nutritional and sensory properties (17).

### 5.2 EPS as encapsulating or delivery agents for probiotics, drugs, or bioactives.

Exopolysaccharides (EPS) produced by probiotic bacteria have gained significant attention as natural encapsulating and delivery agents for probiotics, drugs, and various bioactive compounds. Their biocompatibility, biodegradability, and gel-forming abilities make EPS ideal candidates for protecting sensitive molecules from harsh environmental conditions such as gastric acidity, enzymatic degradation, and oxidative stress (18). By forming a protective matrix around probiotics or bioactives, EPS enhance their stability, viability, and controlled release within the gastrointestinal tract, thereby improving their therapeutic efficacy and bioavailability. Moreover, EPS-based encapsulation can facilitate targeted delivery, allowing for site-specific release and reduced side effects. This multifunctional role of EPS not only supports the development of advanced functional foods and nutraceuticals but also opens new avenues in pharmaceutical formulations and drug delivery systems (25, 13).

### 5.3 Role in synbiotics and nutraceuticals.

Exopolysaccharides (EPS) play a crucial role in the development of synbiotics and nutraceuticals by acting as prebiotic components that synergize with probiotic strains to enhance their survival, colonization, and health benefits. In synbiotic formulations, EPS serve as fermentable substrates that selectively stimulate the growth of beneficial gut bacteria, thereby improving gut microbial balance and metabolic activity (9,10). Their bioactive properties, including immunomodulation, antioxidant effects, and cholesterol-lowering potential, add functional value to nutraceutical products aimed at promoting overall health and preventing chronic diseases. Additionally, EPS improve the texture, stability, and shelf life of synbiotic and nutraceutical products, making them more appealing to consumers. By combining the probiotic effects of live microbes with the prebiotic and biofunctional benefits of EPS, these formulations offer a holistic approach to gut health and wellness (26).

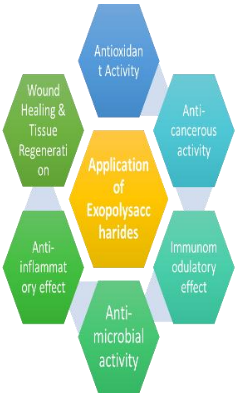


*Figure 2. Showing The multiple applications of Exopolysaccharide in Therapeutics and Pharmaceutical industries.*

## VI. CURRENT CHALLENGES AND LIMITATIONS

6.1 Low yield in production.
One of the primary limitations in the industrial exploitation of EPS is their generally low production yield during microbial fermentation. Many probiotic strains naturally produce EPS in small quantities, which may not be sufficient for cost-effective commercial extraction and formulation. The biosynthesis of EPS is energy-intensive and often tightly regulated within the cell, influenced by environmental conditions and nutrient availability. Scaling up production requires optimization of culture media, fermentation parameters, and potentially genetic engineering approaches to enhance EPS synthesis without compromising bacterial viability or functionality. However, achieving consistently high yields remains a significant bottleneck in manufacturing processes (15,19).

6.2 Strain variability and stability issues.
EPS production is highly strain-dependent, with significant variability observed not only between different species but also among strains of the same species. This variability affects the physicochemical characteristics and bioactivity of the EPS, making it difficult to standardize products for industrial use. Moreover, some EPS-producing strains may exhibit genetic instability over time, leading to fluctuations or loss of EPS production during repeated subculturing or long-term storage. Ensuring the genetic and phenotypic stability of probiotic strains is therefore essential for maintaining consistent EPS quality and functionality (2,32).

6.3 Regulatory and safety concerns.
Despite EPS generally being regarded as safe, regulatory approval remains a major challenge, especially for novel strains or EPS types. Comprehensive safety assessments, including toxicity studies, allergenicity testing, and potential immunogenic effects, are required to meet stringent food and pharmaceutical regulations. The lack of standardized protocols and clear regulatory guidelines for EPS poses additional hurdles for product development and market authorization. Addressing these safety concerns through rigorous evaluation is critical to gaining consumer trust and regulatory acceptance (7,15).

6.4 Lack of large-scale clinical validation.
While numerous in vitro and animal model studies demonstrate the health-promoting properties of probiotic EPS—including immunomodulation, antioxidant activity, and cholesterol lowering—there is a notable scarcity of well-designed, large-scale human clinical trials. This gap limits the ability to conclusively establish efficacy, optimal dosages, and safety profiles in diverse populations. Robust clinical evidence is necessary to validate health claims, guide formulation development, and support regulatory approval for therapeutic and functional food applications (21,29).

## VII. FUTURE PROSPECTS AND RESEARCH DIRECTIONS

The future of exopolysaccharides (EPS) produced by probiotic bacteria is highly promising, with several exciting avenues for research and application. One of the foremost areas of focus is strain improvement through genetic and metabolic engineering. Advances in molecular biology techniques, such as CRISPR-Cas9, provide tools to modify and enhance the biosynthetic pathways responsible for EPS production. By tailoring probiotic strains to produce higher yields of EPS with specific structural features, it will be possible to develop customized bioactive compounds optimized for targeted health benefits or industrial functions (18, 19). In parallel, optimizing production processes and scaling up fermentation systems are crucial for commercial viability. Research aimed at refining culture conditions—such as nutrient sources, pH, temperature, and aeration—can significantly improve EPS yield and quality. Innovations in bioreactor design and continuous fermentation methods may also enhance productivity while reducing costs. Additionally, developing efficient and gentle extraction and purification techniques will be essential to preserve the functional properties of EPS for use in foods, nutraceuticals, and pharmaceuticals (27, 36).

Another important research direction is the comprehensive characterization of EPS structure and its relationship to biological activity. Advanced analytical methods like nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry can unravel the complex molecular architecture of EPS. Understanding how specific structural motifs influence immunomodulation, antioxidant activity, or antimicrobial effects will enable the rational design of EPS with enhanced functionalities. Moreover, investigating the precise mechanisms by which EPS interact with host cells and the gut microbiota will deepen insight into their therapeutic potential (35).

To translate laboratory findings into real-world applications, large-scale clinical trials and thorough safety assessments are imperative. Rigorous human studies are needed to validate health claims associated with EPS-containing probiotics, establish optimal dosages, and ensure long-term safety across diverse populations. Regulatory frameworks will also benefit from comprehensive toxicological data, facilitating the approval and consumer acceptance of EPS-based products. Finally, the potential applications of EPS extend far beyond traditional fermented foods (33). Their unique physicochemical and bioactive properties make them ideal candidates for use as natural thickeners, encapsulating agents for drug and probiotic delivery, and components in biomedical fields such as wound healing and tissue engineering. Exploring these innovative uses will require interdisciplinary collaboration between microbiologists, food scientists, and medical researchers, ultimately leading to new functional foods, therapeutics, and biomaterials (28,32,35).

## VIII. CONCLUSION

Exopolysaccharides produced by probiotic bacteria represent a valuable class of biopolymers with multifaceted health benefits and broad applications in food, pharmaceutical, and biomedical industries. Their ability to modulate the immune system, exert antioxidant and antimicrobial effects, and support gut health underscores their therapeutic potential. Despite challenges such as low production yields, strain variability, and limited clinical validation, advances in strain engineering, bioprocess optimization, and analytical techniques hold promise for overcoming these barriers. Continued interdisciplinary research and well-designed clinical studies are essential to fully realize the potential of EPS as functional ingredients and natural bioactives. With growing consumer demand for natural and health-promoting products, probiotic-derived EPS are poised to become key components in next-generation functional foods, nutraceuticals, and innovative therapeutic formulations.

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