Strategies and Trends for Application Exopolysaccharides of Lactic Acid Bacteria in the Food and Biomedical

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Abstract. Lactic acid bacteria are a significant bioproduct of exopolysaccharides (EPS) in lactic acid. EPS is popular in the food and dairy industries due to its ability to lengthen shelf life, improve technical functionalities, and provide a range of health benefits. The potential of EPS in drug development and diagnostics is also overwhelming. This study carries out an in-depth analysis of many kinds of lactic acid bacteria (LAB)-produced EPS, their classification, as well as current and future applications in different areas, such as food, dairy, baking, cereal-based and functional products. The clinical and pharmaceutical applications of EPS are also described in this article, for example, intelligent drug delivery systems, interpenetrating polymer networks, anticancer drug-targeting, recombinant macromolecular biopharmaceuticals, gene delivery, tissue engineering, and EPS's participation in diagnostics. The article ends with future perspectives on increasing EPS production, diminishing production costs, and utilization in other areas.

Keywords. Exopolysaccharides, Lactic acid bacteria, Food industry, Antimicrobial, Anticancer, Immunostimulatory.

1. Introduction

The excreted form of EPS can be either loosely attached or released to the cell environment and have potential applications in various industries, such as food, biotechnology, cosmetics, and health, as well as consequences for human health [1,2]. EPS are carbohydrate polymers that are found on the surfaces of a great number of microorganisms, both gram-positive and gram-negative bacteria, as capsular Among infectious diseases, it has been reported that some EPS act as virulence factors, but also it has been proven that some of the bacterial EPS are aiming to human health, and the production of exopolysaccharides by some probiotic bacteria (e.g. lactic acid bacteria) with certain traits is found to be closely related to the ability of modulation of host immune response and also inducing [3,4]. In addition, the consumption of EPS supports bacteria producers to overcome intestinal stress, remains in the gut for a long time, and is likely to have a significant role in the interaction between EPS bacteria producers and the gut environment of the host [5]. One of the most widely used definitions of

probiotics is “live microorganisms that, when given in adequate amounts, provide health benefits to the host” [6,7]. The typical bacterial components of probiotic foods or supplements meant to be consumed by humans usually fall under the genera *Bifidobacterium* and *Lactobacillus*. These genera majorly are gram-positive while their phyla vary due to GC content. In contrast to Bifidobacteria which are members of Actinobacteria (GC 50%) and are non-filamentous rods with various morphologies, with the bifurcated or "bifid" shape being the most prominent, Lactobacilli is non-spore-forming rods, or coccobacilli, with a low GC content (50%), The exopolysaccharide-procucing strains of *Lactobacillus* and *Bifidobacterium* have been found in the human gut microbiota during the last few years [8-10]. In this review, we strive to give a depiction of the pathway of EPS biosynthesis, the food and biomedical applications and, the immunomodulatory effect of EPS produced by strains of LAB.

2. Chemical Structures and Molecular Characterization of EPS

Complex carbohydrates made by lactic acid bacteria are called exopolysaccharides (EPS). These consist of units of sugar molecules that repeat. In general, EPS has a sugar backbone with side chains branching off of it; however, the specific molecular structure might differ based on the lactic acid bacterium strain that generates it. The chemical nature of EPS produced by lactic acid bacteria allows for its classification into multiple groups [11], including:

- Homopolysaccharides: EPS composed of a single type of sugar molecule.
- Heteropolysaccharides: EPS composed of two or more types of sugar molecules.
- Linear EPS: EPS with a straight chain of sugar molecules.
- Branched EPS: EPS with side chains branching off from the main chain of sugar molecules.
- Acetylated EPS: EPS with acetyl groups attached to the sugar molecules.
- Succinylated EPS: EPS with succinyl groups attached to the sugar molecules.

Figure 1 shows that various EPS can have distinct activities and characteristics, such as the ability to form gels, digestion resistance, and environmental molecule interaction capability. LAB homoexopolysaccharides (HoPS) are mostly made from sucrose through the polymerization of glucose or fructose and include repeated units of one monosaccharide type and four subgroups of homopolysaccharides: α-glucans, β-glucan, β-fructans, and α-galactan. Both glucan forms are glucose-containing, but the differing connections enable them to be structurally more distinct. When it is associated with glycosidic linkages, the homopolysaccharide of the α-glucan type can be classified as dextran (α -1,6), mutants (α -1,3 and α -1,6), alternan (α -1,3 and α -1,6), or reuteran (α -1,4 and α -1,6). It can also be α-1,2, α -1,3, α -1,4, or α-1,6. Although four distinct kinds of α-glucans exist, only one β-glucan is linked by β -1,2, β -1,3, and β -1,4 glycosidic bonds. Finally, the less common β-galactan is composed of galactose units linked with either β -1,3 or β -1,6, and the fructose units in β-fructans are coupled with either β -2,1 or β -2,6 and glycosidic linkage, which are referred to as levan (β -2,6) and inulin-type (β -2,1), respectively (12, 13). The lactic acid bacteria *Lactobacillus*, *Streptococcus*, and *Leuconostoc* create galactans, fructans, and glucans [14].

The majority of extracellular polysaccharides (EPS) produced by LAB are heteropolysaccharides (HePS), which typically consist of 3-8 units of D-glucose, D-galactose, and L-rhamnose. EPS can also include isoform-specific modifications and monosaccharide derivatives of glucuronic acid (GlcA), N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GalNAc), fructose, mannose, fucose, or substituted monosaccharides (containing phosphate and glycerol) with molecular weights ranging from 8 to 5000 kD) [15–17]. Most strains of Bifidobacteria are also HePS producers, in addition to several mesophilic and thermophilic bacteria (e.g., *Lactococcus lactis* subsp. *cermoris*, *Lc. lactis* subsp. *lactis*, *Lactobacillus casei*, *Lb. rhamnosus*, *Lb. sake*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Lb. helveticus*, and *Streptococcus thermophilus*) [18].

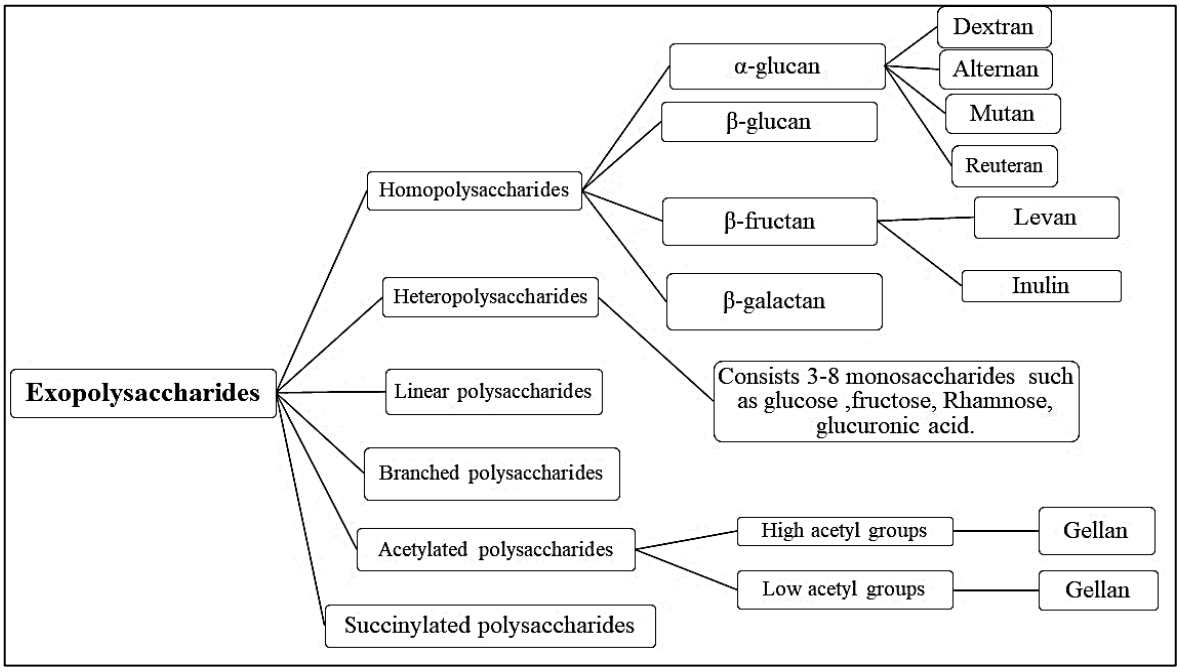

Figure 1. Classification of chemical structure of EPS for LAB.

Enzymes called glycosyltransferase (GTF) and fructosyltransferase (FTF) are attached to the cell wall and play a role in the biosynthesis of homopolysaccharides (HoPS). Fructosyltransferase (FTF) transfers fructose to an expanding chain of homopolysaccharides (Figure 2), while GTF transfers glucose. These enzymes are skilled at breaking down specific types of bonds [18,19].

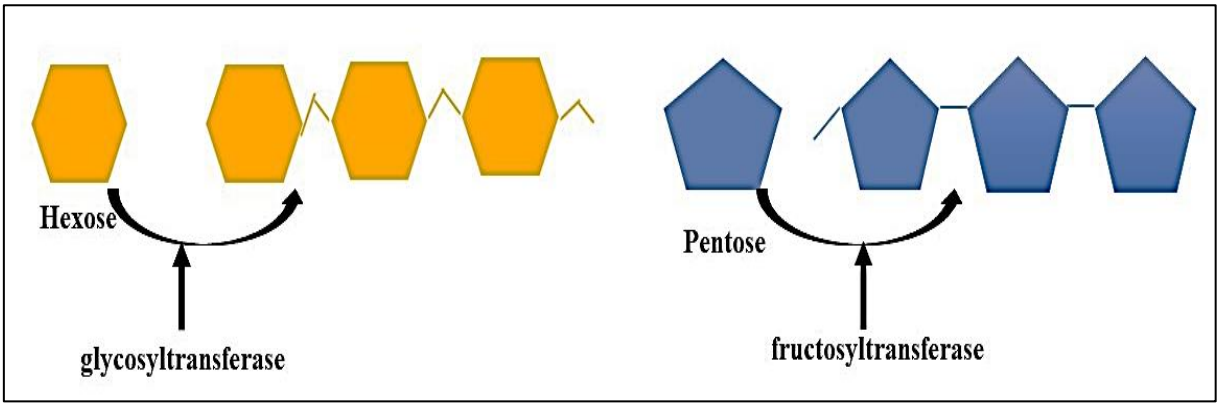

Figure 2. Biosynthesis pathways for homopolysaccharides (HoPS).

For the purpose of heteropolysaccharide (HePS) molecular production in laboratory conditions (Figure 3). The five-step Wzx/Wzy pathway is utilised: (1) Saccharides are phosphorylated to either glucose-6-phosphate or glucose-1-phosphate after being transported into the cell, (2) The intracellular formation of the sugar nucleotides uridine-diphosphate-galactose (UDP-galactose), uridine-diphosphate-glucose (UDP-glucose), and deoxythymidine-diphospho-rhamnose (dTDP-rhamnose), (3) A cell membrane-embedded undecaprenol diphosphate anchor (UDA) serves as the anchor for individual repeating units, which are then synthesised through a number of GTFs to generate repeating units,(4) The repeated sugar units are transferred to the outer membrane by the Wxz flippase protein, finally (5) The sugar molecules are polymerized into heteropolysaccharides by the outer membrane protein Wxy and discharged into the extracellular environment [20-22].

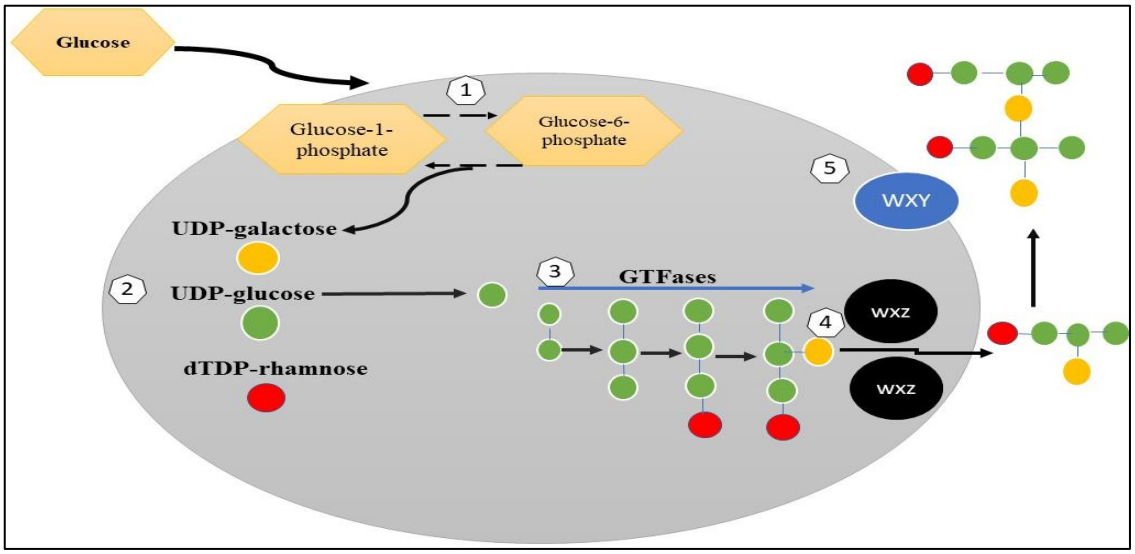

Figure 3. Biosynthesis pathways for heteropolysaccharide (HePS).

3. Applications of Lactic Acid Bacteria EPS

3.1. Application in the Food Industry

LAB-EPS have drawn specific attention as safe and useful additives used in the food industry to produce products with favourable rheological and sensory qualities (Table 1 and Figure 4). They can be utilized in the dairy sector as thickeners, stabilizers, and emulsifiers without adding undesired mouth feel or flavor, and can increase the viscosity and water-holding capacity of yoghurt [22]. Additionally, EPSs can improve cheese's ability to retain water and fat, which increases cheese yield and results in a softer, creamier end product. Additionally, without the use of any stabilizer, EPS produced by *Streptococcus thermophilus* strains can give ice cream a highly viscous and pseudoplastic non-Newtonian fluid behavior [22, 23]. In addition to the numerous uses of probiotic EPSs in dairy products, EPSs can also improve the volume and moisture content of a loaf of bread, giving it a more open and supple structure, whether it contains gluten or not, because of their ability to bind water. They can also prevent starch retrogradation, which lowers the rate of staling and extends the shelf life of products [24].

EPSs can improve the texture of sausage, resulting in a harder, less sticky, and tougher product; pure EPSs are typically not utilized directly as food additives, owing to their poor production; instead, they are frequently employed in the food sector, particularly in yoghurt; food fermented with strains that produce EPSs can have additional health-promoting effects in addition to these technological ones [25]. For instance, EPS-producing LAB ruins alcoholic products, including beer, ciders, and wines. For instance, beer spoilage is mostly caused by *Lactobacillus brevis* TMW 1.2112 strain β-glucan production. Additionally, the production of EPSs causes the development of dental plaque, and the development of EPS-induced biofilms exacerbates hygiene issues in the food sector [26,27].

Table 1. Show various functional food applications for EPSs from some different isolated LAB strains.

Lactic acid bacteria	Type of EPS	Structure subunit	Food application	Ref.
Leuconostoc mesenteroides subsp. *dextranicum* *Lb. casei*, *Lb. saki*, *Lb. fermentum*, *Streptococcus* spp.	Dextran	glucose monomers linked by, α-1,6 glycosidic bonds	Ice cream, Cheese, use in Butter Improves softness, crumb texture, and loaf volume, Prevents sugar crystallization in jelly Candie	[22,23]

Lactic acid bacteria	Type of EPS	Structure subunit	Food application	Ref.
Lb. reuteri	Reuteran	Glucose monomers linked by α-1,4 linkage, also α-1,6 glycosidicbonds	Used in baker	[22,23]
Lb. johnsonii NCC 533 *Lb. reuteri 121*	Inulin	β-1,2 glycosidic bonds	Substitute of fat in food products	[22]
Lb. delbrueckii subsp. *bulgaricus*	Kefiran	Glucose and galactose monomers form variable acidic bonds	Improve viscoelastic properties of acid milk gels, antimicrobial	[24]
Pediococcus damnosus 2.6 *Lactobacillus* spp. G-77	β-D-glucan	D-glcose monomers linked by β-glycosidic bonds	Starter culture	[23,24]
Weissella cibaria MG1 *Lb. reuteri* ff2hh	Glucan	(α1,6 and α-1,4) glucan and fructooligosaccharide	Adjunct culture in cheese	[25]
Leuconostoc mesenteroides	Alternan	α-1,6 and α-1,3 glycosidic bonds	Prebiotics, bulking agent and extender in foods	[23,26]
Streptococcus salivarius	Levan	Fructose monomers linked by β-2,6 osidic bonds, β-2,1 linked side chains	Bio-thickener in food industry, water solubility and Adjunct culture in cheese	[26,27]

3.2. Biomedical Application of EPS

EPS produced by LAB are associated to a variety of functional roles and health benefits, as discussed below, some of which are summarized in Table 2 and figure 4.

3.2.1. Immunostimulatory Activity

Ancestral research suggests that EPS may influence the physical properties of the produced polysaccharides via immunomodulatory activities that differ across strains [28]. Figure 4 shows that the form of the EPS appears to be important since even little molecules of EPS can activate immune cells when they contain a phosphate, which gives them a negative charge. The stimulating effects of a phosphate-containing extracellular soluble protein (EPS) from *Lc. lactis* subsp. *cremoris* were demonstrated in mouse spleen macrophages by inducing the cytokines interferon-gamma (IFN-) and interleukin-1 (IL-1). Separation of high-molecular-weight EPS produced by a strain of *Lb. delbrueckii* subsp. *bulgaricus* encouraged a high concentration of INF-generated by mouse splenocytes, as was described in a different study [29,30]. The stimulatory effect did not seem to be dose dependent, as it was achieved in approximately the same levels at concentrations ranging from 20 to 500 μg/mL [31].
Yoghurt fermented with *Lb. delbrueckii* subsp. *bulgaricus* was another method of administering EPS to mice. In mice given EPS directly, a dose-dependent increase in natural killer cell activity was seen, with the highest response occurring at a maximal dose of 30 mg/kg along with a minor rise in INF-γ and a slight fall in IL-4, similar outcomes were attained for the mice that consumed yoghurt, the stimulation of tumor-necrosis factor α (TNF-α), interleukin IL-6 and IL-1β production by EPS in macrophages has been demonstrated, similar to kefiran, EPS made from *Lb. kefira* was given to mice, and the result.
On the other hand, at high concentrations of IL-10 produced by RW-9595M, TNF-α, IL-6, and IL-12 were hardly detectable. Hydrolysis of EPS altered the molecular weight but had no effect on the electrical charge. This finding adds to the evidence that EPS influences the signalling pathways that initiate cytokine induction in relation to its mass [36,37]. Prior research has proposed that certain strains of *Bifidobacterium* that produce extracellular soluble protein (EPS) could have

immunostimulatory or immunosuppressive effects depending on the dosage. In general, research has demonstrated that higher molecular weight EPS can suppress the immune system, whereas lower molecular weight EPS can elicit a stronger response from the immune system [38, 39].

3.2.2. Antimicrobial Activity for EPS

In addition to actively rejecting harmful bacteria from the gastrointestinal tract (GI), EPS from LABs have been linked to antibacterial characteristics against gram-positive and gram-negative dietary pathogens. EPS-Ca6, derived from *Lactobacillus* spp., inhibits the growth of *Micrococcus luteus* and *Salmonella enterica* ATCC 43972. Furthermore, EPS-DN1 derived from *Lb. kefiranofaciens* DN1 exhibited significant bactericidal action against harmful bacteria, such as Salmonella enteritidis and *Listeria monocytogenes*, in a manner that was dose dependent. An EPS-C70 derivative produced by *Lb. plantarum* and isolated from camel milk effectively suppressed the growth of *Staphylococcus aureus* and *Escherichia coli* [39,40]. Furthermore, it has been suggested that the EPS of *Lb. Johnsonii* FI9785 may promote the colonisation of beneficial bacteria in the host GI tract by replacing harmful bacteria through competitive inhibition. Another possible in vitro mechanism for the antibacterial action of LAB-derived EPS is their ability to inhibit the development of pathogen biofilms by interfering with cellular communication and integrity [41].

Chronic and recurring infections, as well as antibiotic resistance, are more likely to occur when pathogenic bacteria are able to form biofilms and remain in the environment [42]. responsible for overseeing food and medical product safety. In spite of EPS's antibiofilm activity, pathogenic bacteria such as *E. faecalis*, *B. cereus*, and *P. aeruginosa* have proven to be resistant to it [43].

Leuconostoc citreum, which was isolated from sausages, has been found to synthesise EPS with significant antibiofilm activity, recent studies have demonstrated that EPS from *Lb. paracasei* M7 inhibits a wide range of bacteria, including *Enterococcus faecalis* (64.27%), *Bacillus subtilis* (63.84%), *B. cereus* (62.89%), *S. aureus* (61.45%), *Klebsiella* sp. (59.42%), *P. aeruginosa* (58.88%), and the probiotic *W. confusa* from Romanian yoghurt ,it has been proposed that EPS can prevent the initial auto-aggregation of biofilm-forming bacteria by disrupting cell-to-cell communication or weakening cell membranes, the likelihood of LAB colonisation in the gut and its resistance to harmful bacteria, on the other hand, have been suggested to be enhanced by EPS [44].

Although this theory has not been tested with LAB-produced EPS just yet, recent reports [45–47] describe how EPS released by Lb. fermentum UCO-979C reduced *Helicobacter pylori* adhesion by 30% in both in vitro and in vivo experiments and improved the immune response in H. pylori infections. The potential of EPS derived from LAB to inhibit biofilm formation offers a potential alternative antimicrobial strategy in light of the growing body of evidence showing that pathogenic bacteria are becoming increasingly resistant to conventional antibiotics, which poses a serious threat to both public health and food safety [44, 45].

In addition, research has demonstrated that probiotics and their byproducts can help protect against viral infections by strengthening both innate and adaptive antiviral immunity. This, in turn, reduces the severity of symptoms, the frequency of episodes, the amount of virus shed, the restoration of normal gut permeability, and the production of antibodies specific to the virus. In vitro studies have found that extended polysaccharides (EPS), especially sulfated polysaccharides like dextran, have antiviral properties that work against enveloped viruses. These EPS are notorious for blocking different retroviral reverse transcriptase and preventing viruses from entering host cells [46].

In addition, there is some evidence that the EPS layer can hinder the growth of harmful Candida species. Since probiotic bacteria are known to limit Candida's growth, it was believed that interference from EPS was responsible for Candida's inhibitory effect on hyphal formation and adhesion [48,50].

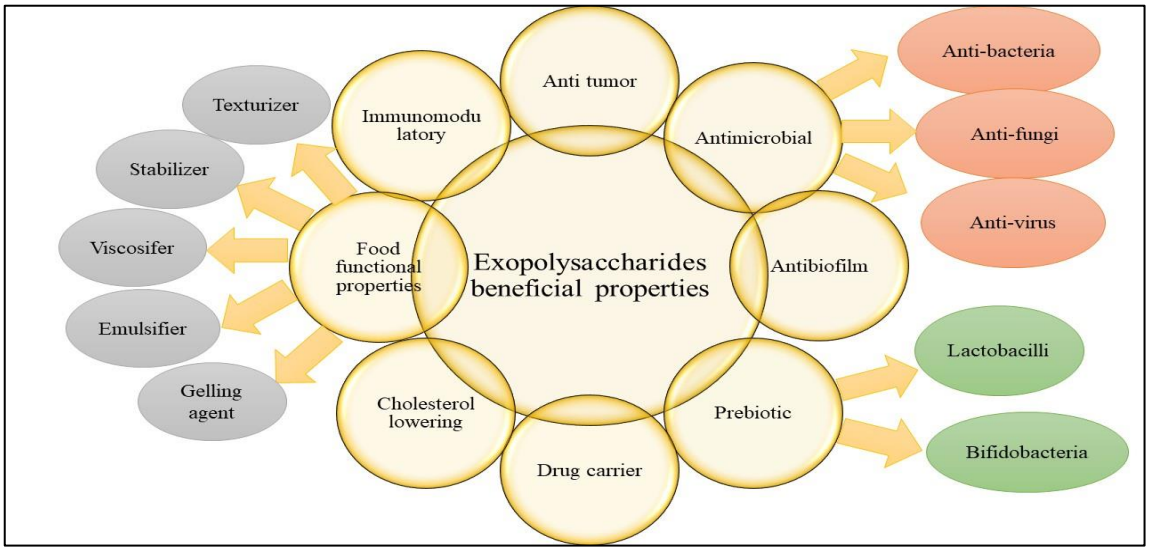

Figure 4. Some exopolysaccharides produced by LAB may have beneficial properties.

3.2.3. Anti-Biofilm Characteristics

Biofilms (slime layers) are extracellular matrices that attach to surfaces and are made up of a complex mixture of lipids, proteins, polysaccharides, and nucleic acids, which create biofilms, many bacterial species, particularly pathogenic bacteria, and increase their resistance to extracellular stress, environmental stress can cause the development of biofilms in a number of harmful bacteria, increasing adhesion and providing protection from the host response; consequently, biofilms are crucial to pathogenesis [51,52].

The ability of *S. typhimurium* to adhere to surfaces and create biofilms is a major factor in its ability to colonize the oviducts and gastrointestinal tract of chickens. It has been demonstrated that certain EPS contribute to biofilm production in human epithelial cell lines (Hep-2), numerous EPS extracted from LABs have the potential to reduce or inhibit microbial biofilms, according to mounting scientific evidence, as a result, they may be used to develop novel approaches to address bacterial biofilm-associated infections and food safety concerns [53].

It has been demonstrated that many EPS of lactobacilli can interfere with the production of biofilms or disperse those that pathogens have already created. A number of pathogens, including enterohemorrhagic *E. coli* and *S. enteritidis*, have shown that the EPS produced by *Lb. acidophilus* prevents them from forming biofilms [54]. Previous research has shown that the EPS from *Lb. acidophilus* A4 and *Lb. plantarum* YW32 had anti-biofilm efficacy against both gram-positive and gram-negative infections. In *B. cereus* RSKK 863 and *L. monocytogenes*, HePS from *Lb. fermentum* LB-69 and *Lb. gasseri* FR4 demonstrated the highest biofilm inhibition [55].

Other LABs have also shown anti-biofilm activity *Candida albicans* SC5314 has been shown to be resistant to the antibiofilm effects of dextran produced by *W. confusa*, and *Lb. citreum* isolated from beef sausages also produces dextran and levan. These polysaccharides show strong antibiofilm activity and biofilm inhibition [21,56]. The quorum sensing (QS) system is a mechanism by which bacteria produce molecules (typically oligopeptides in gram-positive bacteria and acyl-homoserine lactone in gram-negative bacteria) through which they can measure the size or density of the other bacteria around them and control the development of the biofilm [44, 57].

It has been proposed that EPS can operate as a signalling molecule and control the activation of genes involved in the production of biofilms, or it can change the bacterial coat and prevent the attachment of bacteria to surfaces because, as mentioned in the previous section, some sulfated EPS showed a stronger inhibitory effect against a variety of gram-positive and gram-negative pathogens than those without sulfate, one explanation for this might be the disruption of the signals that regulate the formation of biofilms or obstruction of the water-soluble protein efflux pathway due to damage to the cell membrane [55,58].

3.2.4. Antioxidant Properties

The antioxidant activity of EPS derived from LAB has been found to be superior to that of synthetic antioxidants, which can also cause cancer and cytotoxicity. Eliminating massive amounts of reactive oxygen species is essential for oxidative stress regulation. According to in vitro research on the extracellular polysaccharides (EPS) produced by *Bacillus coagulans*, *Weissella cibaria*, *Lb. plantarum*, and *Lb. paracasei*, these polysaccharides can exhibit antioxidant and free radical scavenging action. Other animal models that have investigated this antioxidant effect include a hyperlipidemic rat model and a senescent mouse model that has been exposed to oxidative stress using EPS from Lb. plantarum [59,60]. Evidence of their antioxidant impact on cells was shown by increased glutathione peroxidase and superoxide dismutase enzyme activity and lower levels of malondialdehyde, an indicator of oxidative stress, in both *Lb. plantarum* and *Lb. casei* EPS [61,62].

3.2.5. Anti-Tumor Activities

There are indications that EPS could be incorporated into traditional cancer treatments in the future, despite the fact that studies investigating its anti-tumor effectiveness are still in their early phases. Administering 10 mg/mL EPS from a strain of *Lb. acidophilus* inhibits the development of colon cancer cells while having no effect on normal colon cells [63]. On gastric cancer cell lines, EPS fraction dosages of 10 mg/mL from *Lb. helveticus* and breast cancer cell lines of 10 mg/mL from Lb. plantarum exhibited the same anti-proliferative effect on cancer cells. On the other hand, hepatic cancer cell lines demonstrated modest activity at 5 mg/mL whereas colon and stomach cancer cells benefited greatly from a lower dose of Lb. plantarum EPS at 600 g/L [64]. Lyophilized extracellular protein (EPS) from the lactobacilli *Lb. plantarum*, *Lb. rhamnosus*, *Lb. brevis*, and *Lb. delbrueckii* subsp. *bulgaricus* caused 40% cell death and reduced colon cancer cell viability, according to a recent study [65].

3.2.6. Cholesterol Lowering Activities

The capacity of EPS from LAB to regulate serum cholesterol levels via intestinal adsorption of this molecule is a topic of increasing research because high blood pressure and excessive blood cholesterol are two important risk factors for cardiovascular illnesses. An *in vitro* enzymatic reaction and a polysaccharide precipitation procedure were used to demonstrate cholesterol. The mechanism by which EPS produced by LAB reduces blood cholesterol levels has been shown in animal and human studies [66,67]. While some research has shown that EPS isolated from *Lb. plantarum* RJF4 can lower LDL, another study indicated that mucilage EPS produced by *Lc. lactis* subsp. *cremoris* can improve rat blood cholesterol levels and cholesterol metabolism. A related investigation also showed that EPS can have antihypertensive effects in an *in vivo* rat model [56,68,69].

3.2.7. Anti-Cancer Activity

Cancer is a malignant growth of cells that leads to an abnormal increase in cell division; highly effective chemotherapy, due to its cytotoxic and immunotoxic properties, influences tumour formation and slows patient recovery; and certain mutations in tumour suppressor and pro-to-oncogene genes cause irreversible DNA damage [70,71]. An effective alternative to synthetic anticancer medications, EPS from reputable natural sources like LAB usually shows little cytotoxicity and adverse effects, and finding new anticancer medicines with minimal immune system side effects is the primary goal of many immunopharmacological studies [72]. The anti-proliferative impact of EPS produced by the *Lb. plantarum* strain and *Lb. casei* on HepG-2, BGC-823, and HT-29 cancer cells was significantly reduced. Evidence of anticancer activity against many cell lines was found in extracts from *Lb. casei*, *Lb. plantarum*, and *Lb. acidophilus*, depending on the dosage [73, 74]. An additional body of research has shown that EPS generated by LAB has antioxidant and antiproliferative effects on HepG2 hepatoma cells, with a sustained release pattern and a 70% decrease in tumour volume [75,76].

3.2.8. Drug Delivery Mechanisms

Due to their bioactivity and ability to transport drugs, EPSs show promise as drug delivery vehicles. Bacterial extracellular polypeptides (EPSs) can perform double duty as bioactive molecules and

transporters of vital pharmaceuticals, such as growth factors and anticancer drugs. Compared to making biological scaffolds loaded with living cells, making EPSs to use as medication carriers is easier. The roles of EPS and other drug transporters are similar, but EPS can be modified to increase the regulated release of drugs, prolong their half-life in the body, and improve their efficacy [71,77]. Some research suggests that kefir may protect ciprofloxacin from gastrointestinal side effects. Another study used bacterially-derived sulfated EPS as a vaccine adjuvant to treat and prevent hepatitis B virus in animals [78].

Table 2. Various biomedical activities for EPSs from some different isolated LAB strains.

Lactic acid bacteria	Type of EPS	Structure subunit	Food application	Ref.
Lb. helveticus LZ- R-5	R-5-EPS Heteropolysaccharide	β-D-Galp-β-D-Glcp- β-D-Glcp-β-D-Glcp- β-D-Glcp	Immunostimulatory activity and Immunomodulatory agent.	[29,30]
Lb. plantarum-12	Hetero-exopolysaccharide (L- EPS)	Composed of mannose, glucuronic acid, galactosamine, glucose, galactose and xylose	Anti-biofilm formation, adhesion and invasion to HT-29 cells of S. Flexner. Prevention contamination of equipment from shigella	[33,34]
Lb. plantarum JNULCC001	Hetero-exopolysaccharide (EPS- 001)	Composed of galactose, glucose, mannose, and arabinose with several functional groups, including carboxyl, hydroxyl, and amide groups.	Excellent capacity for methylene blue (MB) biosorption, act as a stabilizing agent during the biosynthesis of SeNPs, or selenium nanoparticles, also act as nanomaterials.	[29,55,56]
Lb. casei NA-2	Heteropolysaccharide	Rhamnose, glucose and mannose residues.	Prevent biofilm formation and squander the biofilms of B. cereus, *S. aureus*, *S. typhimurium* and *E. coli* O157:H7, as these are foodborne pathogens.	[48,57]
Lb. paracasei H9	Heteropolysaccharide (EPS-S1)	Composed of mannose, glucose, galactose and glucuronic acid	antioxidant activities, and anticancer activities	[59,60]
Lb. acidophillus 20079	LA-EPS-20079 (Penta saccharide).	α-D-Glc-α-L-Fuc-α- D-GlcA- α-D-GlcA-α-D- GlcA.	Anticancer, immunomodulatory effects	[30,57]
Lb. fermentum S1	EPS1, EPS2 and EPS3	EPS2 and EPS3 were homogeneous but EPS1 is composed of glucose, galactose, mannose and arabinose, but with different molar ratio	anti-biofilm activity against foodborne pathogens.	[52,53,61]
Lb. fermentum R-49757	Heteropolysaccharide EPS	[β-D-Glcp -β-D-Manp-β-D-Glcp] n	EPS-based diets are playing role in biomedical applications	[67]
Lb. pentosus 14FE	EPS14FE and EPS68FE are	α, β-glucan with α-	Used for novel bioactive	
Lb. pentosus	Homopolysaccharide.	hexapyranosyl residue and β-	polymers for anticoagulant, antimicrobial, fibrinolytic	[42,44,50]

Lactic acid bacteria	Type of EPS	Structure subunit	Food application	Ref.
68FE		anomeric configuration	anticoagulant, antioxidant	
Bifidobacterium infantis ATCC 15697	Heteropolysaccharide	β-D-Galf- α -D-Galf	Antitumor, antioxidant, anti-biofilm, immunomodulatory	[44,45]
B. adolescentis M101-l4	Heteropolysaccharide	Glu:Ga	Inflammatory/Mitogenic	[40,54,55]
B. longum NB667	Heteropolysaccharide	Glu:Gal:Rha	Anti-Inflammatory (immunomodulatory)	[44,61,64]

4. Exopolysaccharide Purification and Extraction

The techniques that permit the successful isolation, recovery, and cultivation of an EPS-producing LAB are equally crucial to the factors that affect its success. There are a number of suggested strategies, all of which heavily depend on the medium employed in the various production processes and the intended eventual use of the EPS result.

4.1. Recovery and Isolation

It typically takes three steps to recover EPS from culture: First, cells and other unwanted substances are removed, then the polymer is precipitated in the cell-free supernatant, and finally, the precipitated polymer is dried [79].

4.1.1. Pre-Treatments

The easiest way to recover and isolate can be found in research using a predetermined media, a phase of centrifugation to remove cells, and isolation by straightforward water dialysis, it may be required to use some pre-treatment processes for the deactivation of specific enzymes or the removal of specific proteins while recovering from liquid media, which is largely reliant on the use of either complex or defined media. It is common practice to use a heating step of 90–100 C to kill microorganisms and inactivate any enzymes that may be present, especially when using complex media, Additionally, the impact of heat treatment on the ultimate EPS yield prior to separation has been assessed. It was found that the full recovery of EPS required a heat treatment step, leading to noticeably larger yields. Prior to EPS isolation, the fermentation broth has been concentrated using a vacuum rotary evaporator, however this technique is rarely documented [79,80].

The protein fraction is typically precipitated either by the addition of trichloroacetic acid (TCA) at concentrations ranging from 4 to 14% under mixed circumstances, by protease enzymatic digestion, or by a combination of these two methods. The final goal will determine if a TCA step is required, which is typically administered before EPS is taken from the media ,it can be skipped if the objective is quantification, but it is necessary for detailed characterization of the polysaccharide structure, ultrafiltration has been proposed as a quicker and more precise approach for EPS isolation as an alternative to TCA and ethanol treatments, Centrifugation can be used to separate the liquid's cell fraction and coagulated proteins later, microfiltration or ultrafiltration steps can then be used to further purify the product[81].

4.1.2. Precipitation After that Dialysis

Following the application of pre-treatments, the following step is precipitation, the most common solvent for this process being ethanol. There are several methods for isolating EPS, including ethanol precipitation in one or two phases, ethanol precipitation followed by dialysis, membrane dialysis with cutoffs of 6000, 8000, 12,000, and 14,000, and TCA precipitation., due to the fact that leftover lactose was discovered to co-precipitate with EPS and produce falsely high readings, the single-step method with ethanol was insufficient for isolation, the methods of two-step ethanol, ethanol precipitation, and dialysis using membranes with a molecular weight cut-off lower than 8000 all produced comparable outcomes and were approved as effective ways to isolate EPS. [82].

Since TCA treatment eliminates proteins and other impurities that can cause EPS precipitation-which can lead to a loss of up to 50% of the final polymer concentration-the low result from TCA precipitation was thought to be a false-negative. There was no discernible difference in the final EPS when tested at two different ethanol temperatures of 20◦C and 4◦C; however, it is recommended to wash the TCA precipitate at least once. On occasion, acetone or a combination of ethanol and acetone has been used. Important for removing carbs with low molecular mass, dialysis is performed as the last step before drying [82,83].

4.1.3. Drying methods and Characterization

The isolation of EPS is usually finished by spray-drying, spray-freeze drying, or lyophilization to a powder. Rinsing the powder with ethanol or dissolving it in sodium hydroxide and centrifuging the mixture to remove contaminants further refine the EPS lyophilizated if specific EPS characterization rather than measurement is the goal. Research attempting to define EPS generated by LAB also makes use of size exclusion chromatography and ion-exchange chromatography. Nuclear magnetic resonance (NMR), Fourier transform infrared (FT-IR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) are among of the more advanced techniques that can be used to investigate the structure of EPS [84].

4.2. Methods for Quantification

Following the first stages of EPS isolation and recovery, a powdered product is created. Though it may be the most convenient way to measure the product's quantity, weighing the powder yields an erroneous final dry weight since it accounts for any impurities. The most used method for determining carbohydrate levels is the colorimetric phenol-sulfuric acid method, which was initially proposed by Dubois et al. (1956) [85].

Phenol and sulfuric acid are used to give simple sugars, oligosaccharides, polysaccharides, and derivatives an orange-yellow hue. On the other hand, if there are any contaminated carbohydrates in the fermentation broth, this quantification method could be off as well. Additional methods for quantifying EPS include the use of the anthrone reagent, which is a different colorimetric method. Strains with higher productivity often use this method, as it can only be considered accurate for yields above 10 mg/L. Other methods, such as liquid chromatography, anion exchange chromatography, or high-performance anion-exchange chromatography pulse amperometric detection, are also used [86,87].

In addition, proteins can be detected using HPLC with UV detection. Liquid fermentation samples can have lactic acid, lactose, and EPS all quantified at once using near-infrared spectroscopy (NIRS). The yields of lactic acid and lactose were determined using NIRS in conjunction with traditional HPLC, while EPS was determined using ultrafiltration and the phenol-sulfuric technique [88-90].

After that, we compared the two and found that EPS had a coefficient of association of 91% and lactic acid and lactose had a value of 99%. The results show that NIRS could be a fast method for tracking EPS in fermentations; further direct methods for tracking EPS include scanning electron microscopy and transmission electron microscopy [91-93].

5. The Future Prospects of ESP Applications

It is clear that EPSs made by LAB are already significant in several domains. Some of their contributions to the food and pharmaceutical industries have been explained here. Both consumers looking for healthier products and manufacturers vying to meet consumer demands and stand out from the competition have an interest in functional food products and the development of EPS-containing cereal-based foods. The influence and amplitude of EPS produced by LAB in this field can be enhanced by reducing its cost and increasing its output (particularly in situ). Researchers have poured a lot of time and energy into studying EPS over the past decade, with an emphasis on evaluating and clarifying HoPS structures and investigating new uses for HePS. In this regard, and because EPS structures are so intricate, there is a growing demand for more targeted techniques (FTIR, NMR, etc.) to provide convincing evidence of structural analysis. Additionally, it would be beneficial to

demonstrate the structure-activity link of LAB polysaccharides in a real-world setting, like as the food business or another relevant area.

Additional research has to be done into the use of extracellular vesicles (EPS) as vectors for gene transfer if they are to find other uses in the medical and pharmaceutical industries. The progress of EPS applications is crucial to the development of gene therapy, particularly for the treatment of cancer. The use of functionalized EPS as drug carriers to treat tumors or neurological illnesses by bridging the blood-brain barrier is an exciting subject that requires further investigation. The potential of EPS as a vehicle for the transport of vital pharmacological compounds, like steroid hormones, warrants additional investigation. Long-term administration methods Nanoparticles encapsulating these hormones have the ability to alleviate numerous severe adverse effects linked to these medications by enhancing their absorption in the gastrointestinal tract and allowing for higher plasma concentrations with reduced dosages, hence decreasing the occurrence of these side effects. Researchers should devote more time and energy to studying these structures' bioequivalence, pharmacokinetics, and adhesion ability. The anticancer effects of EPS depend on their capacity to stimulate the immune system to attack cancer cells. They are long-lasting, targeted, and versatile, but they work more slowly than more conventional treatments like radiation and chemotherapy. Therefore, immunotherapeutic EPS has the potential to be effective anticancer treatments, both alone and in combination. In order to overcome limits and speed up their action, more study is required.

Significant challenges need to be addressed, including the high expenses associated with EPS synthesis, the low polysaccharide yields, the generation of by-products, and the time-consuming methods required for EPS separation and purification. Researching and understanding microbial physiology, genetics, polysaccharide biosynthesis, suitable fermentation conditions, and separation and purification phases is crucial to increasing EPS manufacturing yields while minimizing costs. The high cost of substrates like glucose and sucrose is the main reason for the expenses associated with EPS synthesis. The use of inexpensive raw sources, like dairy or agricultural waste, should thus be prioritized in order to reduce the cost of fermentative EPS synthesis.

Conclusions

The diversity of EPS produced by LAB has recently surged, capturing the interest of many researchers. The vast array of potential applications for EPSs in food, medicine, and healthcare is made possible by their non-toxic nature. Food products that are made with EPSs from LABs have better rheological, stability, flavour, texture, and shelf-life properties. Recent research has shown that EPSs have a number of physiological and technical benefits, such as impacts that modulate the immune system, lower cholesterol, fight bacteria, and even prevent cancer. Hence, the demand for health-promoting functional foods is met by adopting LAB cultures for in situ EPS generation. Two major problems preventing their widespread use in industry are the low yield and wide variation in EPS production abilities among strains. Consequently, exploring novel ideas to optimize EPS production for possible industrial uses is crucial. Technical and biological activity is significantly affected by molecular weight, monosaccharide concentration, functional groups, and EPS type (HoEPS or HeEPS). Recent studies have focused on evaluating EPS structures and developing new functionalities. There is a lot of complexity to the mechanisms via which EPSs suppress pathogens, modulate the immune system, and cause biofilms to form. To improve functional food formulations and pharmaceuticals, a deeper understanding of the underlying mechanisms is necessary. Then, it will be feasible to design EPSs with desired or targeted properties.

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