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Review Article

Bacterial Exopolysaccharides: Types, its Biosynthesis and their Application in Different Fields

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Abstract

Microbial exopolysaccharides (EPS) are polymeric sugar residues that have a diverse spectrum of forms and activities. Exopolysaccharides are high-molecular-weight secondary metabolites (10 to 30 KD) produced during the stationary phase or late exponential phase under a variety of stress conditions such as pH, temperature, heavy metal, and so on. Bacteria produces and releases polymers with various chemical characteristics using a variety of substrates ranging from simple to complex. They can be employed in unique ways due to their specific features. Bacterial polysaccharides are used in a variety of industries, including food, medicines, agriculture, and the environment (bioremediation, flocculation, etc.). Because of its biocompatibility, biodegradability, and environmental and human safety, EPS are commonly used in high-value applications. This review study covers the basics of their types, biosynthesis pathways, and implications in diverse fields.

Keywords: Bacterial Exopolysaccharide, biosynthesis pathway, bioremediation, biopolymer.

Introduction

Exopolysaccharides (EPS) are high-molecular-weight polymers made up of sugar residues with a variety of structures and functions [30]. The term exopolysaccharide was coined by Sutherland in 1972 [1]. For biopolymer formation, bacteria utilize a range of carbon sources, ranging from simple to complex. Depending on their biological position, biopolymers might be intracellular or extracellular. Depending on their location, microbial EPSs can take one of two forms: 1) capsular (cEPSs), which are tightly linked to the bacterial surface, and 2) free EPSs, which are discharged into the surrounding medium (fEPSs). The vast majority of extracellular biopolymers are polysaccharides, inorganic polyanhydrides (such as polyphosphates), polyesters, and polyamides, which were frequently referred to as mucus and micro capsular polysaccharides [27]. Under nitrogen-limiting conditions, microorganisms produce the most EPS near the end of the log phase. In the interaction between bacteria and their surroundings, EPS plays a pivotal function. Bacterial cells are protected against severe circumstances (dry environments, high temperatures), predation, and antibiotic actions when they are surrounded by an EPS layer. Microbial aggregation, surface adherence, biofilm formation, plant-microbe symbiosis, and environmental remediation are all aided by bacterial polysaccharide [11].

Monosaccharides and non-carbohydrate substituents such as protein, nucleic acids, lipids, acetate, pyruvate, succinate, and phosphate make up EPSs, which have been observed in response to abiotic stress reactions such as drought, temperature, and salinity, as well as survival mechanisms for certain bacteria [7]. Mucoid colonies develop on solid surfaces, indicating the presence of exopolysaccharides. The synthesis of an exopolysaccharide may be indicated by an increase in viscosity in a liquid media or by the material solidifying as a gel. Exopolysaccharides are produced by a variety of microorganisms, including algae, fungus, bacteria, and yeast. By forming a biofilm on solid surfaces, these organisms increase their adaptability to the environment and under harsh and unfavourable growth conditions like metal stress, nutrient deficiency, temperature, pH changes, effect of reactive by-products (superoxide anion radical, hydroxyl radical, and hydrogen peroxide), disinfectants, and oxygen antibiotics. Biofilms are communities of microbial cells that are linked to one other and to the solid surface. These microbial cells are encased in a slimy extracellular matrix made up of extracellular materials (exopolysaccharides, proteins and lipids).

Exopolysaccharides have been used in a variety of fields, including pharmacology, nutraceuticals, functional foods, herbicides, and insecticides, among others, with future applications including anticoagulants, antithrombotic agents, immunomodulation, and anticancer, as well as an inducer of interferon, platelet aggregation inhibition, and colony-stimulating factor synthesis. Industry (textiles, dairy products, cosmetics, etc.), health (medicine and pharmacy), and the environment (remediation, flocculation, etc.) are all areas where bacterial exopolysaccharides are used [27]. Non-toxic and biodegradable, EPS is employed as a raw material in industrial applications such as thickeners, stabilizer, emulsifiers, gelling agents, and water binders in foods such as ice cream [11]. This review covers the fundamentals of exopolysaccharide, its types, the microbial biosynthesis route, and their applications in various fields.

EPS types and composition

EPS are long-chain molecules made up of a sugar and sugar residue chain that is lengthy and branched. Chemical structure, functions, molecular weight, and bonding are used to classify bacterial polysaccharides. Exopolysaccharides are classified into two classes based on their chemical composition: homopolysaccharides and heteropolysaccharides (Figure 1).

Homopolysaccharides contain only one type of monosaccharide and their molecular mass is approximately 10^7 Da. Homopolysaccharides are further classified into four groups on the basis of linkage bonds and the nature of monomeric units such as 1) α-D-glucans 2) β-D-glucans 3) Fructans and, 4) Polygalactan.

Heteropolysaccharides contain the repeating units of D-glucose, D-galactose, and L-rhamnose and also contain N-acetylglucosamine, N-acetylgalactosamine, or glucuronic acid and the molecular mass of HePS is in the range of 10^4 to 10^6Da [35]. Non-carbohydrate substituents such as phosphate, acetyl and glycerol are sometimes present. Bonds between monomeric units at the backbone of the polymers are 1,4--or 1,3—linkages and 1,2—-or 1,6--linkages. The distinctions between homopolysaccharide and heteropolysaccharide are reflected not only in the chemical nature and linkage bonds, but also in synthetic enzymes and synthesis sites. The precursor repeating units of heteropolysaccharide are formed intracellularly and isoprenoid glycosyl carrier lipids are involved in the translocation of the precursors across the membrane for subsequent polymerization extracellularly, whereas homopolysaccharide syntheses require a specific substrate such as sucrose. Both gram-positive and gram-negative bacteria produce exopolysaccharide and are found in different niches [35]. Properties and functional attributes of some bacterial exopolysaccharides are given in table 1 [27].

Figure 1: Classification of exopolysaccharides [25].

Biosynthesis of bacterial exopolysaccharides

Some EPSs are synthesized throughout the cycle of bacterial development, while others are only formed in the late logarithmic or stationary phases. Nutrient imbalances, such as high carbon to

Bacterial EPS	Polysaccharide components	Molecular mass	Application of exopolysaccharide	Bacterial strain	Source????
Cellulose	Glucose	~10^6	Foods (indigestible fiber), biomedical (Wound healing, tissue engineered blood vessels) and audio speaker diaphragms	*Acetobacter* spp.	[25,27]
Dextran	Glucose	10^6-10^9	Foods, Pharmaceutical industry (Blood volume expander) and Chromatographic	*L. mesenteriodes*	[29]
Alginate	Guluronic acid and mannuronic acid	(0.3-1.3) × 10^6	Food hydrocolloid and medicine (Surgical dressings, wound management	*P. aeruginosa* and *A. vinelandii*	[1,25]
Xanthan	Glucose, mannose and glucuronic acid	(2.0-50) × 10^6	Foods, petroleum industry, pharmaceuticals, cosmetics and personal care products	*Xanthomonas* spp.	[44]
Curdlan	Glucose	5 × 10^4-2 × 10^6	Foods, pharmaceutical industry, heavy metal removal and concrete additive	*Rhizobium meliloti* and *Agrobacterium radio-bacter*	[1,25]
Succinoglycan	Glucose and galactose	5 × 10^3-1 × 10^6	Food and oil recovery	*Alcaligenes faecalis* var. myxogenes	[1]
Glucuronan	Glucuronic acid	6 × 10^4-6 × 10^5	Food and cosmetics products	*Sinorhizobiummeliloti* and *Gluconacetobacter hansenii*	[27]
Colanic acid	Fucose, glucose, glucoronate, and galactose	2 × 10^4-6 × 10^5	Cosmetics and personal care products	*E. coli*, *Shigella* spp., *Salmonella* spp. And *Enterobacter* spp.	[27]

Table 1: Properties and functional attributes of some bacterial exopolysaccharides [27].

nitrogen (C: N) ratios, and often sub-optimal incubation temperatures, accelerate the production of most of these polymers [45]. Polysaccharides serve as the backbone of EPS production [13].

In bacteria, EPS is produced in two ways.: 1) intracellular and 2) extracellular. Homopolysaccharides are synthesized extracellularly by enzymes produced by bacteria, whereas heteropolysaccharides are synthesized intracellularly and transferred to the extracellular environment. A diverse set of enzymes is present, each of which plays an important part in bacterial EPS synthesis. Based on their involvement in the last three of the four phases of bacterial EPS production, these enzymes are divided into four groups [3].

- **Group 1:** These enzymes are found intracellularly. Hexokinase is the first enzyme in group 1. Hexokinase phosphorylases the glucose (Glu) molecule into glucose-6-phosphate (Glu-6-p). Phosphoglucomutase is the second enzyme, which transforms glucose-6-phosphate to glucose-1-phosphate.
- **Group 2:** The second set of enzymes is assumed to be intracellular and is responsible for the synthesis of sugar nucleotides, which are the building blocks of EPS biosynthesis. Uridine diphosphate-glucose pyrophosphorylase (UDP-Glucose Pyrophosphorylase) is one of them. The conversion of glucose-1-phosphate to uridine diphosphate glucose is catalyzed by this enzyme (UDP-Glu). In the presence of UDP-Gal-4-epimerase, this sugar nucleotide can also interconvert into other sugars, example UDP-Glu to UDP-Gal.

- **Group 3:** Glycosyltransferases (GTFs), which are found in the cell periplasmic membrane, are included in the third group of enzymes. GTFs transport the sugar nucleotide UDP-Glu or UDP-Gal to the repeating unit linked to the glycosyl carrier lipid, isoprenoid alcohol [24].
- **Group 4:** Polysaccharides modified by enzymatic activities such as acetylation, acylation, sulphation, and methylation are exported to the extracellular surface with the help of hydrophobic enzymes such as flippase, permease, or ABC transporters at the fourth stage of synthesis [24].

Bacterial cells primarily use four general strategies for EPS biosynthesis, which are:

Pathway dependent on Wzx/Wzy

First synthesis of nucleotide sugars, assembly of repeating units, polymerization, and export are all part of this route [36]. Activated sugar molecules are transported to and connected with the carrier lipid molecule isoprenoid alcohol in the first stage. GTFs then join other sugar molecules together to create repeating units. These repeating units are now transported across the cytoplasmic membrane by the Wzx flippase [9,15,33,36]. Translocated oligosaccharide undergoes several enzymatic changes, such as methylation, acylation, and others, before being polymerized into polysaccharide by Wzy protein [15]. ABC transporters deliver the formed polysaccharide to the cell surface [9].

Pathway dependent on ABC transporters

The process involved all three members of the ABC Transporter-Dependent Periplasmic protein, Polysaccharide co-polymerase (PCP), and Outer Membrane Polysaccharide Export (OPX) families [8]. Glycosyltransferase is employed in this process to produce polysaccharides before they are exported from the cytoplasm via the tripartite efflux pump complex. When ATP binding and depolymerization (hydrolysis) action at the nucleotide binding domain (NBD) occurs, conformation changes in the membrane heterooligomeric complex of the OPX protein and PCP occur. This pathway is mainly involved in capsular polysaccharide [8].

Pathway dependent on synthase

The polymer products of the Synthase-Dependent Pathway are homopolysaccharides such as cellulose [47,49]. This pathway can be governed with or without the presence of a lipid acceptor molecule. A single synthase protein and membrane-localized GTFs also manage the polymerization of the EPS precursor and eventual transport of the molecule in this pathway [50]. A complete polymer strand of repeating units is transported across the cell membrane by the flippase enzyme. In comparison to other pathways, this one is largely independent of central carbon metabolism.

Extracellular biosynthesis by sucrase protein

Outside the cellular outer membrane, the extracellular sucrase enzyme transforms sucrose into monomeric units [47]. Dextran, alternant, and Levan are examples of extracellularly generated EPSs. *Leuconostoc mesenteroides* produced dextran with a molecular weight ranging from 15 to 20,000 kDa. According to the reaction below, glycosyltransferase dextransucrase transfers glucose from sucrose to the reducing end of a developing dextran chain.

$$\text{Sucrose} \longrightarrow \text{Dextran + D-fructose}$$

Application of exopolysaccharides

Food industry

Dextran is the first industrial polysaccharide delivered by LAB like *Leu. mesenteroides*. Dextran was first identified in sugar cane and beet syrups, where it was discovered to be a good thickening and gelating agent [29]. It acts as a gelling agent in gum and jelly candies. It acts as a crystallization inhibitor in ice cream and improves the body texture and mouthfeel of pudding combinations. Dextran is also used in several chromatography stationary phases and as a blood plasma extender [29]. Xanthan gum, which is produced by the plant pathogen Xanthomonas campestris, has been termed a "benchmark" product because of its widespread use in both food and non-food applications [44], including dairy by-products, drinks, confectionery, dressing, bakery products, syrups, and pet foods. Because of the high conversion of a substrate (glucose) to polymer (60-70%), xanthan synthesis is reasonably affordable [44]. This polymer demonstrates a high viscosity at low concentrations in solution and strong pseudo-plasticity and is steady over a wide range of pH, temperature, and ionic strength. Fructose oligosaccharides (FOS) are frequently utilized in the food industry because they have a low sweetness relative to sucrose, are calorie-free, and are noncariogenic [29]. Inulin and FOS are commonly employed in food applications due to their prebiotic characteristics. Fructose-based polymers can be digested by gut microflora, resulting in improved intestinal flora and increased mineral absorption [29]. Levan derived from *L. sanfranciscensis* LTH 2590 also has prebiotic properties, as demonstrated by numerous *in vitro* experiments [19]. Fructans play an important role in tolerance to cellular stress in plants by stabilizing membranes [28].

The fitness awareness of consumers generated additional demand, especially for low-fat or fat-free dairy products. However, since milk fat contributes to the development of the taste and body texture of dairy products, the removal of this milk fat leads to structural and functional defects in fermented dairy products. The main problems with low-fat yogurt and dahi are a lack of taste and texture [14]. EPS produced by LAB acts as a thickener is used as a natural, appropriate, and superior substitute for a variety of additives. Instead of chemical additives, these cultures meet consumer requirements for products [18], reduce the total solids required without affecting textural characteristics [48]. Low-fat dahi made using various EPS producing cultures of *L. Lactis subsp. Lactis* PM23, *S. thermophilus* ST and *L. Lactis* NCDC 191 are more acceptable [4]. Dextran is obtained from *Leuconostoc mesenteroides* with application in baking improvers. A study provides evidence that EPS effectively enhances the rheological characteristics of the dough and the quality of the bread [6]. The in-situ production of EPS from sucrose resulted in the formation of other metabolites like mannitol, glucose, and acetate, all of which can help improve bread quality [20].

Pharmaceutical industry and health aspects

Lactic acid bacteria have become increasingly popular as probiotics in recent years. The ability of the LAB probiotic strain to tolerate acid and bile, produce antimicrobial chemicals against pathogenic and cariogenic bacteria and attach and colonize human intestinal mucosa. The capsular polysaccharides might facilitate the adherence of bacteria to biological surfaces, so they stimulate the colonization of different microhabitats. Similarly, *Leuconostoc mesenteroides*-produced dextran has been employed to make one of the most effective plasma substitutes for usage in shock and blood loss [41]. Glycosaminoglycan heparin, the medicine of choice for treating thromboembolic diseases, has been linked to a lack of effectiveness in antithrombin deficient patients, with side effects as bleeding and thrombocytopenia. As a result, sulphated forms of alginate have been proposed as a viable alternative with increased activity. Anticoagulant, antithrombotic, anti-atherosclerotic, anti-angiogenesis, anti-metastatic, and anti-inflammatory are some of the other therapeutic properties related to sulphated forms of alginate [10]. Xanthan and sulphated dextran are applied as antiviral and anticancer tools. Fucopol is recognized as a substance having the potential to be used in anticancer, anti-inflammatory, and immune-enhancer medications due to the high fucose concentration in some EPS [12]. Fungal polysaccharides have traditionally been used to scavenge and treat a wide variety of diseases such as infectious diseases, cancer, and other autoimmune diseases. A water-soluble Morchellaconica polysaccharide (MCP) controls nitric oxide formation in macrophages and increases splenocyte proliferation and acts as a powerful immunomodulatory agent [43]. The unreasonable use of antibiotics leads to more and more drug-resistant microorganisms, which ultimately lead to incurable diseases. It was recently discovered that immunomodulators are a potential substitute for antibiotics. Polysaccharides derived from microorganisms are the main factor in macrophage stimulation to induce the immune system's toll-like receptors [23].

Bioremediation

Environmental contamination has created various social difficulties throughout this period of urbanization. The widespread use of chemicals such as solvents, herbicides, insecticides, and other industrial compounds has contaminated soil, air, rivers, oceans, waste streams, and numerous locations. These environmental pollutants harm a huge number of creatures, causing them to become trapped in food chains. Bioremediation, a biological method of utilizing microbes and plants, can help to the removal of hazardous substances from contaminated sites [37-39].

The use of EPS-producing microbes in the treatment of mining-related environmental effluents is a growing field of biotechnology [16]. The probable role of EPS in the removal of heavy metals from the environment is due to their ability to bind metal ions from solutions. A prominent group of sulfate-reducing bacteria (SRB) is generally located in metal-contaminated wastewaters [42]. In the anaerobic condition, these groups of bacteria are degraded of numerous organic contaminants and the precipitation of heavy metals from wastewater. Other bacteria demonstrating biosorption of toxic heavy metals in bioremediation procedures contain *Enterobacter* and *Pseudomonas* species [40,42]. Fungal-bacterial biofilms (FBBs) were employed for the first time to extract nickel from wastewater. Hexavalent chromium bioremediation in wastewater using FBB is also a unique approach. A study found that established FBBs, glass-wool-attached bacterial biofilms, and their monocultures were effective in removing hexavalent chromium. EPS separated from *Zoogloea* spp. and *Aspergillus niger* support in the degradation of pyrene in contaminated soils [17]. Some EPS-producing bacteria, such as Bacillus cereus possess the capability of biocorrosion of stainless steel and are thus utilized in bioremediation to remove unwanted steel compounds in stainless steel companies [5]. *Sphingomonads* are unique in that they have multiple large pleat-like structures on their cell surface, as well as an extraordinary metabolic capacity to degrade a variety of rigid environmental pollutants, particularly xenobiotics like dioxin, biphenyl, and bisphenol, and the ability to produce valuable biopolymers [1].

The petroleum industry

The petroleum industry uses xanthan gum as a bacterial EPS in oil drilling, fracturing, and pipeline cleaning [1], and it's also useful as a drilling fluid additive because of its salt tolerance and resistance to temperature degradation [46]. Microbial Enhanced Oil Recovery (MEOR) is the process of using microorganisms to extract additional oil from existing reservoirs, hence increasing the petroleum production of an oil reservoir. Select natural microorganisms are introduced into oil reservoirs in this manner, resulting in harmless by-products such as slippery natural compounds or gases, all of which help in the extraction of oil from the well. These techniques help to assemble the oil and facilitate oil movement, allowing for a greater amount of oil to be recovered from the well. Genetically engineered *Enterobacter cloacae* are used in MEOR [1].

Agriculture

The growth of EPS-producing bacteria in the rhizosphere of cultivated plants can improve soil fertility and productivity. Meanwhile, EPS-producing bacteria are present within the roots and surrounding soil, increasing the movement of water and nutrients via the plant roots. The PGPR effect could be linked to very high yields in shoot and root growth of cultivated plants that were produced in a salty environment due to an EPS-forming bacterial inoculation because nutrient uptake and utilization is the limiting method for crop growth and yield in later growth stages [2]. In the terrestrial environment, *Azotobacter* EPS is important for ecosystem function because it regulates nutrient cycle processes, which are necessary for soil productivity, as well as biotic and abiotic pressures in the soil ecosystem [1,36].

Exopolysaccharides are employed as biosurfactants in some cases. Many rhizosphere and plant-associated bacteria generate biosurfactants which are low molecular weight surface-active chemicals, and are important for motility, signal transmission, and biofilm formation, indicating that EPS regulates plant-microbe communication. EPS can be utilized to improve agricultural soil qualities on a wide scale through soil remediation. These biomolecules have the potential to someday replace the hazardous surfactants currently utilized in the multibillion-dollar pesticide industries [34]. The outer surface of rhizobia is made up of complex polysaccharides including lipopolysaccharides (LPS), capsular polysaccharides (CPS), and extracellular polysaccharides (EPS). They can create associations with legumes such as *Trifolium, Pisum, Vicia,* and *Medicago* spp and induce the formation of specific organs on roots and stems called nodules, in which atmospheric nitrogen is converted to ammonia by the nitrogenase enzyme complex [1]. The exopolysaccharides gellan and curdlan have agricultural potential because they serve as a soil improver, which improves the cultivated soil's water retention capacity [26].

Textile industry

Because of its viscosity, stabilizer, and cross-linking capabilities with fabrics, EPS is utilized as a binding agent with color dyes or hydrogel in the textile industry. One of the unique applications of EPS is smart fabrics. Hydrogels based on biopolymers exhibit specific physical properties of swelling and shrinkage that are regulated by external factors such as pH, temperature, solvent, electric field, light, stress, ionic strength, and other external chemical stimuli, among others [21]. Biopolymers like chitosan are combined with synthetic polymers to make a hydrogel that can be successfully implanted on fabric surfaces for smart textile applications. At appropriate temperatures, these fabrics are used as deodorant release agents. As a result, they're used in fabric aroma finishing. With a change in external temperature or pH, the polymer beta-Cyclodextrin can release it. These fabrics can also change color in response to changes in external temperature by including thermochromic elements such as cholesterins [1].

Conclusion

Bacterial-produced exopolysaccharides have a wide range of activities and are not restricted by taxa. The monomeric compositions, linking bonds, and associated conjugates demonstrate some of this complexity, while the functions can be categorized into intrinsic and applied. The essential functions in human use, including morphological, structural, and defensive roles, are evident; Medical, cosmetic, pharmaceutical, dairy and other industrial and environmental items are just a few examples. For microbial EPS production, understanding the biosynthetic mechanism is an important topic for optimizing EPS production yields, improving product quality and properties, and also for designing novel strains. Since most bacterial EPS with unique properties have expensive production costs and economic hurdles to overcome, this valuable biosynthetic information is also important to reduce these costs. Since microbial biopolymer biosynthesis is the result of a complex system of many metabolic processes, system-based approaches to control and optimize production are needed to improve the previously reported yields.

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Conflicts of Interest

We declare that here are no conflicts of interest.

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