The Chemistry of Bacterial Exopolysaccharides

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Introduction

Bacteria synthesize a wide range of biopolymers with unique chemical structures, utilizing simple and complex substrates. Depending on their location within the cell, these biopolymers can be classified as either intracellular or extracellular. Extracellular biopolymers, often called Exopolysaccharides (EPSs), are more diverse and essential for bacterial survival and adaptation than intracellular biopolymers, which are comparatively fewer in number and function.[1] Bacterial EPSs have clear advantages over plant, animal, and algae-derived polysaccharides because they can be produced year-round under controlled fermentation conditions, are easily and cost-effectively recovered from cell-free culture supernatants, and allow tunable yields and polymer properties through genetic or metabolic engineering.[2] Their biological functions are pivotal: EPSs provide physical protection against desiccation, toxic compounds, antibiotics, and phagocytosis, while also mediating adhesion to surfaces and cell-to-cell communication.[1,2] The vast chemical and structural variety inherent to bacterial EPSs, coupled with the precision of microbial fermentation, allows for the tailored production of biopolymers with exceptional and predictable functional properties, positioning them as cornerstones in the modern food, pharmaceutical, and bioremediation sectors. This article focuses on the chemical characteristics that underlie this versatility, highlighting a clear link between their molecular structure and its macroscopic utility.

Structural Architecture and Classification

Bacterial EPSs display remarkable chemical diversity, reflecting the wide range of biological roles they perform. At the molecular level, their architecture is defined by several key parameters, including monosaccharide composition, molecular weight, glycosidic linkages, functional groups, branching patterns, and higher-order microstructure.[3]

The most fundamental distinction arises from the primary structure, specifically the nature of the constituent sugar units. On this basis, EPS are broadly categorized as homopolysaccharides (HoPSs), composed of a single type of monosaccharide, or heteropolysaccharides (HePSs), which contain two or more different monosaccharide residues arranged in repeating units. HoPSs include structurally simpler polymers such as curdlan, while HePSs often consist of repeating oligosaccharide blocks ranging from disaccharides to heptasaccharides, resulting in greater structural complexity.[1,4] Common monosaccharides such as glucose, galactose, and fructose are frequently encountered, but many EPS also incorporate rarer sugars like rhamnose, fucose, xylose, and mannose, as well as uronic acids and amino sugars, contributing to their functional versatility.[4]

In addition to monosaccharide composition, EPSs are further classified according to the glycosidic linkages that join individual sugar units. These bonds are defined by the carbon atoms involved, most commonly C1–C3, C1–C4, or C1–C6, and by their α or β configuration.3 In the polymer backbone, β-(1→4) and β-(1→3) linkages generally produce more rigid structures, whereas α-(1→2) and α-(1→6) linkages result in greater chain flexibility.[1] These bonding patterns strongly influence the overall shape of the EPS molecule. Polysaccharides with straight, unbranched chains, such as pullulan, are classified as linear EPS, while those containing side chains and bends, such as EPS-W1 from *Lactobacillus plantarum* W1, are referred to as branched EPS.[4] Additional chemical features, including the presence of neutral or charged functional groups and non-carbohydrate substituents such as phosphate, acetyl, or glycerol moieties, further expand the classification framework by imparting ionic character and altering physicochemical behavior.

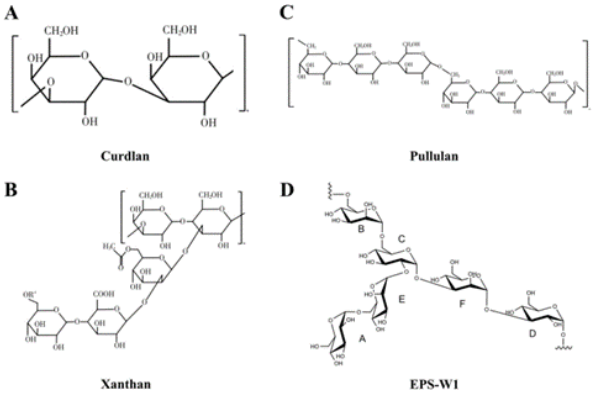

Figure 1: EPS structure of homopolysaccharides [e.g., curdlan (A)]; heteropolysaccharides [e.g., xanthan (B)]; linear polysaccharides [e.g., pullulan (C)]; and branched polysaccharides [e.g., EPS-W1 (D)].[4]

Chemical Modification of EPS

Chemical modification of EPSs has gained increasing attention as a means to tailor their molecular structure and enhance targeted biological functions. These modifications are achieved by introducing functional groups onto the polysaccharide backbone.[4] Carboxymethylation introduces carboxymethyl groups through etherification reactions, increasing water solubility and improving biological performance.[5] For example, carboxymethylated EPSs from *Lachnum* fermentation broths significantly reduced fasting blood glucose and serum triglyceride levels while improving insulin sensitivity in diabetic mouse models, highlighting their potential use in functional foods and therapeutic formulations.[6] Acetylation modifies the steric configuration of EPS by adding acetyl groups, which have been shown to improve reducing power and antioxidant capacity.[7] Phosphorylation is another effective modification strategy, introducing phosphate groups that increase negative charge density and strengthen biological interactions. Phosphorylated EPS from *Lactococcus lactis* and Lachnum species demonstrated significantly improved antioxidant activity both in vitro and in vivo, indicating potential applications in nutraceuticals and biomedical materials. Similarly, sulfonation improves solubility and chain flexibility, resulting in stronger antioxidant and antimicrobial effects, as observed in EPSs from *Enterobacter cloacae* and *Streptococcus thermophilus*.

The Chemistry of EPS Biosynthesis

In bacteria, EPSs are produced through four principal pathways.8 In all intracellular pathways, EPS biosynthesis relies on a common chemical principle: the generation of activated sugar precursors, followed by enzyme-catalyzed glycosyl transfer reactions that construct the polymer backbone and its substituents.

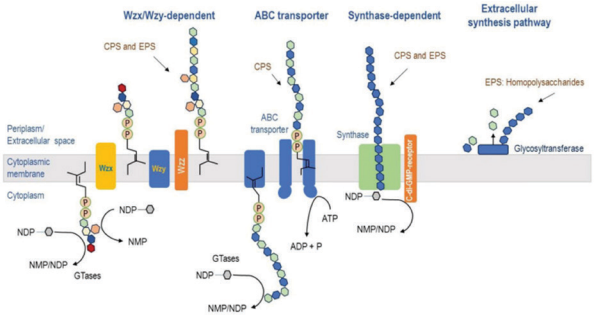

Figure 2: Biosynthesis of polysaccharides in microorganisms.[8]

The chemical foundation of EPS biosynthesis lies in the formation of sugar nucleotides, which act as high-energy donors for glycosylation reactions.[9] Simple sugars derived from central metabolism, such as glucose-6-phosphate or fructose-6-phosphate, are enzymatically converted into sugar-1-phosphates and subsequently activated through reactions with nucleoside triphosphates, typically UTP, GTP, or TTP. This process yields sugar nucleotides such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, GDP-mannose, and TDP-rhamnose.[8] The cleavage of the phosphodiester bond during glycosyl transfer provides the thermodynamic driving force for forming new glycosidic linkages.[10] The nucleotide moiety also serves as a molecular recognition tag, ensuring that each glycosyltransferase incorporates the correct monosaccharide at the appropriate position, thereby preserving the fidelity of EPS structure.

Among EPS biosynthetic routes, the Wzx/Wzy-dependent pathway is the most chemically complex and mainly produces HePSs. Repeating oligosaccharide units are assembled on the cytoplasmic face of the inner membrane on undecaprenyl phosphate (a C55 isoprenoid lipid acts as a mobile chemical carrier), modified by specific glycosyltransferases, flipped across the membrane by Wzx, and polymerized in the periplasm by Wzy to yield high-molecular-weight EPSs

such as xanthan.[8] In contrast, the ABC transporter–dependent pathway, primarily associated with capsular polysaccharide (CPS) biosynthesis, exports completed polymers through an ABC transporter and generates CPSs with a glycolipid terminus containing phosphatidylglycerol and a poly-keto-deoxyoctulosonic acid (Kdo) linker, which anchors the polysaccharide to the cell envelope.[11] The synthase-dependent pathway is a simpler strategy in which polymerization and secretion occur simultaneously through a single synthase enzyme or complex.[12] This pathway mainly produces HoPSs such as cellulose, curdlan, alginate, and hyaluronic acid, where activated sugar nucleotides are directly added to the growing chain as it is extruded across the membrane.[8] While some polymers, like cellulose and curdlan, consist of a single type of sugar and linkage, others, such as alginate, are further diversified by post-polymerization modifications, such as alginate which is first synthesized as polymannuronic acid and later modified by epimerases to generate varying mannuronic and guluronic acid sequences.[13] In contrast, some EPSs are synthesized entirely outside the cell by secreted glycosyltransferases that use substrates like sucrose, producing polymers such as dextran and levan. This extracellular mechanism bypasses sugar nucleotides and membrane carriers, representing a distinct chemical route to EPS formation.

The Rheological Chemistry

Many EPS contain uronic acids (such as glucuronic and galacturonic acid) and pyruvate ketals, which introduce carboxyl groups into the polymer backbone.[14] At neutral pH, these groups are ionized, making EPS highly polyanionic molecules. The resulting negative charge density causes the polymer chains to adopt extended conformations and promotes strong interactions with water through electrostatic forces and hydrogen bonding. This explains the high hydration capacity of EPS and their ability to form viscous solutions even at low concentrations. In addition, the negatively charged sites readily bind metal ions such as Ca^{2+} and Mg^{2+}, a property that contributes to biofilm stability and is widely used in food stabilization and water treatment.[15]

EPS are mostly high-molecular-weight polymers, often ranging from 10^5 to over 10^7 Da. Their large size leads to significant chain overlap and physical entanglement in solution, which results in high viscosity. Their large size leads to extensive chain overlap and physical entanglement in solution, producing high viscosity. Unlike simple liquids, EPSs solutions usually show non-Newtonian flow behavior, where viscosity decreases with increasing shear stress. This shear-thinning behavior is especially evident in HePSs such as xanthan. Xanthan adopts an ordered double-helical structure in solution, forming a weak but extensive network at rest that results in high viscosity and yield stress. Under shear, the helices align with the direction of flow and temporarily disentangle, causing a reversible drop in viscosity. When shear is removed, the original structure rapidly reforms, allowing viscosity to recover.[16] Xanthan's rheological behavior is further influenced by acylation and salt concentration: pyruvate groups stabilize the ordered structure, while acetyl groups tend to destabilize it and reduce synergistic gel formation.[17]

In contrast to xanthan, dextrans form aqueous solutions that behave largely as Newtonian fluids, with viscosity dependent on concentration, temperature, and molecular weight.[18] Native dextrans are polydisperse, with molecular masses typically ranging from 10^6 to 10^9 Da. Controlled acid hydrolysis produces fractions with defined molecular weights, a property that, combined with their low immunogenicity, has enabled widespread clinical and pharmaceutical use, including as blood plasma expanders and chromatography matrices.[15]

Some EPSs also undergo gel formation through specific molecular associations. In polysaccharides such as curdlan and gellan, gelation occurs when polymer chains transition from flexible random coils to ordered helical structures that aggregate into stable junction zones; this process is often thermoreversible.[19] In contrast, gelation in alginate is driven by ionic interactions rather than temperature. Alginate chains contain guluronic acid-rich regions that selectively bind divalent cations, particularly Ca^{2+}, forming strong ionic cross-links between adjacent chains via the well-known "Egg-Box" mechanism.[20] The resulting hydrogels are mechanically stable and widely used in food, pharmaceutical, and biomedical applications.

Engineered Chemistry and Future Outlook

The functional versatility of bacterial EPSs is closely linked to their finely controlled chemical architecture. Unlike many plant-derived polysaccharides, EPS possess highly regular repeating units, variable molecular weights, and chemically active substituents such as uronic acids, acetyl groups, and pyruvate residues. Due to these features, EPS have found widespread use in food systems, pharmaceuticals, cosmetics, and environmental applications. For example, EPS produced by probiotic Lactobacillus species contributes not only to desirable texture in fermented dairy products but also supports beneficial health effects such as immune regulation, reduction of cholesterol levels, and possible antitumor activity.[1] Similarly, bacterial EPS emerge as safe and biodegradable alternatives to synthetic flocculants in water and wastewater treatment, where they effectively aggregate suspended particles without the health risks associated with aluminium salts or synthetic polymers.[1]

Looking ahead, advances in bioengineering and synthetic biology are transforming EPS production from a largely natural process into a tunable chemical platform. By modifying genes involved in EPS biosynthesis, such as glycosyltransferases, epimerases, and acetyltransferases, researchers can tailor monomer composition, chain length, and degree of substitution to achieve desired material properties. Emerging strategies, including domain swapping of biosynthetic enzymes and the design of synthetic gene clusters, offer the possibility of producing entirely new EPS structures with novel functions. As understanding of structure–function relationships deepens, engineered bacterial EPSs are expected to play a central role in developing sustainable biomaterials for healthcare, environmental remediation, and next-generation biotechnological applications.

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Cover Page

The cover page graphic illustrates the four main phases of wound healing: hemostasis, inflammation, proliferation, and remodeling. It shows how the body first stops bleeding, then removes bacteria and damaged tissue, builds new tissue and blood vessels, and finally strengthens the repaired area to form mature scar tissue. In relation to the guest article titled "Chemical Mediators of Inflammation: Targets for Wound Repair," the diagram highlights the importance of inflammatory mediators in coordinating each stage of healing and influencing successful tissue repair.

(see pages 16 - 20)