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REVIEW ARTICLE OPEN ACCESS

Bacterial Extracellular Polymers: A Review

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Abstract

Prokaryotic microbial cells especially bacteria are highly emphases for their exopolysaccharides (EPS) production. EPS are the higher molecular weight natural extracellular compounds observe at the surface of the bacterial cells. Nowadays bacterial EPS represent rapidly emerging as new and industrially important biomaterials because it having tremendous physical and chemical properties with novel functionality. Due to its industrial demand as well as research studies the different extraction processes have been discovered to remove the EPS from the microbial biofilm. The novelties of EPS are also based on the microbial habitat conditions such as higher temperature, lower temperature, acidic, alkaliphilic, saline, etc. Based on its chemical structure they can be homopolysaccharide or heteropolysaccharide. EPSs have a wide range of applications in various industries such as food, textile, pharmaceutical, heavy metal recovery, agriculture, etc. So, this review focus on the understanding of the structure, different extraction processes, biosynthesis and genetic engineering of EPS as well as their desirable biotechnological applications.

Keywords: Exopolysaccharides, Biosynthesis, Genetic engineering, Industrial applications

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INTRODUCTION

Exopolysaccharides are the key feature of most of the bacterial sufaces[1]. The formation of biofilms takes place through the attachment of bacterial cells to the substratum or cells embedded in a protective extracellular matrix[2]. It is a complex structure of a heterogeneous matrix consists of various molecules[3]. These natural polymers have emerged as a new alternative to synthetic polymers with marvellous physical characteristics, so they have vast industrial applications. This term was first used by Sutherland in 1972 in his exclusive work on marine bacteria producing EPS[4]. EPS finds in two forms viz. EPS (capsular EPS) and soluble EPS (slime)[5,6]. From the last decades, industries are more emphases on natural polymers production and these natural polymers are used by various pharmaceuticals, food and other industries which are developing remarkable interest in polysaccharides produced by microorganisms[7]. The total EPS yield depends upon microorganisms used and cultivation conditions provided to them[8,9]. Bacterial EPS's have been utilized as bio-absorbents, bio-flocculants and heavy metal removal agents[10]. The main aim of writing this review is to give an overview of the structure, extraction process, biosynthesis, genetic engineering and applications of microbial EPS.

Structure of EPS

Based on the monomeric unit, EPS's are classified as homopolysaccharides and heteropolysaccharides[11]. Homopolysaccharides contain monosaccharides while heteropolysaccharides are composed of more than one type of monosaccharide[12]. EPS is classified based on the number and nature of monomers, bonds between them and the type of linkage[11]. EPS are generally polyanionic due to the presence of uronic acids or ketal-linked pyruvate or inorganic substances like phosphate or sulfate. Some are also neutral macromolecules[13]. A few EPSs may even be polycationic, e.g. polymer obtained from *Staphylococcus epidermidis* strains[14]. However, the physicochemical factors also affect the production of exopolysaccharides which includes pH, temperature, incubation time, and the constituents of culture media (with various organic and inorganic carbon (C) and nitrogen (N) sources)[15].

Extraction of EPS

Various extraction processes have been developed to recover EPS from biofilm of microbes from different environments to identify the contents of EPS, to analyze various properties (chemical, physical and physicochemical) and to observe the functions of EPS[16,17]. The various extraction methods include chemical or physical and physical and chemical, and analytical methods. It is estimated that extraction methods are dependent on the water solubility of the EPS separated. The extraction methods of EPS should be cost-effective, eco-friendly and do not damage the structure of EPS[16]. The extraction efficiency is calculated as the overall amount of EPS separated from the entire microbial biomass for a particular sample[5].

Physical method

The physical process mainly involves three extraction techniques i.e. centrifugation, sonication and heating[18]. Researchers explored the reason behind variations in extraction efficiency with different physical methods. These variations in results are because during the extraction procedure by heating, the components of EPS might be hydrolyzed, for this particular case, the proteins and polysaccharide content of EPS[18]. Another study showed that the heating allowed extracting the capsular EPS to flocs[19]. However, several studies suggested that the high EPS extraction yield by the heating methodology could also be thanks to meaningful cell lysis which can lead to high protein content in EPS[20,21].

Chemical method

In the chemical method, different chemicals are used which could break the linkage in the matrix so that EPS can be easily released to the external medium containing water. NaOH treatment can cause the ionization of a great number of charged groups, for instance, carboxylic groups in proteins and polysaccharides. Due to this, a strong repulsion occurs between EPS which enhances its solubility. A lot of polymers can suffer alkaline hydrolysis[22]. In glycoproteins, disulfide bonds can be broken if exposed to basic environments (pH above 9), which will promote the extraction of these compounds[23]. The repulsion and solubility between the compounds of the EPS matrix are resultant of the exchange of

divalent cations with mono-valent cations. Resin, EDTA or EGTA are used for the removal of divalent cations. A high concentration of sodium chloride can also be used to carry out cation exchange. This process has been used in *Pseudomonas* sp. for the extraction of adhesive exopolymers[24,25]. The extraction of EPS could be increased by destabilizing biofilm in an enzymatic digestion process[26]. Furthermore, ethanol from activated sludge has been used to extract lipids[27].

Combination of physical and chemical method

Some studies proposed that the chemical extraction method could be of better performance once it is combined with the physical method i.e. defined shear. The shear is often provided by heat, sonication, or stirring under pre-established conditions. The alkaline and heat treatment has been combined to extract capsular EPS from varied microbial species[28]. On the other hand, shear (stirring) and ion exchange by a Dowex extraction have been used in conjunction to extract EPS from activated sludge and biofilms[16,29]. Formaldehyde (CH_2O) and NaCl was applied in combination with ultrasonication, to extract EPS from an anaerobic sludge[30]. The formaldehyde is added to minimize cell disruption during the extraction process. However, formaldehyde (CH_2O) has the capacity to changes the properties of many EPS components[31].

Analytical method

Colorimetric analyses

The complex composition of EPS in biofilms is made up of carbohydrates, lipids, humic substances, proteins, nucleic acids, etc. Colorimetric analyses are may be used to quantify the components in EPS[32]. The measurement of carbohydrate contents performed by two methodologies i.e. the anthrone method or the phenol–sulfuric acid method. The content of protein might be measured by the Lowry technique, the Press-Man technique, or the total N-content technique[16]. The m-hydroxydiphenyl sulfuric acid method has been used to measure uronic acid content in EPS[33]. To measure the nucleic acid content, three different methodologies could be used, which are the 4,6-diamidino-2-phenylindole (DAPI) fluorescence method[16], the UV absorbance method[34], or the diphenylamine method[21].

Innovative methods

EPS structure, functions and conformation examinations are very difficult to work due to their complex composition. However, recent studies in analytical chemistry develop new techniques such as transmission electron microscopy (TEM)[35], scanning electron microscopy (SEM)[35], atomic force microscopy (AFM)[36], confocal laser scanning microscopy (CLSM)[37], fourier transform infrared spectroscopy (FTIR)[38], X-rayphotoelectron spectroscopy (XPS)[38], nuclear magnetic resonance (NMR)[39] and 3-dimensional excitation-emission matrix fluorescence spectroscopy (3D-EEM)[40] which will help in examining the properties of EPS as well as their nature. The qualitative and quantitative analysis of EPS compositions was reported by using chromatography, mass spectrometry and their combination[16].

Biosynthesis of EPS

Biosynthesis of homopolysaccharides and heteropolysaccharides take place in different-different pathways.

Synthase dependent pathway

The synthesis of homopolysaccharides through synthase, a dependent pathway is quite complicated, but specific enzymes make this process easier, by making modifications in initially synthesized homopolysaccharides such as alginate (Table 1) via the polymerization of GDP-mannuronic acid monomers, the biosynthesis of alginate takes place too. The enzymes involved in alginate biosynthesis are epimerases, lyases and acetylases[41]. Diverse alginate epimerases (AlgE1-7) outer of the cell are there that alter the final chemical polymer characteristics through the selective insertion of specific β-D- mannuronic acid (M) and α-L-guluronic acid (G) blocks[41,42]. In recent years, the significance of *P. aeruginosa* was represented as depressingly induces alginate production. The DalgL variant had maximum yields of alginate of equal MW. The higher O-acetylated alginate and lower molecular weight were observed after the overexpression of AlgL. Both are highly focused factors for the pathogenicity of *P. aeruginosa*[43]. Therefore, the biosynthesis of alginate functions as a motley-enzyme complex[44]. A pure EPS of biofilm is bacterial cellulose which is a linear glucan β-(1,4)[45].

The UDP-activated cytosolic glucose monomers follow the cellulose synthase complex that incorporates a preserved catalytic subunit denominated BcsA. It is related to the GT2 family which is known by performs the polymerization

process through upend the mechanism. The structures of the principal subunit and another subunit, BcsA and BcsB both were identified from specific bacteria that was *Rhodobacter sphaeroides*, show the cell domain of the BcsAB comprised the GT activity and a PILZ domain for interaction with activator c-diGMP[46]. The periplasmic domain is narrowly linked to a flavodoxin-like domain[47,48]. The entire cell envelope is permeated by cellulose synthase complex and its productivity is very high[49]. The interesting phenomenon of biosynthesis of cellulose was recently described[46].

Dextrase/Sucrase dependent pathway

The homopolysaccharide dextran and levan are formed and assembled from the cleavage of sucrose molecules by the action of the extracellular sucrase. After that, the monosaccharide unit is transported to a primer molecule, which may be ramified at distinct levels[50,51]. Further, using different primer molecules leads to high oligosaccharide production[52].

Wzx/Wzy pathway

In this pathway activated sugar nucleotide monomers are transferred by an enzyme called glycosyltransferases (GTs). In this manner, the number of GTs available will determine the sequence of the final polymers. Both the side chain and substituents are incorporated adjacent to the backbone, before the completion of the polymer assembly, but the stage at which this incorporation occurs is not clear[53,54]. The Wzx gene encodes the flippase protein which transfers the repeating unit by H+-dependent antiporter mechanism[55]. Different numbers of trans membrane sequences are shown by the structures of the several Wzx proteins, besides lack in similarity. It points out that different types of Wzx protein exist[56]. Evidence has been found to support the preference of the substrate Wzx on kindred O-units further than the first sugar[55-57]. As soon are transferred the repeating units towards the periplasm another enzymes, the polymerase will recognize it and helps in the polymerization of repeating units. This procedure is carried out by the polymerase, sometimes accompanied by a co-polymerase, which may be associated with the process of determining the length of the polymer[58,59]. The Wzx / Wzy pathway is defined by the involvement of the main protein in the transportation and polymerization of specific repeating units, on which the final structure of EPS depends. Various EPS such as xanthan and succinoglycan are synthesized through this pathway[60-62] (Table 2).

ABC transporter pathway

Two synthesis strategies dependent on the ABC transporter have been identified. One of these strategies is combined with the synthesis and export of cytosolic glucans[63,64]. The second is the synthesis and export of uncoupled glycans by modifying the non-reducing terminus of the polymer attached to Und-P that ends the chain extension. At this point, the terminator determines the glycan chain length and simultaneously serves as an export signal recognized by the transporter[63]. A terminal residue linked by the WbdD protein[65] and the end process depends on the chain size and the stereochemistry of the WbdD-WbdA complex[65,66]. To assemble the glycan chain, the domains of GT activity are carried by the WbdA protein. Recently, a protein was described in *Raoutella terrigena*, that was observed the significant role for polymerization, termination, and quality control within its protein structure[67]. These types of findings show the complexity behind the biosynthesis mechanism. Additionally, specific domain scans could be given within the protein complex. Hence, it was possible to understand an important phase of CPS biosynthesis, whose role is crucial in human pathogens. Recently, in *Campylobacter jejuni*, the ABC PglK transporter mechanism was described, which is highly dependent on ATP to achieve the transport of lipid-linked oligosaccharide units[68]. The drop-interface-bilayer systems have been novel techniques that have helped to gather the most recent knowledge about the Wza homologs for the export of CPS, allowing to know the complexity of this transport mechanism[69] and in turn, contributing to the construction of promising perspectives[70,71].

Engineering strategies

The specific operons present on the genome which are encrypted in genes for the biosynthesis of EPS. Thus, the number of open reading frames (ORFs) may differ from one to more than 30 ORFs[86]. EPS is composed of all the genes that are essential for its biosynthesis of polysaccharides units, the turning of the repeating units, as well as the polymerization and the final

Table 1. List of homopolysaccharides and producing bacteria

S.No.	EPS	Localization	Polymerisation enzyme	Precursors	Micro-organism	Ref.
1.	Dextran	Extracellular	Dextransucrase	Saccharose	*Leuconostoc* sp.	72
2.	Pullulan	Extracellular	UDPG-pyrophosphorylase	UDP–d-glucose	*Aureobasidium Pullulans*	73,74
3.	Levan	Extracellular	Levansucrase	-	*Bacilluslicheniformis*, *Acetobacter* sp., *Halomonas* sp.	75
4.	Curdlan	Extracellular	Curdlan synthase	UDP–glucose	*Rhizobium* spp.	76
5.	Cellulose	Extracellular	Cellulose synthase	UDP-d-glucose	*Acetobacter Xylinum*	77

polymer transport (Table 3). Besides these, there are some specific genes present on the operon which are involved in sugar precursors synthesis, whereas other genes that provide nucleotide sugar are spread all over the chromosomes[62]. It was observed that most of the bacterial genes could encode more than one polysaccharide biosynthesis pathway[87]. The production of EPS depends upon the regulatory effects and cultivation conditions[87,88]. The various type of carbon (C) and nitrogen (N) sources can affect the polysaccharide's expressions in bacteria[89]. It was found that cdi-GMP has an impact on the biosynthesis of EPS, moreover, overexpression resultant as production of novel EPS[90]. The new binding sites could be affecting the EPS biosynthesis[91,92].

Applications of EPS

Agriculture

EPS has a wide range of applications in agricultural fields and is known to have the ability to increase productivity. EPS's secreted by microorganisms play a crucial role during soil development[102]. It is also capable of entrapping nutrients and provides protection to microbes against unfavorable environmental conditions by forming niches[102-104]. EPS also plays an important role in protecting a crop against desiccation and predation by other organisms[105]. EPS also protects the seedlings from drought. The ability to freeze water, technically called ice nucleation activity (INA), is widely used in biotechnology, for example for the production of energy-saving artificial snow and ice. Additionally, in industries such as food processing, it has been used during ice-cream production and freezes concentration efficiently avoiding loss of flavor[106].

Heavy metal degradation

It has been reported in several studies that EPS has a high affinity for heavy metals present in wastewater[107]. The binding affinity of EPS towards heavy metals depends upon the composition and binding sites present in EPS[108]. EPS is associated with the surface so that it protects micro-organisms from heavy metal toxicity[109]. It has been reported that the adsorption capacity of most of the heavy metals such as copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn), etc. depends on the components of EPS, which shows that the main reason for the adsorption performance of EPS is the protein[110].

The major issues of heavy metal, contamination have been seen in agricultural soils because heavy metals can easily enter into the food chain and possess serious health hazards to the humans as well as ecosystem[111]. Toxic heavy metals include cadmium, lead, copper, zinc and manganese[112].

Biomedical applications

EPS's have a wide range of applications in the biomedical sector. It is used as a plasma volume expander for controlling wound shock[113,114], as an antiacid stomach protector in capsules and as a stabilizing agent in pharmaceutical suspensions and emulsions[115]. It is also used during eye surgery, in wound healing, used in cosmetics, in the treatment of osteoarthritis[116], as a drug-controlled release carrier[117] and also used in skin repair[118].

Food applications

The need of today's hour is the healthier food without compromising with the safety of

food[119]. There are a lot of microbes producing EPS for eg. lactic acid bacteria mainly produce EPS which improves the quality, texture and safety of various food products and also inhibits the growth of disease-causing organisms in food[120,121]. The EPS in the food industry has been used as an emulsifier, stabilizer and thickener. It is also used in the packaging of food products. Mostly xanthan, gellan and cellulose which are secreted by bacteria other than lactic acid bacteria, are predominantly used in the food industry[122].

CONCLUSION

As described in this review, It is now widely considered that bacterial EPS plays a very important role in various industrial applications. Moreover, EPSs biosynthesis is a complicated process through which various alterations occur and resultant many number of EPSs produces on bacterial cell surface, which have a valuable range of physicochemical properties and highly promising commercial applications. However, the EPS extraction methods from cell surface still required the some novel techniques or tricks that will be easy to handle, time consuming and more effective for understanding of mechanism involved in synthesis and excretion. This study showed the role of EPS in the food, pharmaceutical, heavy metal recovery and agriculture field, but there is still much to learn about their functions in the environment. To understand more about biopolymers synthesis, it will be necessary to explore insight into the some extremophiles from extreme condition these EPSs are highly stable at various physical as well as on chemical parameters more than mesophilic bacterial EPSs. On the other hand genetic engineering is the new tools for changes in the properties of molecules that will be possible by genome annotation and construction of EPS biosynthetic pathways in bacterial cell to understand about how they will incorporate and how they will be affected. The big research gaps still remain that no method exists to extract all microbial polysaccharides but in upcoming scientific studies it could be possibility to explain about EPSs with their specific structure and functions.

Table 2. List of heteropolysaccharides and producing bacteria

S.No.	EPS	Localization	Polymerisation	Precursors enzymes	Micro-organism	Reference
1.	Succinoglycan	Extracellular	Phosphoglyco syltranferase	UDP-glucose,UDP-galactose	*Sinorhizobium meliloti*	78,79
2.	Hyaluronic acid	Extracellular	HA synthase	UDP-glucuronic acid,UDP-N-acetylglucosamine	*Psudomonas aeruginosa*, *Streptococcus* sp.	80,81
3.	Gellan	Extracellular	Gellanlyase	UDP-glucose,TDP-rhamnose, UDP-glucuronic acid	*Psudomonas elodea*, *Sphingomonas paucimobilis*	82,83
4.	Alginate	Extracellular	Glycosyl tranferase	GDP–mannuronic Acid	*Pseudomonas* sp.	84
5.	Xanthan	Extracellular	Xanthan polymerase	UDP–glucose, GDP–mannose and UDP–glucuronate	Xanthomonas Campestris	85

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Table 3. List of genes responsible for EPS production

EPS	Name of the Bacteria	Gene	Reference
Xanthan	*Xanthomonas Campestris*	gum D	93
Hyaluronan	*Streptococcus zooepidermicus*	has A	94
Cellulose	*Acetobacter xylinus*	bcsA	95
Levan	*Erwinia amylovora*	rlsA	96
Gellan	*Sphingomonas paucimobilis*	pgm G	97
Alginate	*Pseudomans aeruginosa*	AlgD, AlgC, AlgR ,AlgB, AlgZ	98,99,100,101

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ETHICS STATEMENT

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