

A Review on Biosynthesis Mechanism and Application of Microbially Produced Exopolysaccharide Substance (EPS)

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ABSTRACT

Recent discoveries in science increases use of microorganism and microbial products in various commercial, industrial and environmental applications. Microbial exopolysaccharide substance(EPS) are produced by bacteria, fungi and algae. Study of biosynthesis pathway for EPS production provides idea regarding composition of polymers and in-situ view to engineered pathway to get desired polymers. EPS is composed of carbohydrate, protein, uronic acids, humic substance, lipids etc. EPS can help microbes in survival against different environmental conditions. EPS released by microbial cells provides defense against harsh conditions of starvation, pH, and temperature. Due to its structure and efficient role in nature EPS can be utilize in the field of medicine, environment, food industry, cosmetic industry and agriculture.

INTRODUCTION

Microorganisms resembles natural polymers that are tightly associated with cell surface or they may be liberated into outer environment as exopolysaccharides substance(EPS) having wide variety in structural and functional aspects. Due to increasing demand of natural polymers, microorganisms are widely used for production of biopolymers. Certain carbohydrates are produced by microorganisms as extracellular polysaccharide having wide applications in food industry, pharmaceutical industry and environmental pollutant removal. These polysaccharides are produced by microorganisms as attached capsule with

bacterial cell wall or liberated into the medium as ropy polysaccharide or mucus (Behareet *et al.*, 2009; Broadbent *et al.*, 2003; Yang *et al.*, 2018). Exopolysaccharides are biosynthetic organic polymers secreted outside the cell responsible for binding of microbial cell and particulate material known as cohesion and when binds with substratum known as adhesion (Salama *et al.*, 2015). During environmental stress condition, certain microorganisms produce extracellular polysaccharide as a self defense which not only protects them from toxic substances and dewatering but also serves as source of carbon and energy (Patel *et al.*, 2014; Gupta & Diwan, 2017). EPS also plays important role in biofilm formation and in host pathogen interaction

(Schmid, 2018). These biopolymers resemble exemplary physiological, rheological and physio-chemical properties which makes them suitable for various clinical, industrial and environmental applications. Besides polysaccharide backbone, EPS possesses multiple structural diversifications by altering various side chains, functional groups, non-carbohydrate substitution as well as diverse bonding and linkages results in generation of different homopolysaccharides as well as heteropolysaccharide. EPS present in peripheral surface of microorganisms can defend them against infiltration of toxic metal ions. Sequestration of metal ions depends on structural and compositional makeup of EPS and due negative charge of EPS, which combines with positively charged heavy metal resulting in biosorption of heavy metals. Due to this property of EPS from last few years has been widely used in reduction of heavy metal contamination. (Gupta and Diwan, 2017).

COMPOSITION AND TYPES OF EPS

Biosynthetic polymer EPS is majorly composed of carbohydrates. Second most important component of EPS is protein which accounts for 10-60%(W/W). In matrix of EPS other molecules such as lipids, nucleic acids, uronic acids and some other inorganic materials are also found which together with carbohydrate & protein help in adhesion, aggregation, adsorption, storage and protection of microbial cell (Chenet *et al.*, 2021; Salama *et al.*, 2015). Based on repeating units of monosaccharides, EPS can be classified as homopolysaccharides and heteropolysaccharides. Homopolysaccharides consist of single type of monosaccharides synthesized by anchored or secreted transglycosylases which are able to catalyze transfer of corresponding glycosyl moiety. Dextran, levan, mutan are examples of homopolysaccharides produced by *Lactobacillus* sp., *Streptococcus* sp., *Leuconostoc dextranicum* respectively. Heteropoly-

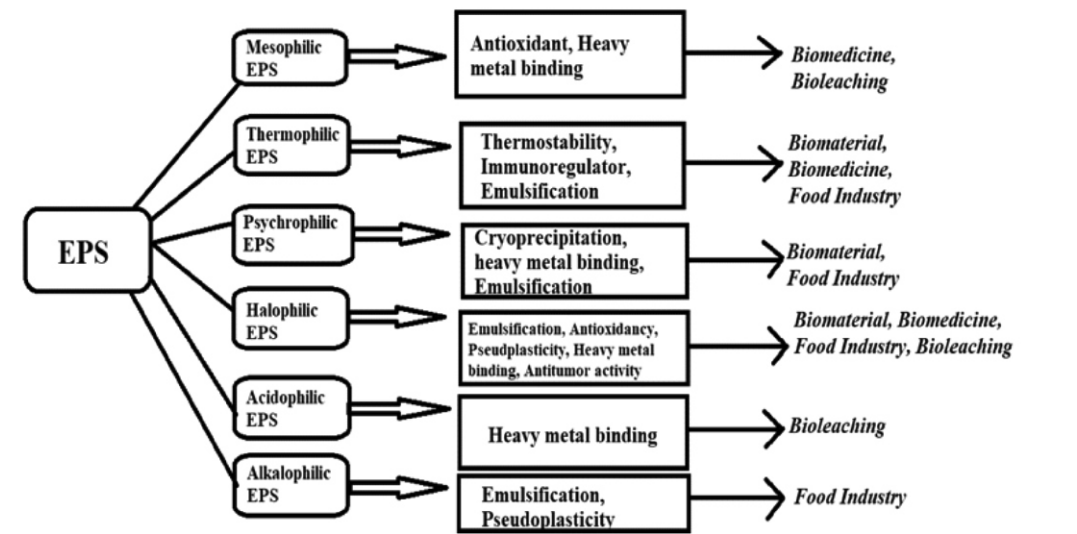


Figure 2.1: EPS secreted by different types of microorganisms and its function (Wange *t al.*, 2019)

saccharides are synthesized by repeating unit of branched and unbranched chain of different monosaccharide units majorly of glucose, galactose and minorly of rhamnose, mannose, N-acetylglucosamine, N-cetylglucosamine, glucuronic acid etc. Unlike homopolysaccharides, heteropolysaccharides are synthesized intracellularly at cytoplasmic membrane catalyzed by glycosyl transferases which transfer sugar nucleotide from activated donor molecules to specific acceptor molecules, thereby forming a glycosidic bond (Behare *et al.*, 2009; Nadzir *et al.*, 2021; Broadbent *et al.*, 2003).

BIOSYNTHESIS OF EPS

Exopolysaccharides are carbohydrate polymers that help organism to withstand extreme conditions. As it resists extreme environment, it offers novel microbial diversity which produces novel types of EPS (Satpute *et al.*, 2010). Biosynthesis of EPS occurs when various activated sugar monomers, derivatives of sugars such as sugar alcohol or aminated sugars make complex near the cytoplasmic membrane of cell resulting in formation of homopolysaccharides and heteropolysaccharides (Nichols *et al.*, 2005). Depending upon physiological condition of microbial cell, EPS can be synthesized by four general mechanisms such as Wzx/Wzy-Dependent Pathway, ABC Transporter-dependent pathway, Synthase-Dependent pathway & Extracellular Biosynthesis by Sucrase. Homopolysaccharides are generally synthesized by synthase dependent pathway and extracellular biosynthesis by

sucrose protein whereas heteropolysaccharides are synthesized by Wzx/Wzy-dependent pathway and ABC transporter-dependent pathway (Nadzir *et al.*, 2021).

3.1 Wzx/Wzy-Dependent Pathway:

The Wzx/Wzy-Dependent Pathway is a major pathway for EPS synthesis which consists of three stages: (i). synthesis of nucleotide sugars, (ii) assembly of repeating units, and (iii) polymerization and export. In first stage repeating units are constructed through appropriate transfer of monosaccharide units towards undecaprenyl phosphate (Und-P) anchored at the cytosolic side of the inner membrane. In second stage, glycosyl transferases (GTs) link more sugar units followed by its translocation across cytoplasmic membrane by Wzx flippase encoded by *wzx* gene. Side chain and other functional groups such as acetate, glycerol, pyruvate, succinate, sulfate will be incorporated before final polymerization event takes place, but at which stage it incorporates is still unclear. In third stage, translocated polysaccharide chain undergoes various modifications such as acetylation, methylation *etc.* and polymerized to polysaccharides by Wzy protein (Vuyst *et al.*, 2001; Nichols *et al.*, 2005; Wang *et al.*, 2019). Wzx gene encodes flippase protein which transfers repeating units in H⁺ dependent antiporter mechanism. Different Wzx proteins lack similarity at sequence level indicated by various numbers of transmembrane sequences which conclude diverse type of *wzx* proteins exist. Wzx proteins are major target proteins for polysaccharide production. During the final construction of EPS, depolymerization occurs. Depending on the proteins involved

in depolymerization process, name of the pathway given as Wzx/Wzy pathway (Schmid, 2018).

3.2 ABC Transporter Dependent Pathway:

ABC Transporter dependent pathway is mainly involved in synthesis of capsular polysaccharides. Name ABC transport pathway comes from ATP Binding cassette dependent pathway in which active sugar units are transported to Und-P molecule in inner membrane of cell to form Und-PP sugar molecule by action of GTs and polymerizing enzymes located at cytoplasmic side of inner membrane. Synthesized polysaccharides are then translocated cross inner membrane by tripartite efflux pump complex (Nadzir *et al.*, 2021). Two strategies for ABC transporter dependent pathway have been identified. One is observed in *Klebsiella pneumoniae* O2a in which competition occurs between ABC transporter and assembly chain, which finalizes length of glycan chain. Second observed in *Escherichia coli* O9a in which uncoupled glycan synthesized and exported by non-reducing terminal modification of Und-P linked polymers that results in termination of chain extension. Glycan length dictated by terminator and ABC transporter recognized export signals resulting in accumulation of polysaccharides outside the inner membrane of cell (Schmid *et al.*, 2015).

3.3 Synthase-Dependent Pathway:

In contrast to ABC/Wzx-Wzy-dependent pathway, synthase based pathway synthesizes homopolysaccharides which made up of single type of monosaccharides such as glucose, maltose, galactose, *etc.* The major examples of homopolysaccharides are bacterial cellulose or

alginate. In biofilm forming bacteria, cellulose is a major polymer made up of linear beta-(1,4)-glucan. Glucose monomers are activated by assembly of cytosolic UDP followed by specific mechanism of cellulase synthase complex which includes conserved catalytic subunit BcsA (bacterial cellulose synthase subunit A) belonging to GT2 family and performed processive mechanism of polymerization after which polymers transported to outside the cell. In *Rhodobactersphaeroides*, BcsAB complex show GT activity and PILZ domain to interact with allosteric activator c-diGMP. Cellulose synthesis pathway is a highly variable and species specific (Wang *et al.*, 2019; Vuyst *et al.*, 2001). Alginate biosynthesis occurs via polymerization of GDP-mannuronic acid monomer by GT polymerase Alg8 and Alg44 which also act in polymerization. Complex of alginate biosynthesis consist of epimerases (AlgG), lyases (AlgL) and acetylases (AlgIJFX). In periplasmic complex, several extracellular alginate polymerases are present resulting in final modification of polymer characteristics (Krasteva *et al.*, 2017).

3.4 Extracellular Biosynthesis by Sucrase:

In this pathway, extracellular sucrase cleaves sucrose to monomeric unit outside cellular outer membrane. Polymerization of monomeric unit occurs by GTs forming dextran and levan with distinct branches. Molecular effect and branching effect depends on reaction condition and engineered sucrose variant. Synthesized EPSs are then realized into extracellular environment (Rehm, 2010; Patel *et al.*, 2012).

EPS PRODUCTION

Major component of EPS is carbohydrate. Hence it is required for EPS production in culture medium. During metabolism, intracellular accumulation of polysaccharides secretes exopolysaccharide into culture medium. Microbes can produce EPS by solid state and submerged fermentation. After EPS production cultural biomass can be separated from fermentation broth followed by EPS extraction using solvents like ethanol, isopropanol, chloroform, acetone etc. In final step of EPS production, purification and drying is carried out to get pure EPS (Tabernero and Cardea, 2020; Chen *et al.*, 2021).

4.1 EPS producing microorganisms

Certain bacteria, fungi, algae can able to secrete extra polymeric substance in outer environment. Presence of various environmental factors, such as medium composition, temperature and pH greatly affects production and composition of EPS. Different environmental condition produces different kind of EPS based on specific enzymes secreted by microorganisms. These enzymes are responsible for polymerization of EPS (Behare *et al.*, 2009; Nouha *et al.*, 2017). Following tables provides idea regarding EPS produced by specific kind of microorganism.

Table 4.1: Various types of EPS produced by different types of microorganisms

Type of EPS	Micoorganisms	Reference
Dextran	<i>Leuconostocmesenteroides</i>	Broadbent <i>et al.</i> , 2003
Glucan	<i>Streptococcus mutans</i>	Broadbent <i>et al.</i> , 2003
Fructan	<i>Streptococcus salivarius</i>	Behare <i>et al.</i> , 2009
Xanthan	<i>Xanthomonas campestris</i>	Behare <i>et al.</i> , 2009
Acetan	<i>Acetobacter xylinum</i>	Behare <i>et al.</i> , 2009
Gellan	<i>Sphingomonaspaucimovilis</i>	Behare <i>et al.</i> , 2009
Alginate	<i>Pseudomonas aeruginosa</i> , <i>Azotobacter vinelandii</i>	Gupta and Diwan, 2017
Hyaluronan	<i>Pseudomonas aeruginosa</i> , <i>Pasteurella multocida</i>	Gupta and Diwan, 2017
Galactopol	<i>Pseudomonas oleovorans</i>	Gupta and Diwan, 2017
Fucopol	<i>Enterobacter sp.</i>	Gupta and Diwan, 2017
Kefiran	<i>Lactobacillus hilgardii</i> , <i>Lactobacillus rhamnosus</i> , <i>Lactobacillus kefir</i>	Nadzir <i>et al.</i> , 2021

APPLICATIONS OF EPS

Various properties of EPS such as pseudoplasticity and high viscosity make it resistant to various extremities of temperature, pH and salinity. Due to these properties EPS act as thickening, coagulating, adhesion, stabilizing and gelling agent used in various industrial and

environmental applications. Novel EPS contains high uronic acid and sulfate contents in its structural composition that increases its use in biotechnology. (Satpute *et al.*, 2010).

5.1 In food industry:

EPS improves physical property of fermented products by acting as food stabilizer, viscosifier, texturizers, emulsifier

or gelling agent. Heteropolysaccharides produced from *Lactobacillus* sp. used as food additives, function in starter culture results in safe and natural product. EPS improves rheological property, texture, stability of food material and have higher water retention power which increases demand of novel EPS producing microorganisms. Various types of EPS are used in food industry for example, incorporation of dextran in dough results in improvement of texture. Addition of softening agent glutan in dough ultimately increases specific volume and shelflife of bakery product. EPS produced by *Lactobacillus* sp. improves functioning of human gastrointestinal track and contribute to food fibre which increases it's use in food industry (Vuyst *et al.*, 2001; Behare *et al.*, 2009). Pullulan produced by *Agrobacterium* sp. have attractive rheological, thermal gelling and thickening property which increases its use in food and pharmaceutical industry. Xanthan produced by *Xanthomonas campestris*, an anionic heteroglycan which even at low concentration have pseudoplasticity and highly viscous due to which it is used as stabilizer, thickening, and suspending agent in food industry. (Seviour *et al.*, 2011; Li *et al.*, 2014).

5.2 Biomedical Applications:

Increases in commercialization have some adverse effects on human life. It leads to increase in certain metabolic diseases such as cancer, heart blockage, increase in blood glucose *etc.* Microbially synthesized biomolecules such as EPS provides certain health benefits against metabolic diseases. EPS produced by *Lactobacillus* sp. exhibits health benefits to humans by lowering cholesterol, anticarcinogenic,

immunomodulating activity and antitumoral effect. Anticoagulant activity of EPS produced by *Alteromonas infernus* have similar effect like heparin, therefore used in a treatment of lipemia and arteriosclerosis. Extremophilic EPS induces apoptosis in tumor cells by coupling with specific surface receptors with less cytotoxicity, hence used as anticancer agent. In vitro study of EPS produced by thermophilic bacteria *Geobacillus* sp. TS3-9 was found to inhibit proliferation of hepatoma carcinoma cell in dose dependent manner. Addition of non-carbohydrate functional group may increase biological activity as compared to naive EPS. Modified levan produced from halophilic organism show biocompatibility to non-tumor cells and anticancer activity in-vitro (Wanget *al.*, 2019; Jouault *et al.*, 2001). Sulfated EPS such as dextran sulfate has antiviral activity against enveloped viruses by interfering with absorption and penetration of virus into host cell, and also inhibits viral reverse transcriptase enzyme. Immunoregulatory effect of EPS from *Bacillus licheniformis* strain B3-15, induce expression of proinflammatory cytokines such as IL-4, IL-10, IL-18 and TNF- α resulting in improvement of immune surveillance of PBMC (peripheral blood mononuclear cells) against viral infection. Due to higher activity against virus infection, antitumor activity and anticoagulant activity, use of EPS in biomedical has been increased so far (Arena *et al.*, 2009). EPS also acts as drug carrier, which enhances its use in drug delivery system. Dextran used as coating material in medical devices and surgical sealant due to its biocompatibility and adhesive property respectively (Nadzir *et al.*, 2021).

5.3 Agricultural Applications:

EPS secreted by soil *Rhizobium* sp. helps in development of symbiotic relationship with its host by various mechanisms such as root hair attachment, host specificity in nodulation, perception of nodule oncogenesis, avoid plant defense response, biofilm formation, structural recognition, control of the plant defense systems resulting in attachment of *Rhizobium* sp. on plant surface. In this symbiotic relationships plants in return get nutrients from *Rhizobium* sp. which requires for plant to grow and due to this mode of action of EPS, it is highly recommended in agriculture (Ghose and Maiti, 2016). Absorption of EPS on soil leads to precipitation and binding of soil particles results in modification of soil porosity which favors water and nutrient extraction by plants from drying soils. These properties of EPS enhance its use in agriculture (Pandit *et al.*, 2020). Due to good biocompatibility, biodegradability, and mechanical strength EPS act as good biomaterial in biomedical engineering. Mixture of gellan with hydroxyapatite and with Hyaluronic acid used in bone tissue engineering and growth, and attachment of chondrocyte in tissues respectively. Gellan along with hydroxyapatite and Hyaluronic acid used for bone tissue engineering and growth and attachment of chondrocyte respectively (Nadzir *et al.*, 2021).

5.4 As an antioxidant

Excessive production of reactive oxygen species (ROS) and oxygen-derived free radicals may damage cells and tissues leading to metabolic diseases. Oxidative stress plays crucial role in occurrence of mutation in DNA, protein through generation of free radicals. Reportedly free

radical cause severe diseases such as atherosclerosis, diabetes, several types of cancers, neurodegenerative disorders, disorders and alteration in the immune system. Antioxidants are agents which resolves oxidative stress by acting as protective agents, therapeutic agents, functional foods additives in the preservation of nutritive products. EPS act as antioxidant can neutralize effect of free radical by various mechanisms. Antioxidant molecule undergoes self oxidation leading to elimination of unpaired electrons, make it less reactive resulting in elimination of dangerous diseases. Thus, EPS shown to play crucial role by acting natural antioxidant (Tabernero & Cardea S., 2020; Li *et al.*, 2014).

5.5 In oil recovery

Microbial EPS act as emulsifying agent by forming emulsion. Emulsion is high molecular weight heteropolysaccharide protein containing repeating trisaccharide of N-acetyl -D-galactosamine, N-acetyl galactosamineuronic acid and an unidentified N-acetyl amino sugar used for cleaning of oil contaminated vessels, oil spills and microbial enhanced oil recovery (Satpute *et al.*, 2010). Certain EPS such as, scleroglucan increases the viscosity, hence the hydraulic pressure of sea water or brine is used to extract oil. Scleroglucan lubricates the drill and controls the backpressures created during drilling. In addition, scleroglucan exhibited high thermostability in drilling fluids when it was associated with polyglycols through intermolecular interactions resulting in oil recovery (Yadav *et al.*, 2014).

5.6 Heavy metal removal

Heavy metals are compounds which are having slightly higher density than water.

Trace amount of heavy metals are required for normal physiological function of living cell but same heavy metal in higher concentration harm structure of DNA and protein, disturbance in ion channels living tissues instigates bronchitis, cancer, dermatitis, hypo 1 kerotosis, livercirrohosis, mental disturbance, ulcer etc in individuals. Modern industrialization increases heavy metal discharge in environment resulting in heavy metal pollution which became major concern nowadays. Microbes and microbial component such as EPS act as good

absorbing agent which increases its use in heavy metal pollution. Earlier it was thought that heavy metal have inhibitory effect on microbial cell but instead microbes develop certain metal resistant mechanism hence, it is used in heavy metal removal (Gupta & Diwan, 2017; Naveed *et al.*, 2019). Various mechanism are employed by microbial EPS to remove heavy metal that including biosorption, biomineralisation, biotransformation, sequestration etc. as shown in following figure along with its mode of action.

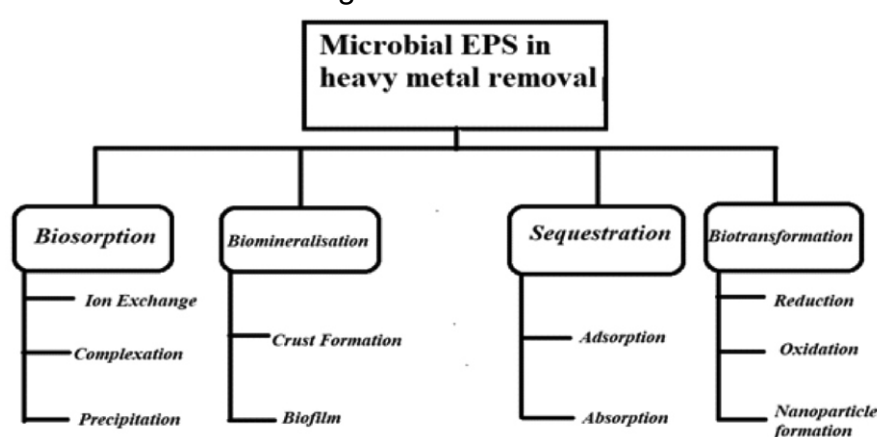


Figure 5.1: Mechanism of heavy metal removal by microbial EPS (Naveed *et al.*, 2019)

Conventional methods for heavy metal removal are cost effective and generate toxic by-products which limit its use in heavy metal removal, instead microbial EPS use as an alternative. Microbes develop protecting mechanism against infiltration of heavy metals by actively or passively interacting with them. EPS have certain properties including physisorption (*i.e.*, physical contact and electrostatic attraction), ion-exchange function, the binding sites of EPS, and chemical precipitation makes them suitable as biosorbant (Feng *et al.*, 2021).

5.6 Cosmetic industry:

Gelling and antioxidant property of EPS enhances its use in cosmetic industries. Certain microbial EPS such as glucan is used in skin care preparation, creams and protective lotions. Antioxidant property of EPS increases its use in commercial application of skins that protects against ultraviolet rays and damage caused by free radicals, therefore used in anti-aging creams. Hyaluronan is used as ingredient for enhancing collagen biosynthesis in skin cells (Sharmila *et al.*, 2014)

CONCLUSION

New microbiological research focuses on use of microbial product in various industries and environmental pollution control that shows remarkable effects on human life. One of major microbial biomolecules is exopolysaccharide substance (EPS) secreted by various kinds of microorganisms including bacteria, fungi, algae etc. EPS can be produced in laboratory by submerge fermentation as well as solid state fermentation. Different organism follows different pathways for secretion of EPS that will decide structural and functional composition of EPS. Microbes employed major 4 different biosynthetic pathways in making of EPS that enhances complexity in EPS structure. EPS have wide range of applications in field of food industry, cosmetic industry, biomedical, environmental and agricultural applications. Several techniques are employed to remove heavy metal contamination from environment, but they reside certain disadvantages. Instead EPS is used as it contains negative charge that makes them good sorbent agent to absorb heavy metals resulting in eco-friendly biosorption in environment. Due to gelling property of EPS, its use in cosmetic industry is increased. Reducing property of EPS makes it suitable as an antioxidant to remove oxidative species. Anti-inflammatory, anticancer, immunomodulator activity of EPS increases its use in field of medical research. EPS helps in attachment of microbes on surface and increases secretion and attachment to plant surface that enhances its application as plant growth promoting substance in agricultural field. Hence EPS become

wonderful molecule for new findings and applications in field of biological sciences.

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