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Review article

# Microbial polysaccharides and their classification: a brief study on fundamental biochemical properties

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## ABSTRACT

Microbial polysaccharides are typical poly-carbohydrates those are abundant in many types of microorganisms. Because of their polycarbohydrate nature microbial polysaccharides consist of long chain polymeric carbohydrates composed of monosaccharide units joined by glycosidic linkages. These microbial polysaccharides have high molecular weight and react with water by using amylase enzymes as catalyst. However, microbial polysaccharides are water soluble biopolymers but their rheological characteristics make them act like binders, coagulants, emulsifiers, biofilms, hydrogels, lubricants, stabilizers and thickening or suspension molecules.



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Microbial polysaccharides are derived from different microorganisms like bacteria, yeast, fungi and algae. Some of the examples of microbial polysaccharides are Gellan gum, Xanthan gum, Scleroglucan, Pullulan, Hyaluronic acid, Fucogel, Cholanic acid, Alginic acid, Bacterial cellulose, Dextran, Curdlan, Levan polysaccharide etc. Microbial polysaccharides are non-toxic modules with diverse physical properties such as, viscosity, mechanical strength, elasticity and biocompatibility. Bacterial polysaccharides are obtained from several species of bacteria including *Alcaligenes sp, Agrobacterium sp, Rhizobium sp, Bacillus sp, Cellulomonas sp, Bifidobacterium sp, Streptococcus sp, Klebsiella sp, Enterococcus sp, Staphylococcus sp*, etc.

Microbial polysaccharides are classified into three main categories: capsular polysaccharides (CPSs), lipopolysaccharides (LPSs) and exopolysaccharides (EPSs). According to morphological localization microbial polysaccharides can be divided as intracellular and extracellular polysaccharides. On the basis of structural formation, polysaccharides which contains only one kind of monosaccharide unit are called homopolysaccharides. Similarly, polysaccharides consisting two or more types of mono-saccharides are called hetero-polysaccharides. Such as, cellulose, dextran and pullulan are homo-polysaccharides and xanthan and hyaluronic acid are known as hetero-polysaccharides.

Keywords: Microbial Exopolysaccharides (EPS), Capsular polysaccharides (CPS), Fucose polysaccharides (FPS), Lipopolysaccharides (LPS).

## **INTRODUCTION**

Despite having greater manufacturing costs than conventional polysaccharides like cornflour and cellulose-derived products, which dominate the market, microbial polysaccharides have found extensive use in the chemical, culinary, and pharmaceutical sectors. Numerous bacteria make water-soluble biopolymers called microbial polysaccharides <sup>[1, 2]</sup>. The process of making molecular sieves uses dextrans. *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* are the main producers of them. Extracellular phosphorylated mannans produced by *Hansenula, Pichia,* and *Pachysolen* species are immune to microbial assaults. In Japan, pullulan, which is made by *Azotobacter pullulans*, is utilised as a filmwrap material for food packaging. *Acetobacter xylinum* uses molasses as a carbon source to manufacture bacterial cellulose (BC).

The pathogenicity of bacteria and variables that promote virulence are mostly linked to CPSs. The outer membrane contains LPSs, and a number of bacteria create a virulence factor. EPSs are high-molecular-weight polymers and extracellular polysaccharides. The primary functions of microbial (prokaryotic and eukaryotic) EPSs are cell adhesion and environmental protection. EPSs also act as energy and carbon stores. Monosaccharides and non-carbohydrate substituents such acetate, phosphate, pyruvate, and succinate often make up EPSs <sup>[3, 4]</sup>. They fall into one of two categories: homopolysaccharides or heteropolysaccharides. Heteropolysaccharides, like xanthan and hyaluronic acid, include two or more types of monosaccharide units. whereas homopolysaccharides, like cellulose, dextran, and pullulan, only have one kind. Because of their ability to create viscous pseudoplastic liquids, EPSs produced by microbes have unparalleled rheological qualities. They may be used as an immobilisation matrix, a microcarrier in tissue cultures, a stabiliser, a viscosifier, an emulsifier, and a moisture retainer in the food sector.

Extracellular polymeric compounds called microbial polysaccharides, which can be soluble or insoluble and are produced

by bacteria, yeast, algae, fungus, and other microorganisms, are valued for their many uses. Microorganisms produce EPS as metabolic byproducts. They are high molecular weight substances made up of carbohydrates (sugar residues) and non-carbohydrate substituents including acetate, glycerol, pyruvate, sulphate, carboxylate, succinate, and phosphates, as well as proteins, DNA, and phospholipids <sup>[5, 6]</sup>. The Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) define probiotics as "live microorganisms, which when consumed in adequate amounts confer a health benefit on the host." Because it is Generally Regarded as Safe (GRAS) and used in many applications, EPS generated by probiotic lactic acid bacteria (LAB) is one of the microbial polysaccharides that has been selected.

Microbial polysaccharides are used as bio-flocculants, bioabsorbents, and drug delivery agents in the food, cosmetic, and pharmaceutical sectors because of their intriguing and alluring properties. Polysaccharides are utilised in the environmentally friendly manufacturing of silver nanoparticles since they are superior to synthetic polymers. The interaction of metal ions with EPS's hydroxyl groups significantly affects a nanoparticle's size and form. EPS are widely found in natural sources and are biodegradable, non-toxic, and biocompatible. Lactobacillus, Lactococcus, Bifidobacterium, Leuconostoc, Pediococcus, Streptococcus, Enterococcus, and Weissella sp. are the most common LABs that produce EPS. Probiotic bacteria are among the many microorganisms that humans eat; they can live in the presence of bile, low pH, gastric juices, and colonise the gastrointestinal tract's epithelial layer [7, 8].

The natural microbial flora's antagonistic action against pathogenic germs is diminished when antibiotics are used. Consequently, probiotic EPS are used as supplements to treat human conditions such obesity, autoimmune illnesses, colon cancer, gastric ulcers, inflammatory bowel diseases, and cardiovascular diseases. Pharmacological uses of EPS, such as anticoagulants, anti-allergic, antithrombotic, immunomodulatory, blood cholesterol-lowering, and

Nutraceuticals, have been thoroughly investigated. EPS possesses enormous swelling and gelation capabilities, as well as a significant capacity for water binding and retention. On the surfaces of bacterial cells, EPS such as xanthan, sphingan, alginate, and cellulose encourage the development of biofilms as a protecting barrier <sup>[9]</sup>.

According to reports, the EPS generated by Lactobacillus acidophilus, Lactobacillus gasseri, Lactobacillus plantarum, and Lactobacillus rhamnosus that were isolated from different sources have antioxidant and anticancer properties. By blocking the aglucosidase and α-amylase enzymes in vitro, recent research on EPS generated by Lactobacillus plantarum C70 and L. plantarum RJF4 of various origins demonstrated its anti-diabetic efficacy. Leuconostoc citreum, Lactobacillus johnsonii 142, Bifidobacterium sp., and Lactobacillus plantarum all exhibit strong immunomodulatory activity, and their extracellular polymeric substances (EPS) can trigger the innate immune response. EPS generated by Bifidobacterium sp. was found to lower cholesterol levels in diet induced obese mice. The origins, structure, categorisation, physical characteristics, biosynthesis and diverse biological potential of extracellular routes. polysaccharides (EPS) generated by probiotic bacteria are the main topics of this study [10].

#### **Polysaccharide Types and Chemistry**

Microbial polysaccharides are mainly linear molecules with side chains of various lengths and complexity connected at regular intervals. They can be either ionic or nonionic. The majority of microbial polysaccharides are linear hetero-polysaccharides, which are repeating units made up of three to seven distinct monosaccharides grouped in groups of ten or less. The monosaccharides might be uronic acids, pentoses, hexoses, or amino sugars, among others. The chemistry and functions of many polysaccharide types derived from microbiological sources have been reviewed <sup>[11]</sup>.

#### **Chemical Structure**

D-glucose, D-galactose, and D-mannose; L-fucose and Lrhamnose; and N-acetylhexosamines, N-acetyl-D-glucosamine and Nacetyl-D-galactosamine, are the most prevalent monosaccharides found in microbial polysaccharides. Certain microbial polysaccharides may include uronic acids, such as D-glucuronic and D-galacturonic. Microbial polysaccharides frequently contain acyl substituents, such as ketal-linked pyruvate or ester-linked acetate. Another crucial element in microbial polysaccharides is ketalpyruvate. D-glucose, Dgalactose, and D-mannose; L-fucose and L-rhamnose; and Nacetylhexosamines, N-acetyl-D-glucosamine and N-acetyl-Dgalactosamine, are the most prevalent monosaccharides found in microbial polysaccharides <sup>[12]</sup>.

Classification of Microbial Polysaccharides based on Structural Assimilation

1. Lipopolysaccharides of Gram-negative Bacteria

Extremely polydisperse molecular weights are present in *A. vinelandii* polymers and the molecular weight distribution may be influenced by the extracellular atginate lyase. There is a great deal of structural diversity, and the O-specific side chains dictate the bacteria's O-antigenic specificity. Our grasp of this topic was founded on research on *Salmonella sp.* LPS, which led to the identification of 3,6-dideoxyhexoses and a deeper comprehension of the nature of immunological determinants <sup>[13]</sup>.

#### a) Lipid A

Along with other biological characteristics, lipid A possesses the endotoxic qualities of LPS. Two 2-amino-2-deoxy-D-glucose residues make up the backbone of a  $\beta$ -D-(1-6)-linked disaccharide found in Salmonella sp.'s lipid A. Lipid A is connected to the core by 3-Deoxy-D-manno-octulosonic acid (KDO), which is connected to 0-3'. The remaining sites are acylated with fatty acids (C12, C14, and C16) on oxygen and 3-hydroxy-D-myristic acid [(R)-3hydroxytetradecanoic acid] on nitrogen. With the exception of some molecules having pyrophosphate connected to O-1, the structure of lipid A from an *Escherichia coli* LPS is identical. The *Vibrio cholera*e lipid A possesses a structure that is quite similar to that found in *Enterobacteriaceae*, although structural investigations of *Salmonella minnesota*'s lipid A show a slightly different structure <sup>[14]</sup>.

#### b) Core Structure

It is usually possible to cleave the glycosidic links of the KDO residues without harming other glycosidic linkages in the LPS because these linkages are more susceptible to acid hydrolysis. The core and the O-specific side chains are thus isolated from the lipid A by this process. The bacteria's so-called R forms, or rough forms, produce incomplete LPS that eventually lacks portions of the core as well as the O-specific side chains. The Ra core of Salmonella LPS is the most thoroughly researched structure. Hep is an acronym for L-glycero-O-manno-heptose in this structure. The Anglo-American regulations state that this residue is L-glycero-a-O-manno-heptopyranosyl. The incomplete E. coli B core is thought to have several structural characteristics with the heptose and KDO areas that have not yet been identified for the Ra core <sup>[16]</sup>.

#### c) O-Antigen specific Bacterial Polysaccharides

Pyruvic acid is acetalically connected to the 0-4 and 0-6 of the  $\beta$ -D-galactopyranosyl residue in the *Shigelladysenteriae* type 9 Oantigen. While they are ubiquitous in other bacterial polysaccharides, such acetals are rare in LPS. NMR spectroscopy can be used to determine the acetalic carbon atom's absolute configuration. It is always R when connected to galactose and S when connected to the other sugars in the situations that were examined. Proteus species' Oantigens have a number of peculiar structural characteristics. There have been reports of P. mirabilis strain D 52 O-antigen. A number of *Y. pseudotuberculosis* O-antigens have been suggested to have

in other Enterobacteriaceae LPS. O-antigens from various Pseudomonas aeruginosa strains have been studied <sup>[17]</sup>.

<b>Figure 1:</b> Microbial Polysaccharides and their Structural and Chemical Properties <sup>[15]</sup>	5]
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Туре	Origin	Monomers	Charge	Characteristics of chemical structure	MW	Properties
Bacteria						
Alginate	Pseudomonas aeruginosa, Azotobacter vinelandii	Guluronic acid, Mannuronic acid	Anionic	Blocks of $\beta$ -1,4-linked d-mannuronic residues, blocks of $\alpha$ -1,4-linked l-guluronic acid residues, and blocks with these uronic acids in random or alternating order	1000 - 1400 KDa	Hydrocolloid, Gelling capacity, Film forming
Curdlan	Alcaligenes faecali, Cellulomonas flauigena	Glucose	Neutral	β-1,3-D-glucan	5x10 <sup>4</sup> - 2x10 <sup>6</sup>	Gel forming ability Water insolubility Edible and non-toxic Biological activity
Dextran	Leuconostoc mesenteroides	Glucose	Neutral	$\alpha$ -d-glucan linked by $\alpha$ -1,6-glycosidic bonds; some 1,2-,1,3-, or 1,4-bonds are also present in some dextrans	10 <sup>6</sup> - 10 <sup>8</sup>	Non-ionic, Good stability, Newtonian fluid behaviour
Gellan	Sphingomonas paucimobilis	Glucose, Rhamnose, Glucuronic acid	Anionic	Partially O-acetylated polymer of d-glucose-1,4- $\beta$ -d-glucuronic acid-1,4- $\beta$ -d-glucose-1,4- $\beta$ -l-rhamnose tetrasacharide units connected by $\alpha$ -1,3-glycosidic bonds	5 x 10 <sup>6</sup>	Hydrocolloid-stable over wide pH range Gelling capacity Thermoreversible gels
Hyaluronan	Pseudomonas aeruginosa, Pasteurella multocida	Glucuronic acid, Acetylglucosmine	Anionic	Repeating units of $\beta$ -1,4-linked disaccharides of $\beta$ -d-N- Acetylglucosmine- $\beta$ -1,3- d-Glucuronic acid	5000Da to 20MDa	Biological activity Highly hydrophilic Biocompattible
Levan	Bacillus subtilis, Zymomonas mobili, Halomonas sp.	Fructose	Neutral	β-2,6-D-fructan	<108	Low viscosity, High water solubility, Biological activity, (anti-tumour & anti- inflammatory activities), Adhesive strength, Film- forming capacity
Xanthan	Xanthomonas campestris	Glucose, Mannose Glucuronic, Acid, Aceate, Pyruvate	Anionic	$\beta$ -1,4-d-glucan with $\beta$ -d-mannose- 1,4- $\beta$ -d-glucuronic acid-1,2- $\alpha$ -d- mannose side chain. Approximately 50 % of terminal mannose residues are pyruvated and the internal mannose residue is acetylated at C-6	2.0-50 x 10 <sup>6</sup>	Hydrocolloid, -High viscosity yield at low shear rates even at low concentrations, Stability ver wide temperature, pH and salt concentrations ranges
Pullulan	Aureobasidium pullulans	Glucose	Neutral	$\alpha$ -1,6-linked $\alpha$ -1,4-d-triglucoside maltotriose units	5-900 x 10 <sup>3</sup>	

## 2. Extracellular Polysaccharides of Gram-negative Bacteria a) M-Antigen Specific Bacterial Polysaccharides

All *Enterobacteriaceae* create the mucous polysaccharide known as the M-antigen when cultivated in particular conditions, such as low temperatures and high concentrations of salt. The group acetalically connected to either 0-3 or 0-4 or to 0-4 and 0-6 of the terminal  $\beta$ -D-galacto pyranosyl group may differ, but the carbohydrate backbone (72a) is the same for M-antigens generated by different strains. It may be formaldehyde, acetaldehyde, or pyruvic acid <sup>[18]</sup>.

## b) V-1 Antigen Specific Bacterial Polysaccharides

The Vi-antigen, so named because it was mistakenly thought to be associated with the virulence of the bacteria, is another antigen that is not type specific. This homopolysaccharide, which is made up of residues of 2-acetamido-2-deoxy-a-D-galactopyranosyluronic acid, has been detected in strains of *Salmonella, Citrobacter*, and *E. coli*. Vi-Antigens at 0–3 without the acetyl group also have been found <sup>[19]</sup>.

## c) K-Antigen Specific Bacterial Polysaccharides

Several *E. coli* strains elaborate the Kl antigen, also known as colominic acid, which can exist with or without O-acetyl groups. Neuraminic acid residues of the structurally similar K92 antigen are connected alternately by 0–8 and 0-9. Both the K12 and K82 antigens include O-acetyl groups and have the same carbohydrate backbone. *Klebsiella* K20 and *E. Coli* K30 have the same antigen. Lastly, the *Haemophilus influenzae* type b capsular antigen and the K100 antigen share structural similarities <sup>[20]</sup>.

## d) B-1 Antigen Specific Bacterial Polysaccharides

Structure 59, which has a terminal paratofuranosyl group, is the repeating unit in the serotype I B antigen <sup>[21]</sup>.

## e) A-1 Antigen Specific Bacterial Polysaccharides

The paratosyl group is connected to 0-3 of a 6-deoxy-a-Dmanno-heptopyranosyl residue in the serotype IA antigen, which most likely has the same terminal <sup>[22]</sup>.

# 3. Polysaccharides of Gram-positive Bacteria

## a) Micrococci

Various species of Micrococcus that lack lipoteichoic acids generate LPS in their cell walls. The polysaccharide component is an  $\alpha$ -D-mannan, which has 50-60 sugar residues connected in 2:2:1 ratios by 0-2, 0-3, and 0-6. A glycerol residue, which is also esterified with fatty acids, is glycosidically connected to the polysaccharide after being acylated with succinic acid <sup>[23]</sup>.

## b) Streptococci

Based on group and type specificities, the genus *Streptococcus* has been categorised serologically. The carbohydrates that make up the group-specific cell wall antigens are frequently teichoic acid-type carbohydrates. Proteins and carbohydrates make up some of the type-specific antigens. *Streptococcus pneumoniae* (*Diplococcus pneumoniae*) antigens have been thoroughly investigated, and the structures of a number of them have been identified. But *S. pyogenes*, among other species. The type 1 *Streptococcus pneumonia* and *Shigella sonnei* LPS and the C substance both contain the identical 2-acetamido-4-amino-2, 4, 6-trideoxy-D-galactose as the antigen <sup>[24]</sup>.

## c) Bacilli

*Bacillus licheniformis* cell walls contain bacillosamine, also known as 2, 4-diamino-2, 4, 6-trideoxy-D-glucose, as a 4-7V-acetyl derivative. The cell walls of *Bacillus licheniformis* have yielded a teichuronic acid <sup>[25]</sup>.

## d) Coryne bacteria

Tetrasaccharide repeating units make up the extracellular polysaccharide of *Corynebacterium insidiosum*. Composed of trisaccharide repeating units, *Arthrobacter viscosus* elaborates a viscous extracellular polysaccharide of possible economic importance. Additionally, it has O-acetyl groups, which make up 50% of the total acetylation theoretical value <sup>[26]</sup>. Microbial polysaccharides, in addition to plant extracts and functional hydrocolloids derived from animals, show a lot of promise as renewable resources. However, the physiochemical characteristics of microbial polysaccharides might not be sufficiently accessible in hydrocolloid form. To improve their technical and functional qualities for use in food, medicine, and pharmaceuticals, these polysaccharides should be modified. Covalent bonds are created between the various polymer chains by chemical cross-linking, creating intramolecular or intermolecular connections. Cross-linking duration and agent concentration are crucial variables for chemical cross-linking; a high cross-linking agent concentration accelerates the process <sup>[28]</sup>.

## Physical Crosslinking a) Ionic Interaction

The ability of anions to cross-link polycations is essential for ionic interaction. Proranolol hydrochloride is a hydrophilic medication that may be encapsulated in gellan gum in an aqueous environment using the ionotropic gelation process. Using the same technique, diclofenac sodium-containing cross-linked Ca+2 gellan beads can affect the formation and characteristics of the beads based on the drug (e.g. polymer ratio, pH of cross-linking solution, and speed of agitation) <sup>[29]</sup>.

Additionally, this interaction strengthens the Al+3/gellan network. The ensuing coordination of the Al+3 ion with oxygen atoms of hydroxyl groups in gellan chains around the cation is caused by the favourable cation size and supramolecular structure of the tetrasaccharide unit. This discovery could prompt more research, such as examining the impact of additional trivalent ions on the hydrogel network <sup>[30]</sup>.

## b) Crystallisation

Stereocomplex-formation has been used to create a unique hydrogel system that regulates the release of medicinal proteins. Two distinct batches of lactic acid (PLLA) and D-lactic (PDLA) were linked to dextran. To create the biodegradable hydrogel shown in Figure 3, L-lactic-acid oligomer and D-lactic-acid oligomer grafted to dextran were further combined. Stereocomplex-formation crystallisation has the benefit of self-assemble linking, which eliminates the necessity for a cross-linking agent <sup>[31]</sup>.

## c) Protein-Polysaccharide Interaction

Food-related systems frequently include interactions between polysaccharides and proteins, which are essential for giving food items their gelling, stabilising, thickening, and emulsifying qualities. Co-solubility produces a stable biopolymer mixture in a diluted system where mixing entropy predominates. This is due to the steric interaction of the negatively charged micelle surface by the glycomacropeptide portion of kappa casein, also known as the "hairy layer," and the complementary interaction between the negatively charged constituents of kappa-carrageenans and positively charged constituents of kappa casein <sup>[32]</sup>.

## Modification of Microbial Polysaccharides

COOH

HO

ÇOOH

H2OH

ÓH

CH-OH

Ół

ÓН



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Figure 3: Structures of Klebsiella pneumoniae CPS Repeating Units <sup>[33]</sup> [3)- $\alpha$ -D-Galp-(1-2)-[3,4-O-(1-carboxyethylidene)]- $\alpha$ -L-Rhap-(1- $\rightarrow$ 

 $\rightarrow$ 3)- $\beta$ -L-Rhap-(1 $\rightarrow$ 4)- $\alpha$ -L-Rhap-(1 $\rightarrow$ ]<sub>n</sub>

## $K32^{a}$

$$[3)-\alpha-L-Rhap-(1\rightarrow 2)-\alpha-L-Rhap-(1\rightarrow 4)-\alpha-D-GlcpA-(1\rightarrow 3)-\alpha-D-Galp-(1\rightarrow 3)-\alpha-D-Gal$$

## $K40^{b}$

## **K60**<sup>c</sup>

$$\begin{array}{c} [3)-\alpha-L-Rhap-(1\rightarrow 3)-\beta-D-Glcp-(1\rightarrow 2)-\alpha-L-Rhap-(1\rightarrow 2)-\alpha-L-Rhap-(1\rightarrow 3)_{n}\\ &\uparrow\\ &&1\\ \beta-D-GlcAp-(1\rightarrow 3)-\alpha-L-Rhap\\ &&2\\ &\uparrow\\ &&1\\ \beta-D-Glcp\end{array}$$

# K71<sup>d</sup>

## Radical Polymerization Chemical Cross-linking

In the presence of cross linking agents like ethylene glycol dimethacrylate, the poly (2 hydroxyethyl methacrylate) (pHEMA) hydrogel system provided a clear illustration of the cross linking process via radical polymerisation approach. Since then, other hydrogel systems have been created by applying comparable techniques. Consequently, a sophisticated method for creating methacrylated dextran has been suggested. A thorough assessment of subsequent research on the chemically cross-linked dextran hydrogel system has been provided. The ultrasound polymerisation method, however, has not yet been documented <sup>[34]</sup>.

Ultrasound research has been widely applied in many polymer-based applications as a unique tool for emulsion

polymerisation and polymer synthesis. Very little polymer was generated during the sonification of an aqueous solution of the monomer 2-hydroxyethyl methacrylate (HEMA). Monomers including 2-hydroxyethyl methacrylate (HEMA), poly(ethylene glycol) dimethacrylate (PEG-DMA), ethylene glycol dimethacrylate (AA/EGDMA), and bis-acrylamide (Am/Bis-Am) were also utilised in the hydrogel manufacturing process, along with macromers such dextran methacrylate (Dex MA) <sup>[35]</sup>.

#### **Biosynthesis of Microbial Polysaccharides**

A small number of exopolysaccharides are produced extracellularly, but the majority are thought to be produced by a cellular process that is identical to or comparable to that of cell wall production. Synthesis outside of cells. Fructose is released when the

glucosyl component of sucrose is transferred to the non-reducing end of the dextran molecule by glucosyl transferases, which synthesise the dextrans. The molecular weights of microbial exopolysaccharides have not been thoroughly studied. On gel electrophoresis, *Azotobacter vinelandii*'s alginate looks polydisperse, with molecular weights ranging from 20 to 800 x 103. Lower rates of exopolysaccharide formation in developing bacteria may result from rivalry between cell wall synthesis and the carrier, which is shared by cell wall polysaccharide and exopolysaccharide synthesis <sup>[36]</sup>.

Substitutes for pyruvate and acetate are composed of normal basic repeating oligosaccharides, many microbial exopolysaccharides also contain variable modifiers such succinate half esters, pyruvate ketals, and acetate esters. The control of the degree of replacement and the function of these substitutes in vivo are mostly theoretical. It has been hypothesised that the acetate groups on the mannuronic acid residues of the alginate produced by Azotobacter vinelandii inhibit the mannuronic acid groups' epimerization to guluronate. When the ionic strength of the environment increases, such as from 0 to 1% NaCI, the exopolysaccharide xanthan, which is derived from Xanthomonas campestris, usually exhibits an increase in viscosity at concentrations higher than 0.5%. The degree of substitution of the pyruvate ketal on the terminal mannose of xanthan gum varies with strain and growth conditions, but the viscosity of the deacetylated product in distilled water and salt solution changes when the acetate ester is removed from the inner mannose of the side chain [37].

One in three side chains of xanthan are said to be pyruvylated. Compared to xanthans with all side chains containing the acid 17, it is said to have lower viscosities in salt and water. The precursory process of microbial alginate biosynthesis by *Azotobacter vinelanclii* and *Pseudomonas aeruginosa* is depicted as the creation of microbial alginate is discussed in detail below. It has been discovered that several *P. aeruginosa* alginate negative mutants are deficient in this enzyme. Additionally, calcium ions can be used to change the ratio of mannuronic/guluronic acid residues in polymers made by *A. vinelandii*. *A. vinelandii*'s polymers have extremely polydisperse molecular weights and the extracellular atginate lyase might be involved in determining the molecular weight distribution. The xanthan biosynthesis pathway is believed to be comparable to that of other microbial exopolysaccharides, despite the fact that it has not been determined <sup>[38]</sup>.

## Bacterial, Fungal and Algal Polysaccharides Dextran

A homopolysaccharide of glucose, dextran has a molecular weight ranging from 10 to 2000 kDa with a-(1-6) links. *Leuconostoc, Streptococcus, Weissella,* and *Lactobacillus* species may synthesise this chemical outside of the microbial cell from sucrose using dextransucrase enzymes. The ideal temperature range is between 25 and 30 degrees Celsius, and the pH should be between 6.9 and 6.9. *L. mesenteroides* and *L. dextranicum* generate the commercial dextran. Two processes are used by the dextransucrase to produce dextran: (i) hydrolysing the sucrose and binding the glycosyl moiety; and (ii) using an insertion mechanism to build up the dextran. The microbial strain's dextransucrase is used to synthesise the dextran's size and structure, which explains why different dextrans have different molecular weights and sizes  $^{[39, 40]}$ .

## Xanthan gum

Xanthomonas campestris, Xanthomonas pelargonii, Xanthomonas phaseoli, and Xanthomonas malvacearum are the gramnegative, plant pathogenic bacteria that make this heteropolysaccharide. D-glucose, Dmannose, D-glucoronic acid, acetal linked pyruvic acid, and D-acetyl groups with large molecular weights ranging from 2~106 to 20~106 Da make up this polymer's repeating units<sup>[41, 42]</sup>.

#### Gellan gum

Kelco was first commercialised the gum after it was discovered in 1978. With repeated units of a-rhamnose, two residues of b-Dglucose, and b-Dglucuronate, this gum is an anionic linear heteropolysaccharide with exceptional viscosity and heat stability. Alcohol is used to create a precipitation during the 30- to 60-hour fermentation process, which is carried out at 30°C and pH between 6.0 and 7.0. During this time, the extraction of gellan gums with varying levels of esterification can be produced by adjusting certain circumstances. Microbial growth and gellan gum synthesis are directly correlated, and things that adversely impact microbial growth can decrease gellan gum output. It is well known that the highest amount of gellan gum is produced when yeast extract is used as a source of nitrogen and sugar as a source of carbon <sup>[44, 45]</sup>.

#### **Fucose Containing EPS**

L-fucose, D-glucose, and D-galactose units make up Clavan, which is produced by *Clavibacter* strains, particularly *C. michiganensis*, and has the ability to form solutions with a high viscosity. *Enterobacter* produces FucoPol, which has a high molecular weight and is primarily made up of fucose (32–36 mol %), galactose (25–26 mol %), glucose (28–37 mol %), and glucuronic acid (9–10 mol %). FucoPol's film-form capability enables its application as an inner layer of a multilayer packaging material with hydrophilic qualities, ductile mechanical qualities, and strong gas barrier qualities when employed with a low water content. Chitosan was used to improve these qualities, allowing for a wider variety of foods to be used <sup>[46, [47]</sup>.

#### Hyaluronan

Also known as hyaluronan, HA is a linear heteropolysaccharide that is highly hydrophilic. It is composed of units of b-D-glucuronic acid and b-D-N-acetyl-glucosamine residues

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connected by b-(1-4) and b-(1-3)-glycosidic linkages that are generated by members of group *C. Streptococcus* bacteria. These polysaccharides are mostly utilised in the medical and cosmetics industries because of their excellent water retention, biocompatibility, and viscous behaviour <sup>[48, 49]</sup>.

#### Levan

Various microbes, such as *Bacillus subtilis*, *Pseudomonas syringae*, and *Brenneria goodwinii*, employ levansucrose to efficiently biosynthesise levan. Resuscitation of biocatalysts, levan's neutral charge, low viscosity, high water solubility yet non-swelling, and biological activity which includes anti-tumour, antioxidant, anti-inflammatory, and cholesterol-lowering effects are some of its primary characteristics <sup>[50, 51]</sup>. **Pullulan** 

Pullulan is made by aerobic fermentation, which is carried out in a batch or fed-batch setting with high aeration rates, an ideal pH of 4.5, and a temperature range of 24 to 30 degrees Celsius for 100 hours. While a variety of carbon sources, such as sucrose, glucose, fructose, maltose, starch, or malto-oligosaccharides, can be used, ammonium and complex nitrogen sources are the best sources of nitrogen. While successive maltotrioses are joined by a-(1-6) glycoside bonds, pullulan is a linear homopolysaccharide made up of maltotriose units joined by a-(1-4) glycoside linkages. The yeast-like fungus *Aureobasidium pullulans* produces this polysaccharide on an industrial scale during the late exponential and early stationary phases. Its average molecular weight is between 362 and 480 kDa <sup>[52, 53]</sup>.

Figure 4: Structural Configuration of Microbial Polysccharides. A) Pullulan, B) Levan and C) Dextran<sup>[43]</sup>



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Figure 5: Structural Configuration of Microbial Polysaccharide's. A) Scleroglucan, B) Alternan and C) Elsinan [56]



A)







## Schizophyllan (SPG) or Sizofiran

The fungal beta-glucan polysaccharide schizophyllan (SPG) or sizofiran is made up of b-(1-3)-D-glucopyranose backbone with a single b-(1-6) branchThe edible fungus *Schizophyllum commune* produces -D-glucopyranose residue at every third glucose unit. It has a triple helical shape and a molecular weight that ranges from 100,000 to 200,000 Da. produced via submerged fermentation with a pH of 4.8 in a glucose-rich medium, this glucan is a water-soluble and nonionic polymer that precipitates after 4–8 days when water-miscible organic solvents (like methanol) are added <sup>[54, 55]</sup>.

*Scleroglucan:* Sclerogluan Produced mostly by *Sclerotium glucanicum*, the scleroglucan is a fungal beta-glucan with a triple helical conformation and, as a result, anticancer action. It shares a similar structure to schizophyllan (b-D-glucan 1 to 3 and branch at 1 to 6). *Sclerotium glucanicum* grows as pallets encased in a scleroglucan layer, which lowers the mass transfer rate to/from cells and, consequently, the formation of polysaccharides<sup>[57, 58]</sup>. **Welan Gum** 

Sphingomonas sp. and a mutant strain of Alcaligenes produce Welan gum, an anionic, non-gelling heteropolysaccharide that belongs to the sphingans class. It has the following structure [to 3].-b-Glcp-(1 to 4)b-D-GlcpA-(1 to 4)-b-D-Glcp-(1 to 4)-a-L-Rha-(1 to], where L-rhammose or L-mannose are substituted on C3 of each 1,4linked glucose repeating unit, where Glcp stands for glucose, GlcpA for glucuronic acid, and Rha for rhamnose<sup>[59, 60]</sup>.

## CONCLUSION

Due to the great variety in EPS molecular structure, which produces a wide range of features and prospective applications, microbial EPS applications are quite diverse, ranging from the food to medical industries. EPS is mostly utilised in the dairy industry and can be employed as a water-binding agent, stabiliser, emulsifier, gelling agent, or viscosity agent. The most well-known and widely used EPS in the food business is gellan gum, which serves as the primary example. In addition to enhancing the texture and physical stability of food products, this gum has been successful in providing sufficient, regulated flavour release throughout a broad pH range. Most wellknown for their therapeutic qualities, EPS derived from fungal strains (such as lentinan, schizophyllan, and glucan) are frequently referred to as "Biological Response Modifiers." It has been demonstrated that EPS from Fomitopsis pinicola has excellent DPPH and hydroxyl radical scavenging capabilities as well as protective effects on yeast cells against oxidative damage caused by UV and H2O2. Additionally, the antioxidant activity of Cordyceps gracilis EPS was investigated, demonstrating strong DPPH and ABTS radical scavenging, iron chelating, and reducing power. Additionally, Ganoderma neojaponicum EPS demonstrated promise as immunomodulating agents that boost the immune system's ability to combat infectious illnesses.

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