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Rheological Behavior and Pharmaceutical Applications of Bacterial Exopolysaccharides

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ABSTRACT

Over the last few decades, numerous bacterial exopolysaccharides have been extensively studied for their composition, structure, biosynthesis and functional properties, owing to their unique properties over traditional plant-derived gums and synthetic polysaccharides. They have been employed in food processing industries as rheology modifiers, gelling agents, in oil drilling and cement industries. But their full-fledged commercialization is yet to be achieved and their scope and possibilities in pharmaceutical field and optimized drug delivery are yet to be explored. The present review outlines, critically evaluates and summarizes the research outcomes on structure, solubility, solution behavior, rheological characterization, gelation mechanism and potential industrial and pharmaceutical applications of curdlan, polysaccharide 13140, Fucopol, levan, sphingans, succinoglycan and several other bacterial exopolysaccharides in their native form and also their derivatives. It also aims to identify and propose potential drug delivery systems where gelling and non gel-forming exopolysaccharides can be utilized successfully for development of stable, acceptable and effective dosage form.

INTRODUCTION

Polysaccharides obtained from bacteria, may accumulate inside the cells displaying storage function such as glycogen, may be present as structural components in the form of a slime layer as capsular polysaccharides e.g. K30 O-Antigen or secreted outside the cells as extracellular bacterial polysachharides or exopolysaccharides (EPS) e.g. xanthan, bacterial alginate, sphingan etc. (Manivasagan and Kim, 2014; Schmid *et al*., 2015). Most of the microbial polysaccharides are complex molecules, branched or unbranched and are homo- or heteropolysaccharides, consisting of ramifications of varying lengths and different degrees of complexity, with molecular weights varying between 10 and 1000KDa. Depending on the occurrence of D-glucose, fructose or D-galactose as the monomeric units and on the basis of linkage bonds, homopolysaccharides can be further classified as α-D-glucans, β-D-glucans, fructans and polygalactans. In case of heteropolysaccharides like sphingans, the repeating units may consist of D-glucose, D-galactose, rare sugars such as L-rhamnose, fucose and occasionally, N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc) or glucuronic acid (GlcA) (Nwodo *et al*., 2012). Bacterial EPS have demonstrated diverse functional properties which can be attributed to their unique rheological behavior in aqueous medium, quite different from that exhibited by plant-derived gums or synthetic polymers (Hou *et al*., 1996). Explosive growth is being witnessed in the use of polysaccharides in different fields and industries where gels find wide acceptance as in food, tissue engineering, paper, textile, paints, cement, oil drilling and other industries where viscosity modifier is an essential ingredient. Under different circumstances, these gels may act as gellant, thickener, stabilizer, emulsifier, coagulant, flocculant, film/membrane former etc. Since 1940, food processing industry started using modified starches and high fructose sweeteners as additives.

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Popularity of polysaccharides as components of edible items is mainly due to their strong water-binding and water-retention capability in addition to their immense swelling and gelation potential. Bacterial polysaccharides are currently being used as adjuvants in skin care regimens, preferably because of their positive effects on skin hydration and also their capability in stimulating cell renewal and biosynthesis of several skin constituents. Marine bacterial EPS have been investigated for their potential to be used as nanopatterned scaffolds, where attachment of specific ligands can promote cellular function and differentiation of embryonic stem cells (Roca *et al*., 2015; Lindstrom *et al*., 2012; Baruah *et al*., 2016; Venugopal, 2011; Camargo *et al*., 2012; Moscovici, 2011; Tako *et al*., 2016).

Exhaustive review on the different structural variants of bacterial EPS and multi-faceted applications of polysaccharides has provided the impetus to delve deep into the source, structure, linkages, factors affecting aqueous solubility and finally rheological characterization of the EPS in aqueous environment under different conditions of temperature, pH, salt concentration etc. Proper rheological study will only facilitate categorization of the microbial exopolysaccharides into gel-forming and non-gel forming ones.

The present review article aims to focus on the source, structure and detailed studies on solubility, solution rheology and gelation mechanism of several gelling and non-gelling bacterial exopolysaccharides in aqueous medium. Rheological characterization of aqueous dispersions of polysaccharides becomes more significant from pharmaceutical point of view as flow behavior affects manufacturability, processing, consumer acceptance, stability, quality and performance of a pharmaceutical product. However, native EPS sometimes may fail to fulfill the consumer demands with respect to their viscosity or desired use. Native polysaccharides can be subjected to customized structural modifications through substitutions under controlled reaction conditions.

These newer derivatives usually possess optimized solubility, rheological characteristics and other tunable properties leading to newer avenues for their novel uses. Therefore, a section has been added to highlight strategies for production of bacterial exopolysaccharides with tailor-made properties and enhanced functionality. The present review highlights on recent trends and findings on solubility profile, rheological behavior, reported and expected pharmaceutical possibilities of exopolysaccharides of bacterial origin such as curdlan, levan, Fucogel and sphingans. Some miscellaneous heteropolysaccharides such as succinoglycans and EPS obtained from Lactic acid bacteria have also been discussed.

ORIGIN AND CHEMICAL COMPOSITION OF BACTERIAL EPS

Bacterial exopolysaccharides have been broadly classified as homopolysaccharides or heteropolysaccharides depending on the occurrence of single- or multiple types of sugar residues in the linear or branched polymeric backbone.

Homopolysaccharides

(1→3)-β-D-glucans

Three different structural classes of (1→3)-β-D-glucans have been found to occur in the capsular material of various microbial species, with roles in aggregation, pathogenicity and storage (McIntosh *et al*., 2005) . (1→3)-β-D-glucans are linear or branched polysaccharides, consisting of trisaccharide repeating unit with two (1→3)-linked β-glucopyranosyl units in the main chain with either (1→6) or (1→2)-linked side chains of varying length and distribution (Puertas *et al*., 2014).

Curdlan is a linear, unbranched, neutral, water-insoluble exopolysaccharide secreted by *Agrobacterium biovar* 1 (identified as *Alcaligenes faecalis var. myxogenes* at the time of discovery) or *Agrobacterium radiobacter*, with an average degree of polymerization (DP) of 450. It owes its name to its ability to "curdle" when heated. The monosaccharide present in this linear (1→3)-β-D-glucan is D-glucose linked in (1→3) manner. The average molecular weight has been found to vary between 5.3 X 10^4 to 2.0 X 10^6 Da in 0.3 M NaOH (West and Peterson, 2014; Curdlan monograph; Zhang, 2015).

There is report of a curdlan-like, water-insoluble, gel-forming polysaccharide, Polysaccharide 13140, being synthesized by *Alcaligenes faecalis var. myxogenes* strain NTK-u. Similar to curdlan, it is composed almost exclusively of (1→3)-glucosidic linkages. It possesses compatibility with most of food ingredients, is highly palatable, can retain and bind water for long and thus finds application in food processing industry (Kimura *et al*., 2016).

β-(2→6)-D-fructans

Levan is a non-structural, branched homopolymer containing fructose residues with molecular weight reported as 5.0 X 10^5 Da. The monosaccharide residues are formed into compact nanospheres which can resist depolymerisation up to 70°C, even when placed in 1N HCl (Oner *et al*., 2016; Levan website). The exopolysaccharide is produced by large number of bacteria belonging to different genera such as *Bacillus*, *Halomonas*, *Pseudomonas*, *Aerobacter*, *Streptococcus*, *Microbacterium*. It is a member of β-(2→6)-D-fructans where some short β-(2→1)-branches are synthesized from sucrose by the bacterial enzyme, levansucrase and may contain one fructose residue. A strain of *Paenibacillus polymyxa* is reported to produce levan esters such as acetyl, phosphoryl and benzyl esters. The final conformation of levan in solution depends on the existence of the carbohydrate in furanose form (Moscovici, 2011; Gupta *et al*., 2011).

Therefore, bacterial homopolysaccharides belonging to the class of homopolysaccharides containing either glucose or fructose residues, may be linear or branched and are usually water-insoluble. Gel-forming ability of the exopolysaccharides depends on the linkages and monomer residues.

Heteropolysaccharides

Heteropolysaccharides of bacterial origin have been reported to be composed of different monosaccharides such as glucose, galactose, fucose, mannose, rhamnose or glucuronic acid. These monomeric residues may occur in various combinations and

same monomers may combine in different molar ratios giving rise to heteropolysaccharides of diverse physicochemical nature, ionic behavior and rheological characteristics.

Fucose-containing heteropolysaccharides

Several species of *Enterobacter* are known to produce fucose-containing EPS. For example, *Enterobacter* sp. CNCM 12744 secrete an EPS where the molar ratio of fucose, galactose, glucose and glucuronic acid is 2:2:1:1. Other EPS contain either glucuronic acid as the main sugar component with 8-10% fucose as in *Enterobacter* sp. SSYL (KCTC 0687BP) or is a heteropolysaccharide consisting of glucose, galactose, fucose, mannose, glucuronic acid and pyruvyl, obtained from *E. amnigenus* (Vanhooren and Vandamme, 2000).

Fucogel, produced by *Klebsiella pneumoniae*, is a linear anionic trisaccharide, the repeating unit being formed by galacturonic acid, L-fucose and D-galactose. It is structurally similar to polysaccharides obtained from *Klebsiella* bacteria type I-156, K63 and K42 (Dumitriu, 2004). Fucopol secreted by *Enterobacter* A47 is a heteropolysaccharide composed of almost equimolar amounts of fucose, glucose, galactose along with minor percentage of glucuronic acid. It also contains acyl groups – acetate, pyruvate and succinate.

Rhamnose-containing heteropolysaccharides

Sphingans are a group of exopolysaccharides, containing rare sugar, rhamnose and are secreted by gram-negative bacteria belonging to the genus, *Sphingomonas.* Some of the commonly known sphingans reported in various literature include heteropolysaccharide S-7, welan gum (S-130), rhamsan gum (S-194), RMDP-17, diutan gum (S-657), S-88 and S-198. Sphingans are characterized by the occurrence of a common linear tetrasaccharide repeating unit composed of Glc-GlcA-Glc-Rha or Man. The terminal sugar residue is different in different sphingans. All of them contain the glucuronic acid as the second monosaccharide unit except S-7 where 2-deoxy glucuronic acid is present. Welan, an anionic polysaccharide is produced by *Alcaligenes* ATCC 31555. It carries only one acetyl group as substituent and a side chain of α-(1→3)-linked rhamnose or mannose in the ratio of 2:1 present at the first glucose of the repeating unit. Owing to the presence of D-glucuronic acid in its chemical structure, it acts as a polyelectrolyte. Rhamsan, obtained from *Alcaligenes* ATCC 31961, has two D-glucosyl side chains, linked by (1→6) linkages on O-6 of the 3-O-substituted β-D-glucopyranosyl unit. This particular linkage is different from (1→3)-linkage in welan. Diutan, obtained from *Sphingomonas* sp. ATCC 53159 possesses dimeric L-rhamnose side chain linked to the first glucose residue of the growing repeating unit and two acetyl groups are attached at C2 and C6 positions of the second glucose in the repeating unit. Other monosaccharides present in the repeating backbone structure include glucose and glucuronic acid. The carboxylate group linked to the glucuronate imparts anionic charge to diutan and the rhamnosyl side chains protect the carboxylic acids, preventing cross-linking by divalent calcium ions. The polysaccharide gum, S-198, is synthesized by *Alcaligenes* ATCC 31853 (Schmid *et al*., 2015; Coleman *et al*., 2008; BeMiller and Whistler, 2012; Thomas *et al*., 2013; Schmidt *et al*., 2013).

Miscellaneous

Succinonoglycan is a branched acidic heteropolysaccharide which is obtained from different genera of bacteria such as *Rhizobium*, *Agrobacterium*, *Alcaligenes* and *Pseudomonas* strains. The monomers present are β-linked glucose and galactose in the ratio of 7:1. Among pyruvate, succinate and acetate substituents, pyruvate occurs in stoichiometric amount but the amounts in which the other two exist depend on origin of strain and fermentation conditions. Its molecular weight lays in the range of 5.0×10^3 and 1.0×10^6 Da (Schmid *et al*., 2015; Nwodo *et al*., 2012).

Lactic acid bacteria (LAB) belonging to the genera of mesophilic and thermophilic *Lactobacillus* species are known to secrete extracellular heteropolysaccharides (HePS). A particular HePS obtained from *Lactobacillus plantarum* YW11 consists of glucose, galactose and N-acetylated sugar residues in a molar ratio of 2:71:1. Another strain, *Lactobacillus plantarum* YW 32, is reported to produce HePS composed of mannose, fructose, galactose and glucose in a molar ratio of 8.2:1:4.1:4.2 with a molecular weight of 1.03 $X10^5$ Da (Baruah *et al*., 2016).

SOLUBILITY, SOLUTION RHEOLOGY AND GELATION MECHANISM OF BACTERIAL EXOPOLYSACCHARIDES

Polysaccharides adopt a secondary structure or a suitable conformation governed by the monosaccharide composition and linkages thereof. Helical conformation is the most common and helices may also aggregate together to form a network-like structure giving a particular order to the system. However, all polysaccharide systems do not exhibit ordered conformation in solution system. Presence of ions of appropriate valency and charge also can induce some degree of order to the system. It is to be kept in mind that, in solution, most polysaccharides undergo a transformation from an ordered to a disordered state which may be either thermally induced or induced by removal of specific ions. Change in order of system is reflected in enhanced solubility of the polysaccharides. Ion-binding capability and ability to form an ordered structure with a specific conformation for polyanionic polysaccharides depends greatly on the occurrence of uronic acid residues or acyl substituents such as pyruvate, ketals or succinyl half-ester groups. (1→6)-linked homoglycans possess some aqueous solubility owing to extra degrees of freedom provided by the rotation about C-5 to C-6 bonds (Whistler, 1973). Galactoglucan polysaccharides produced by *Rhizobium meliloti*, show high degree of flexibility and always exist in a disordered conformation. Lack of intra-chain hydrogen bonds and location of anionic pyruvic ketal along with acetyl groups on the periphery of the helical structure of the polysaccharide are responsible for the absence of an ordered conformation (Wingender *et al*., 2012). Therefore, molecular conformation and flexibility, chemical

structure and composition, molecular weight, presence of ionizable groups and ions in the system, nature and number of intra- and intermolecular interactions play a significant role in determining the solution properties of the polysaccharide polymers. Polymers with degree of polymerization as low as 20-30 have also been found to be water-insoluble and poorly soluble at neutral pH (Cumpstey, 2013; Karaki *et al*., 2016).

Not all the polysaccharides can form gels although they may produce solutions of extremely high viscosity that can tolerate wide variations in temperature, pH and salt concentrations. Solution behavior of the polysaccharides affects the intrinsic viscosity, critical overlap concentration of the molecule and finally, the coiling tendency of the polymer (Freitas *et al*., 2011). Gel-forming ability arises from stiffness of the polymer chains and ability to produce a three-dimensional network as a result of cross-linking and intermolecular bonding between ordered zones of the constituent chains or entanglements, sometimes in a specific ionic environment (Sutherland, 2001; Thomas *et al*., 2013; Banerjee and Bhattacharyya, 2012). Functional groups of the polysaccharides that take active part in hydrogen bond formation include hemiacetal oxygen, hydroxyl group, methyl group, sulfuric acid group, carboxyl group etc. Apart from hydrogen bonds, van der Waals forces of attraction, ionic interactions or electrostatic forces of attraction also contribute to the phenomenon of gelation. Intra-molecular linking may also be responsible (Tako, 2015).Two non-gel-forming polysaccharides may form a gel due to synergistic action of the optimum concentrations of the individual polysaccharides (Wingender *et al*., 2012). It has been observed that linear, ionic homoglycans can readily form gels due to association at several junction zones. Gel cannot flow but can undergo reversible stretching on deformation. The consistency of the gel increases with development of a strong tertiary structure at the junction zones. Gel stability is controlled by concentration of polysaccharides, ionic status and co-existence of other macromolecules (Lindstrom *et al*., 2012; Sutherland, 2001; Whistler, 1973). From the above discussion, it is clear that presence of an ordered conformation and occurrence of suitable functional groups in the exopolysaccharide structure explains the formation of a stable gel in most of the cases. Study of solution behavior of microbial polysaccharides is essential for exploring their uses and commercial applications in various industries. Rheological characterization and analysis helps in elucidating the structure-function relationship of the compounds at molecular level in aqueous solvents (Tako *et al*., 2016).

It is obvious from above discussion that rheological understanding of bacterial exopolysaccharides is essential to exploration of their possible applications in pharmaceutical industry in modifying viscosity, stabilizing preparations and controlling drug release. Final product of optimum viscosity and performance will exhibit improved efficiency when administered by suitable routes. In addition to the effects of solvent nature, pH, ionic strength, temperature and co-solvents on polysaccharide-solvent interactions, length of time is crucial in maintaining consistency of flow behavior of pharmaceutical preparations.

The following section emphasizes on the probable conformations and conformational shift of the gel-forming and non-gel forming bacterial exopolysachharides when introduced into aqueous as well as non-aqueous medium.

Gel-forming bacterial EPS

Curdlan is water-insoluble at room temperature and insoluble in ethanol. However, it is reported to possess better solubility in other organic solvents [e.g. dimethylsulfoxide (DMSO), formic acid, and some aprotic solvents such as N-methylmorpholine-N-oxide (NMMO) and dimethylacetamide containing lithium chloride(DMAc/LiCl)] compared to other naturally occurring polysaccharides. Gelation has been observed for this poorly soluble bacterial EPS and deacetylated rhamsan in aqueous media at a low concentration range of 0.1-1.0% w/v at room temperature. Hydrogen bond formation between the solvent molecules and polymer-solvent molecules led to ice-like structure and finally transition from liquid to gel state. L-rhamnosyl residues in deacetylated rhamsan participate in gel formation (Roca *et al*., 2015; Tako *et al*., 2016; Tako, 2015). Curdlan with higher degree of polymerisation (approximately DP of around 200) exhibited gel formation on heating. Detailed study of viscoelastic properties of curdlan aqueous suspensions has revealed occurrence of two distinct types of gels, depending on heating temperature: low-set thermal reversible gel (~55 °C) and high-set thermal non-reversible gels (~80 °C). High-set, thermostable elastic gel can be obtained by heating 2% aqueous suspensions of curdlan in a boiling water bath for 10 mins. This type of high-set, thermostable gel finds wide applications in food industry, especially in enzyme immobilization (Bhatia, 2016). Curdlan exhibited pH-dependent structural morphologies in alkaline medium, varying from microfibrils to spindle-shaped fibrils of different lengths and also thin lamellar hexagonal crystals (Puertas *et al*., 2014). It demonstrated a helical, ordered structure, formed of single and triple helices, at low concentration of sodium hydroxide but highly alkaline pH induced a significant alteration in conformation. At moderate concentration of sodium hydroxide, curdlan becomes completely soluble and assumes the structure of random coils. Similar ordered conformation is also observed when curdlan is added to other non-solvents such as 2-chloroethanol, dioxan or when water is added to dimethyl sulfoxide solution (Roca *et al*., 2015; Tako *et al*., 2016; Tako, 2015).

Aqueous suspension of polysaccharide 13140 has been found to produce thermally irreversible elastic gel on heating. The gel showed simultaneous stability in acidic pH. Hence, it can be used in situations to enhance viscoelasticity (Kimura *et al*., 2006).

Due to excellent thermostable rheological behavior in aqueous solvent, Fucopol can serve as a comparable or even better alternative to Fucogel, xanthan gum, guar gum and alginate (Freitas *et al*., 2011). It has demonstrated shear-thinning behavior with recovery of viscosity as soon as shear rate was reduced. Viscous behavior of the polysaccharide is attributed to polymer chain entanglement. Aqueous solution of Fucopol at low concentration (0.5%wt) has been shown to act as a stabilizer in the

preparation of o/w emulsions prepared with cooking oils such as sunflower oil, rice bran oil, corn oil (Texeira and Vicente, 2013). Fucopol when dissolved in biocompatible, ionic liquid, choline acetate, displayed non-Newtonian rheological behavior as viscous modulus was always found to be greater than elastic modulus (Candido, 2015). Bilayer films of Fucopol and chitosan demonstrated superior swelling behavior in aqueous solvent, in comparison to monolayer Fucopol films. Tensile tests on the bilayer films revealed elastic modulus to be 137 MPa (Ferreira *et al*., 2016). No ordered conformation could be detected in Fucogel solution and thus it is moderately effective as a thickener (Dumitriu, 2004). In an investigation to determine the efficiency of polysaccharides such as xyloglucan, galactomannan, sodium hyaluronate, Fucogel and xanthan gum, in formulation of a stable o/w emulsion, the consistency, viscoelastic properties and creep compliance of the emulsion were found to vary. Fucogel was found to be poor in performance compared to xyloglucan and sodium hyaluronate whereas it exhibited better efficiency in contrast to xanthan gum and galactomannan. However, the stability of the emulsion after storage for 20 days was below optimum in comparison to emulsions formulated with neutral polysaccharides (Vianna-Filhoa *et al*., 2013). With Fucopol and Fucogel, polymer chain entanglement accounts for gel formation.

The exopolysaccharides isolated from different species and strains of *Enterobacter* show variable gelation characteristics depending on their chemical structure. Non-acetylated EPS obtained from *Enterobacter* XM6 has been reported to form a highly crystalline structure below 30°C which also melts at that temperature (Sutherland, 2001; Wingender *et al*., 2012).

Therefore, it can be concluded that sol-gel transformation in gel-forming bacterial exopolysaccharides may be either reversible or irreversible with pronounced effect of gel stability. Polymer-solvent interaction and solvent-solvent interaction creates an ordered structure for the solvent and facilitates formation of three-dimensional gel.

Non-gel-forming bacterial EPS

Bacterial polysaccharide, S-198, is an example of a non-gel forming polymer, producing a thermostable, highly viscous solution even at high temperature. At temperature of 0°C and at 0.8% w/v concentration, S-198 did not show any signs of gelation. No change in viscosity and elastic modulus could be observed even if temperature was increased to 80°C. In presence of urea (4.0M), the elastic modulus of S-198 was found to be less than that of its aqueous solution (Tako *et al*., 2016). It can be developed in manufacture of water-based lubricants (Bhatia, 2016).

Levan is a unique polysaccharide in the sense that it does not undergo swelling in water and densely packed, spherical structures are maintained intact. It possesses high solubility in hot water, varying solubility in cold water and insoluble in absolute ethanol. Compared to inulin, it shows better solubility in water at room temperature. Presence of (2→6) linkage and branching account for higher aqueous solubility of levan. Viscosity of levan solution increases with salt concentration but is inversely proportional to temperature (Levan website; Gupta *et al*., 2011). It has been found to facilitate safe and effective delivery of micronutrients (Bhatia, 2016). Low intrinsic viscosity of levan makes it a very suitable pharmaceutical excipient and it has been used as a binder and in design of controlled release matrices (Nakapong *et al*., 2013).

Rheological studies on aqueous solution of a new fucose–containing extracellular polysaccharide revealed high viscosity with increase in temperature. Temperature fluctuations did not have any effect on viscosity and viscoelasticity of the polymer solution even after few cycles of heating-cooling (Cruz *et al*., 2011).

In case of EPS synthesized by *Enterobacter aerogenes* type 54, containing the same repeat unit of D-glucose, L-fucose and D-glucuronic acid, as that of EPS from strain XM6, the carboxylate groups are shielded by side chains due to the presence of O-acetyl groups on every tetrasaccharide or octasaccharide and thus the EPS fails to produce gels which are amorphous in nature. But it can form gels in presence of various ions (Sutherland, 2001; Wingender *et al*., 2012).

Sphingans exhibit unique rheological characteristics depending on the nature and location of the side chains. Welan gum which contains acetyl and L-rhamnosyl or L-mannosyl as a side group does not gel but shows excellent behavior of a typical pseudoplastic fluid at high temperatures (till 150°C) and low shear rate making it an excellent non-gel forming additive for petroleum, food, cement and other industries. Rheological characteristics of the gum is attributed to double helix structure stabilized by interactions between side chain and main chain. In aqueous solution, square of concentration of the gum was found to be directly proportional to elastic modulus. The side chains protect the carboxylate groups and impart additional stability to the ordered, helical structure, which cannot be disturbed or destabilized under any circumstances in aqueous medium. Destabilisation can be induced if DMSO is added and gel formation can be initiated in welan if DMSO concentration is lowered by addition of water (Thomas *et al*., 2013). Because of its thermal stability, it can be used in preparations which need autoclaving. It can also withstand wide variations in pH and calcium salt concentrations. On addition of calcium chloride solution (6.8mM), elastic modulus of welan gum solution was lowered. This is attributed to the presence of side chains which prevent the formation of intermolecular bridges mediated by divalent calcium ion. Solubility of the gum in polar solvents such as ethylene glycol and aqueous DMSO was lowered by addition of salt. Decrease in solubility occurs due to enhancement of electrostatic attraction between negatively charged helices and positive counterions in solvents with dielectric constants substantially lower than that of water (Member *et al*., 1995). Welan gum has also been found to possess excellent suspending ability as estimated from the yield value of 80 dynes/cm^2 at 1% concentration, which is comparable with that of xanthan gum. It is considered superior to xanthan in terms of performance as it presents higher viscoelastic moduli (Roca *et al*., 2015). Compared

to welan, rhamsan, another non-gel forming polysaccharide, has been found to be more viscous at low concentrations and low shear. It is reported to produce an aqueous solution of viscosity, 2000 cP at a concentration of 0.25% w/v and at low shear rates. It can withstand high concentration of salt such as ammonium phosphate. On the contrary, its thermostability is poor and loses viscosity as temperature goes above 100°C. It is a better suspending agent than xanthan gum. Rhamsan gum has shown potential in the field of food, paints, coatings, plastic surgery and suspension fertilizer systems. The microbial EPS, S-7 produces aqueous solution of high viscosity and thus can be used as suspending agent. It can be included as a component of oil drilling fluid. However, it is not resistant to acid hydrolysis. Diutan, another member of sphingan group is considered to be the most thermostable gum and is characterized by high water retention capacity and resistance to the effects of mono-and divalent cations. Viscosity of diutan is attributed to the long side chains resulting in intertwining and entanglement of polymer chains and formation of molecular aggregates at a very low concentration. Increasing temperature from 298K to 348K or high salt concentration did not affect the diutan gum viscosity. Resistance to thermal and salt effects is attributed to double helical structure, location and nature of side chains (Roca *et al*., 2015; BeMiller and Whistler, 2012; Sonebi, 2006; Xu *et al*., 2015; Fink, 2003).

Shear-thinning rheology is demonstrated by the polysaccharide succinoglycan and it also shows temperature-independent viscosity below its transition temperature. It has even been reported to be more effective than hydroxyethylcellulose (Fink, 2015). Heteropolysaccharide isolated from Lactic acid bacteria is capable of forming viscous solutions at low concentrations and can actually enhance the texture, body and mouthfeel of fermented milk drinks. Variables like temperature, pH, ionic strength produced little effect on non-Newtonian, pseudoplastic flow characteristics of the exopolysaccharide. It is compatible with skimmed milk at low temperature and acidic pH and thus can be used to enhance the viscosity and flow properties of fermented milk on a long-term basis. This particular rheological behavior of heteropolysaccharide will prove to be beneficial in pharmaceutical and food industry. Cross-linking of the high molecular-weight HePS with proteins of fermented milk produces a network like structure which actually contributes to high viscosity (Baruah *et al*., 2016). There are reports on gelation ability of a new class of polysaccharides produced by some species of lactic acid bacteria, (1→3)-β-D-glucans. Helical conformation of the molecules has been shown to be disrupted at alkaline pH and also at temperature greater than helix melting temperature. Intermolecular hydrogen bonds are broken followed by dissociation and denaturation of the multi-helical units to single stranded random coils (Puertas *et al*., 2014).

EXPLORING PHARMACEUTICAL OPPORTUNITIES OF BACTERIAL EXOPOLYSACCHARIDES

Structurally related exopolysaccharides may or may not demonstrate similar aqueous solubility profile, rheological behavior, thermostability and acid stability. Minor changes in the molar ratio of the monosaccharides or orientation and position of the side chains can lead to drastic changes in solution behavior of the polymers. Gel-forming polysaccharides have been found to be mostly water-insoluble producing thermostable gel. Non-gel forming polysaccharides may be water-soluble or water-insoluble and some of them like S-198, levan and EPS secreted by *Enterobacter* produce aqueous solutions of low viscosity even at high temperatures exceeding 80°C. Sphingans yield dispersions of good suspending ability even at low concentrations. A common structural feature found in all non-gel forming exopolysaccharides is the presence of protective side chains preventing favourable interactions between solvent molecules and highly active functional groups like carboxylate groups. Lack of polymer-solvent interaction preserves the native structure of the polysaccharides and inhibits gel formation.

Reversible or irreversible sol-gel transformation in bacterial exopolysaccharides can be induced by temperature, pH or addition/removal of organic solvent from the system. Ability of EPS like welan, S-198 to form thermostable solutions of high viscosity without gelling will be of great help in formulation of parenteral and ophthalmic products which require autoclaving. Controlled release in-situ gel forming parenteral drug delivery systems and matrix for enzyme immobilisation can be developed with some exopolysaccharides by utilizing their capacity to form stable thermosetting gels at higher temperature. Curdlan and Fucopol have demonstrated excellent stabilizing action or suspending/emulsifying property at considerably low concentrations. Welan/Fucopol/rhamsan and succinoglycan have been found to be better than xanthan gum and hydroxyethyl cellulose respectively and can thus replace these polymers or can be used in conjunction with these polymers in various dosage forms. Low sensitivity of diutan towards mono and divalent cations enhances its scope to be used in pharmaceutical preparations. Stability of gels or aqueous solutions of high viscosity produced by heteropolysaccharides from lactic acid bacteria and polysaccharide 13140 in acidic pH renders them suitable in fabrication of gastroretentive drug delivery systems.

There are reports of bacterial exopolysaccharides being employed in preparation of nanoparticles of improved efficacy and reduced toxicity in comparison to non-encapsulated drug. The propensity of exopolysaccharides to self-assemble arises from attractive dispersion forces between alkyl side chains and core of the nanoparticles. Amphiphilic nature of levans results in the formation of self-assembled nanoparticles from water (Srikanth *et al*., 2014). Selenium, iron or cobalt encapsulated in levan nanoparticles have been produced with reduced toxicity and protective effect on human intestinal Caco 2 cells (Bondarenko *et al*., 2015). Synthesis of gold and silver nanoparticles capped with levan through green route have made possible availability of highly stable, biocompatible catalysts capable to reduce organic and inorganic substrates (Ahmed *et al*., 2014). Levan has also shown great promise in enzymatic development of nanoparticles for encapsulation of acetylated α-tocopherol (Nakapong *et al*.,

2013). Bovine serum albumin has been successfully entrapped in levan nanoparticles wherefrom drug release occurred via zero-order kinetics (Sezer *et al*., 2013).

STRATEGIES FOR PRODUCTION OF BACTERIAL EXOPOLYSACCHARIDES WITH MODIFIED FUNCTIONALITY

Different strategies may be adopted to yield customized bacterial EPS possessing tailor-made properties and improved functionality. Modifications can be induced by chemical reactions with other biomacromolecules, to alter the nature of side-chains or through cross-linking reactions (Freitas *et al*., 2011a). These new variants are expected to be high-performance products as they are likely to possess desirable material characteristics such as rheological properties for novel applications in various industries and as tissue engineering scaffolds. In most cases, usually, degree of acetylation and pyruvylation has been modified. Self-assembled hydrogel nanoparticles of curdlan derivatives have been found to be efficient in delivery of anticancer drugs (Bhatia, 2016). Partial sulfation of curdlan at O-6 position yields curdlan sulfate of higher aqueous solubility. Degree of substitution has an effect on the thermal stability of the derivative and also on its ability to form macromolecular complexes with polycytidylic acid (Koumoto *et al*., 2004).

Curdlan sulfate has been studied in fullerene chemistry resulting in different variants of C60-substituted curdlan sulfates with different degrees of substitution and of varying aqueous solubility (Ungurenasu and Pinteala, 2007). Carboxymethyl curdlan has been developed with an aim of improving aqueous solubility and functionality. Compared to native curdlan, it was found to possess negligible gelation ability as the hydrogen bonds between water and curdlan were disrupted. In another study, water-soluble derivative was hydrophobised with deoxycholic acid residues to produce self-assembled nanoparticles used for receptor-mediated targeted and sustained delivery of anticancer drug, epirubicin, in murine carcinoma cells. Cytotoxic effect and tumor volume reduction were more pronounced with the derivatised curdlan and tumor distribution was found to be highly selective with negligible accumulation in heart and kidneys in mice (Gao *et al*., 2010; Moscovici, 2011). Phosphorylated curdlan also showed enhanced water solubility and the presence of (1→3)-glucosidic linkages accounted for its higher chain flexibility compared to other phosphorylated polysaccharides. Higher cross-linking degree, higher density and lower swelling degree were obtained with curdlan microgel in contrast to the phosphorylated derivative.

Oxidation of curdlan with nitroxyl radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) is reported to produce water-soluble (1→3)-β-polyglucuronic acid (Zhang, 2015). Regioselective amination of curdlan resulted in higher hydrophilicity and increased affinity towards nucleic acids and was used for delivery of siRNA to mouse embryonic stem cells with significant reduction in levels of eGFP(Han *et al*., 2014) Acetylated, phosphorylated and benzylated levan demonstrated good antioxidant, antitumor and antiproliferative activities (Liu *et al*., 2012).

FUTURE SCOPE

Few microbial polysaccharides discussed in the review have demonstrated excellent thermostability, tolerance to wide pH variations and salt concentrations. Recent developments have been made and are still in progress to fulfill the demand for cheaper microbial nutrients and to minimize the cost of downstream processes. Till date, only few bacterial species have been genetically engineered or subjected to chemical modification to obtain molecules with fine-tuned properties. Thus there is ample scope for entry of new high-performance exopolysaccharide of bacterial origin and creation of a specific market niche (Freitas *et al*., 2011; Vanhooren and Vandamme, 2000). A novel application of microbial exopolysaccharides is in the area of environment management through flocculation of suspended pollutant particles in wastewater and industrial effluent water. Pharmacotoxicological studies are yet to be done on for most of these polysaccharides to establish the status of GRAS (generally regarded as safe) (Nwodo *et al*., 2012; Moscovici, 2011). Moreover, there is a potential risk of interaction between these polymers and other macromolecules occurring in human body. Compatibility studies with commonly used pharmaceutical excipients need to be carried out for their utilization in pharmaceutical industry in design of intelligent therapeutics (Schmid *et al*., 2015).

CONCLUSION

Bacterial exopolysaccharides can therefore be assumed to constitute a diverse class of biomacromolecules with various degrees of flexibility in structure, variations in shape and orientation of the helical conformation, solubility and rheological behavior in aqueous medium. Recent trends in exploring bacterial exopolysaccharides as alternative, renewable resources in different industrial sectors including pharmaceutical manufacturing and drug delivery have focused research on elucidating their rheological behavior in aqueous medium and establishing gelation mechanism. Although their typical flow behavior, thermal stability and tolerance towards the effects of salt or pH favor their utilization for commercial purpose, much studies need to be carried out to reduce their cost of production and also for labeling them as safe for human use and consumption. Continuous efforts should also be directed towards derivatisation of the exopolysaccharides with enhanced solubility profile, rheological behavior and ultimately diverse applications in pharmaceutical field. The entire family of native bacterial exopolysaccharides and their derivatives is expected to capture the global polymer market in near future.

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