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REVIEW ARTICLE

Application of microbial extracellular carbohydrate polymeric substances in food and allied industries

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Abstract

Extracellular polymeric substances (EPS) are biopolymers, composed of polysaccharides, nucleic acids, proteins and lipids, which possess unique functional properties. Despite significant strides made in chemical production processes for polymers, the niche occupied by exopolysaccharides produced by bacteria, yeast or algae is steadily growing in its importance. With the availability of modern tools, a lot of information has been generated on the physico-chemical and biological properties using spectrometric tools, while advanced microscopic techniques have provided valuable insights into the structural–functional aspects. The size of EPS generally ranges between 10 and 10,000 kDa. The wide spectra of applications of EPS as adhesives, stabilizer, gelling, suspending, thickening agent, and surfactants in food and pharmaceutical industries are observed. The health benefits of these EPS enable the improvement of dual function, added value, and green products. This review summarizes previous work on the structural composition, rheological and thermal behaviour, and biosynthetic pathways of EPS and bioprocesses developed for their production. This review also considers each of the above factors and presents the current knowledge on the importance and refinement of available downstream protocols and genetic engineering towards specific food applications, which can help to diversify their prospects in different food and allied industries.

Keywords Biosynthesis · Fermentation · Thermo-stability · Rheology · EPS

Introduction

A microbial cell (both Eukaryotic and Prokaryotic) produces a diverse range of structural, functional and valuable polysaccharides, which may be homopolymeric or heteropolymeric in composition. These polymeric substances are called as exopolysaccharides. The homopolymeric EPS molecules are composed of repeating units of a single monosaccharide, mostly glucose or fructose, whereas heteropolysaccharides are typically branched and composed of repeating units of more than one monosaccharides, mostly galactose, glucose, fructose, etc., with other non-carbohydrate groups. Heteropolysaccharides are produced from intracellular intermediates at a lower quantity and primarily associated with immune modulation. The term "EPS" is used to denote both "extracellular polysaccharides" and "extracellular polymeric substances". Extracellular polysaccharide molecules are composed of sugar (carbohydrate) molecules only, whereas extracellular polymeric substances are composed of repeating units of monosaccharides, proteins, and nucleic acid molecules (Costa et al. 2018b).

These exopolysaccharides (EPS) are associated with microbial cells growing on solid surfaces, and it is a common phenomenon in the natural environment. The term exopolysaccharides (EPS) was proposed by Sutherland in 1972 to describe several forms of microbial polysaccharides found outside their cell walls (Cerning 1995), which may be loosely attached or tightly bound depending upon their structural and functional relationships with the cell. The morphological characteristics of mucoid colony are associated with the secretion of EPS molecules by the respective microorganisms, and also account for the several

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morphological and architectural characteristics of living cells. Therefore, EPS can be defined as a film (in chemical terms as polyelectrolytes adsorbed onto a colloidal particle) covering a living microbial cell surface (Dittrich and Sibler 2010). EPS molecules can be visualized under the light microscope through negative staining or customized stains based on its poly-anionic nature. Scanning electron microscopy (SEM) techniques are frequently used to detect the presence of exopolysaccharides, but the chemical characteristics of EPS cannot be captured using SEM. On the other hand, transmission electron microscopy (TEM) can provide considerable information about the surface structures. Their properties related to facilitating the adherence of cells to surfaces forms the basis of diverse functions, such as protection from engulfment by the predatory protozoa or white blood cells (phagocytes), or from drying or desiccation in bacteria growing in terrestrial habitats or from attack by antimicrobial agents. Most importantly, EPS provides the basis for the successful colonisation of diverse niche, ranging from the inhospitable extreme environment to the nutrient-enriched eutrophic water bodies, playing a key role in the communal life of biofilms (Hibbing et al. 2010).

The high-water retention capability and unique rheological properties of exopolysaccharides are attributable to their high purity and regular structure (Costa et al. 2018a). These biochemical properties have revolutionised its industrial applications and observed immense use as adhesives, stabilizer, gelling, suspending, thickening agent in food industries (Moscovici 2015). Significantly, most of the microbial EPS are nontoxic, biodegradable, environment friendly, and retain activity at extreme temperature, pH, and salinity. Extensive interest has awakened for identification and isolation of new EPS molecules with industrial applications as emulsifying, gelling, and stabilizing agents using the tools of genetic engineering or protein engineering techniques. Such EPS polymers with unique physical and chemical characteristics help in diversifying their use in food and related industries (Elnahas et al. 2017).

With respect to physical and biochemical properties of EPS, the applications of EPS in food and allied industries vary from packaging to food additives. EPS molecules can provide an impetus as they are less hazardous than chemically synthesised polymers, and the production processes are environmentally friendly. One of the relevant examples in this context, LAB, has gained importance as bio-thickeners and texturizers (Silva et al. 2019; Werning 2012). These LABs are recognised as GRAS (Generally Recognised As Safe) status which allow them to be incorporated in food processing (Ami Patel 2013). EPS molecules can also form tough and flexible films; this property can have applications in the production of films or coatings useful in packaging industries for enhancing the shelf life of fruits and other food items. Most importantly, replacing chemical additives can be possible with minimal capital investment in food production processes. Most of the internationally recognized food regulatory bodies, such as the US Food and Drug Administration (FDA), Canadian Food Inspection Agency (CFIA), European Food Safety Authority (EFSA), review their lists of permitted food additives after amending EPS as the food additive. Such interventions may lead to improved or altered specifications of the food-grade polysaccharides, or introduction of different EPS through genetic engineering or novel bioprocessing.

Apart from the food industry, EPS is useful in petroleum industry during drilling and recovery of oil from water. Succinoglycan and xanthan are useful EPS which are used during manufacturing of different kinds of paints. Inclusion of succinoglycan and xanthan with paints provides stability to the suspensions of the pigments, combined with the pseudoplastic behaviour, which facilitates pumping or spraying of paint mixtures. The cognizance and importance of EPS, especially in the agricultural industry, for producing materials which can be useful for soil conditioning or combating environmental stress (osmotic stress, desiccation, UV radiation, etc.) has made EPS a high-demand product from various biological sources, such as bacteria, yeast, and algae (Delattre et al. 2016). Despite the diversity and complexity of EPS, irrespective of the origin, there are certain commonalities which include:

(1) Glucose is the most dominant monosaccharide present in EPS, although other hexose and pentose molecules were also identified. Microbial EPS may also comprise inorganic chemicals, such as phosphate and sulphate. The physical and chemical properties of EPS are largely influenced by the presence of other substances, such as uronic acid increases the poly-anionic nature of the organic acid which leads to increasing the lipophilic quality of the entire polysaccharide (Donot et al. 2012).
(2) EPS molecules have the ability to form a gel with or without the presence of ions. Alginate requires ions to form the gel, whereas curdlan does not require any ions to form the gel (Seviour et al. 2009).
(3) Most of the exopolysaccharides exhibit pseudoplastic flow pattern, i.e. shear thinning in an aqueous environment. The fungal scleroglucan (from *Porodisculus pendulus)* is a neutral polymer which exhibits high viscosity (stable viscosity was reported over a wide range of temperatures, pH, and presence of ions) in aqueous solutions (Alghmadi 2016).
(4) EPS in solution has an ordered helical conformation (single or double helix), which is stabilized by intermolecular hydrogen bonds, such as a well-known and established food hydrocolloid xanthan that forms a double or triple helix. This helical structure makes the EPS semi-rigid. Hence, a solution containing xanthan

molecules exhibits relatively high viscosity (Morris et al. 2001).

(5) EPS also exhibit a good surfactant activity which facilitates emulsifying hydrocarbon in water, thereby forming stable emulsions with aromatic or aliphatic hydrocarbons. As a result of these attractive chemical and physical features with potential applications, a good number of patents have been filed related to its improved production technology and downstream processing throughout the world.

(6) EPS have a proven wide spectrum of health benefits. Kefiran synthesised from *Lactobacillus kefiranofaciens* was reported to suppress an increase of blood pressure and reduce the serum cholesterol levels (Maeda et al. 2004). Antiviral activity and immunomodulation property were also reported from the EPS secreted from *Bacillus licheniformis* (Gugliandolo et al. 2014). Glucan, levan and reuteran isolated from various *Lactobacillus reuteri* strains were reported of their inhibitory effects on enterotoxigenic *Escherichia coli* (ETEC) (Wang et al. 2010). EPS from *Lactobacillus plantarum* (NRRL B-4496) was reported to effectively inhibit the growth of the intestinal carcinoma cell line in vitro as well as in vivo against Ehrlich ascites carcinoma actively (Haroun et al. 2013).

In this review, an attempt was taken to highlight the diversity and applications of exo-polysaccharides produced by various microorganisms through biotechnological route. The focus of this review was to collate the available scientific information concerning EPS composition, production, biosynthesis and industrial applications in a critical manner to permit researchers to comprehend the advances and prospects towards applications of EPS in food and allied industries.

EPS composition and structure

Insights into the structure of EPS molecules are essential to explore their physicochemical properties to a greater extent. Exopolysaccharides are primarily composed of various carbohydrates, which include glucose, galactose, and rhamnose, in different ratios (Wingender et al. 1999). But in addition to various carbohydrates, there may be organic and inorganic constituents present, such as uranic acid, methyl esters, sulphates, pyruvates, etc. (Sutherland 2001). The bacterial EPS are loosely attached or covalently linked to the cell surface of the microorganisms, and usually being acidic in nature, they comprise heteropolysaccharides associated with the functional groups, such as carboxyl, hydroxyl, etc., that are responsible for their high affinity towards metal ions, such as Ca^{++}, Zn^{++}, etc. In a few studies, it was observed that during the growth phase of microorganism, EPS molecules were secreted into their surroundings without any permanent attachment to the surface of the microbial cell. This type of a characteristic differentiates them from the structurally similar polysaccharides known as capsular polysaccharides (CPS), which persist permanently on the surface of microbial cells. EPS can be marked as a homopolysaccharide which contains single types of monosaccharides and heteropolysaccharides, which include repeating units of different monosaccharides. Although homopolysaccharide polymers have the same repeating monosaccharide unit, diversity with regard to branching zones, glycosidic bonds, chain length leads to the difference in their properties. The combination of carbohydrates found in microbial exopolysaccharides was reported to be incredibly diverse, although most of the sugars are those commonly found in animals and plants. Indeed, the composition of EPS is evidently linked to the production parameters and the substituent decoration which may lead to either increase or decrease in their stability (Gupta and Diwan 2017). The hetero-polysaccharides are made of different monosaccharide sugar residues and synthesised due to the joint action of different types of glycosyltransferases (Årsköld et al. 2007). Due to the presence of different carbohydrates with low individual concentrations and their interactions, it becomes difficult to identify the exact chemical nature through biochemical analysis and the selection of downstream processes, i.e. extraction, separation, etc., of EPS molecules. Additionally, the extraction process further demands minimal or negligible disruption or alteration of the EPS. Hence, characterisation of the chemical moieties using advanced spectroscopic techniques, is imperative for the exploitation of EPS effectively.

The biosynthesis of homopolysacchairdes, such as levan and dextran, are catalysed by extracellular sucrose enzymes, such as levansucrase (E.C. 2.4.1.10), dextransucrase (E. C. 2.4.1.5), and, therefore, simultaneously releasing fructose monosaccharide in the culture medium (Franken et al. 2013; Zannini et al. 2016). Synthesis of heteropolysaccharides (e.g., alginate and xanthan) needs sugar nucleotide precursors, and the enzymes involved in their polymerisation also have significant housekeeping functions within the cells (Welman and Maddox 2003).

EPS can be further classified based on the nature of extraction protocols and the positioning of EPS in the debris, such as (1) bound fractions (compounds surrounding the cells), (2) colloidal fractions (polymeric substances excreted in the medium), and (3) residual fractions of EPS (insoluble EPS) (Takahashi et al. 2009). EPS produced by microorganisms, isolated from the marine environment, are mostly heteropolysaccharides made up of a high level of uronic acids with acetyl succinic groups (Poli et al. 2010). The structure of bacterial EPS (*Lactobacillus johnsonii*) is illustrated in Fig. 1a as identified by Dertli et al. (2013).

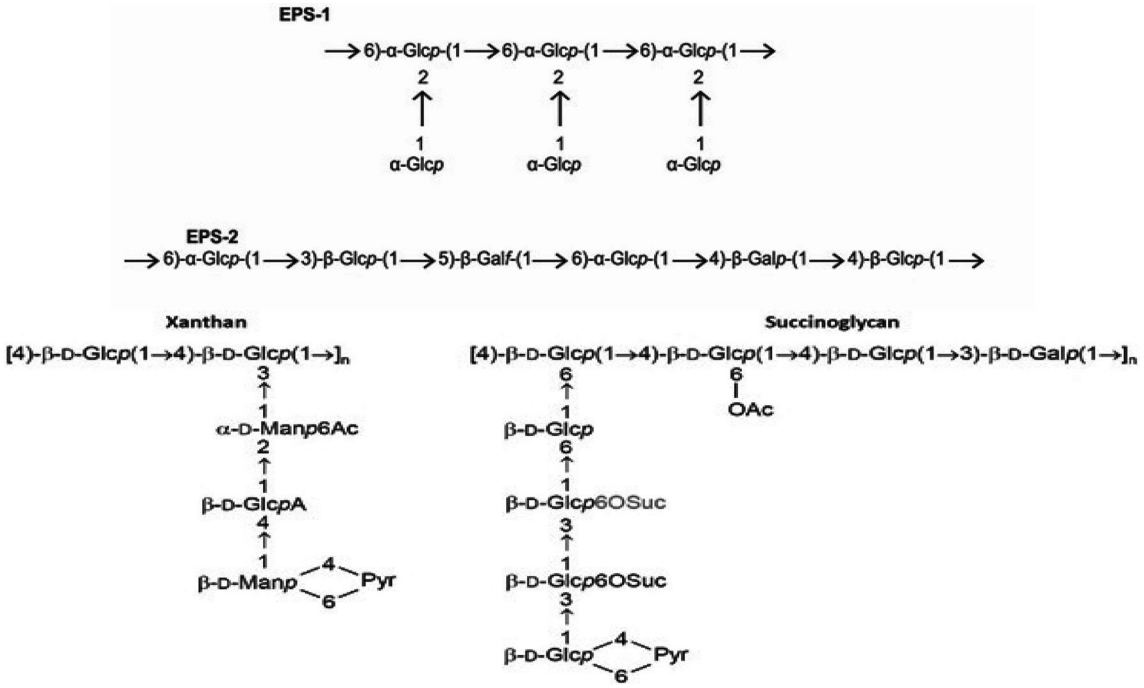

Fig. 1 **a** Structure of exopolysaccharides (Dertli et al. 2013). **b** Repeating unit of xanthan and succinoglycan (*Ac* acetyl group; *Gal* galactosyl group; *Glc* glucosyl group; *GlcA* glucuronyl group; *Man* mannosyl group; *Pyr* pyruvyl group; *Suc* succinyl group) (Becker 2015)

Figure 1b describes the structure of the two most common EPS, i.e. xanthan and succinoglycan. Both these EPS consist of a variable number of repetitions of sugar molecules. Although the basic structure of carbohydrate was found to be uniform, but the non-sugar groups varied along the polysaccharide chain. Chemical analysis of slime layers of a few cyanobacteria revealed an array of neutral sugar and uronic acid residues (Gupta and Diwan 2017). Similar structures were also observed in the bacterial external layers (Hoiczyk and Hansel 2000).

Chromatographic methods are known for their high selectivity, sensitivity and reliability and have been the choice for analyses of the whole EPS, hydrolysed or partially hydrolysed EPS, or their derivatives, respectively. The most popular chromatographic techniques to analyse EPS, used by various researchers, were HPLC, gas–liquid chromatography, size-exclusion chromatography (SEC), and paper chromatography. Spectroscopy methods in conjugation with chromatographic methods, such as gas–liquid chromatography–mass spectrometry (GCMS), FT-IR, and 1D-and-2D NMR spectroscopy, were also reported to be valuable for the analyses of the building blocks comprising the carbohydrates present in EPS (Baldev et al. 2015). Total carbohydrates present in the EPS can be quantified using phenol–sulphuric acid method or orcinol–sulphuric acid method (Moghannem et al. 2018). Solid-state nuclear magnetic resonance (NMR) spectra can help to predict and determine structural components of the EPS.

Fungal EPS exhibit remarkable extent of diversity, which may vary depending upon the mode of growth or the condition of fermentation exposed to the respective fungi. The structural composition of the fungal EPS varies from being pure carbohydrate to carbohydrate merged with protein, phosphate or sulphate. The molecular weight of the same EPS synthesised by different fungi may also vary due to different branching patterns and the length of polymer chains. A special type of pullulan synthesised from *Cryphonectria parasitica* (Forabosco et al. 2006), contrasting with other common pullulans, contains a few α-(1, 6) maltotriose, and this pullulan had more α-(1, 6) maltotetrose subunits. Xu et al. (2006) reported the production of five types of EPS (different molecular weights) in two kinds of bioreactors. Among them, three types of EPS were isolated after fermenting entomopathogenic fungus, *Paecilomyces tenuipes* C240, in a stirred-tank bioreactor. Among those three EPS, two EPS contained glucose as the key monosaccharide, while the other one was composed of mannose. The same organism produced two different EPS when the fermentation process was conducted in an airlift reactor. After purification, one EPS showed glucose, while the other showed arabinose as its primary building block molecule.

EPS biosynthesis pathway

The biopolymers, produced by different types of microorganisms, can be categorized into four significant groups, such as polyesters, polyamides, inorganic polyanhydrides and polysaccharides. Bacterial EPS are typically produced within the cell and secreted into the extracellular environment. The homopolysaccharides like dextran, levan, and mutan are synthesised outside the microbial cells by the catalytic action of enzymes from the respective bacteria, and sucrose acts as the substrate for these enzymes. The proteins which help to transform the substrate into the polymer can be categorized into four groups. The first group is hexokinase (intracellular enzyme) type, which phosphorylates glucose to glucose-6-phosphate. The second group is also a kinase, pyrophosphorylase is among the one that catalyses the conversion of glucose-1-phosphate to uridine-diphosphate glucose, which plays as a vital molecule in the EPS synthesis. The third group of enzymes is glycosyltransferases that are found in the periplasmic membrane of the cell. The glycosyltransferase enzyme transports the sugar nucleotides. The fourth group of enzymes is tangled in the polymerisation of the molecules which are located outside the cell membrane as well as with the cell wall of bacteria.

Nutrient imbalance favours exopolysaccharide synthesis among the microorganisms. In general, microorganisms, in the presence of significant amount of utilizable carbohydrates, boost polysaccharide production. The favourable conditions in the growth medium of microorganisms use C:N, C:P, and C:S. But these favourable conditions are not general in practice. Besides, for one microorganism, it may not necessarily be as favourable or even suitable as it may be for another microbe in the same situation.

The mutual precursors for the biosynthetic pathway of EPS production were reported by nucleotide diphosphates (NDPs) and nucleotide monophosphate (NMP) molecules. These two molecules act as an activated donor during the glycosyltransferase-catalysed transfer of the sugar to a lipid carrier. Bacterial EPS production can occur through four different pathways: (1) Wzx/Wzy-dependent pathway, (2) ATP-binding cassette (ABC) transporter-dependent pathway, (3) synthase-dependent pathway, and (4) extracellular synthesis-mediated by a single sucrase protein. The details of the pathway are illustrated in Fig. 2. It was observed that most of the microbial cell-surface-attached EPS are produced via the Wzx/Wzy-dependent pathway. The Wzx/Wzy was generally named after the proteins involved in this pathway. The lipid-linked sugar units were synthesised by the catalytic action of glycosyltransferase enzymes (highlighted with yellow in each diagram of Fig. 2) located at the interface of the cell cytoplasm and the membrane. The essential constituents of this pathway were the three integral inner-membrane regulatory proteins, namely Wzx flippase, Wzz chain-length regulator, and Wzy polymerase. The lipid-linked repeat

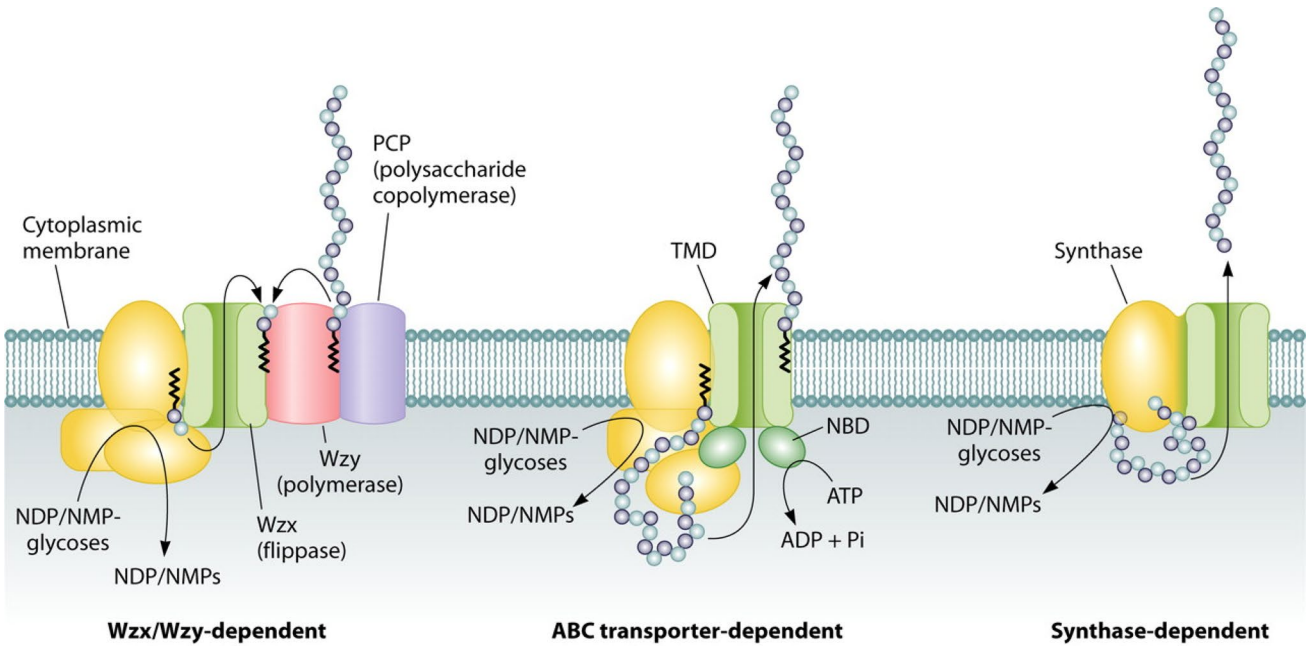

Fig. 2 Schematic diagram (abridged) summarizing the biosynthetic pathways involved in bacterial EPS synthesis by bacteria and their trans-cytoplasmic membrane export. *ADP* adenosine diphosphate; *ATP* adenosine-tri-phosphate; *TMD* transmembrane domain NDP, nucleoside diphosphate; *NMP* nucleoside monophosphate (Cuthbertson et al. 2010)

units were exported via the Wzx flippase and polymerized at the periplasmic region of the membrane. The polymer was expanded in a block-wise process, by amalgamations of repeated units at the reducing end of the glycan chain, through the Wzy polymerase enzyme-catalysed reaction (Islam and Lam 2014).

The carbohydrate chains were extended after the addition of monomers to the non-reducing terminus of a lipid-linked intermediate in the ATP-binding cassette (ABC) transporter-dependent pathway. The chain elongation process was completed in the cytoplasm, followed by the exportation by ABC transporters (Freitas et al. 2011). Although the differences of polysaccharide-assembling mechanism exist between Wzx/Wzy and ABC transporter system, both the secretion pathways were reported to use analogous protein families to facilitate the EPS production. The synthase-dependent exopolysaccharide secretion can occur without any influences of lipid acceptor molecule. Depending on the characteristics of polysaccharide, a single protein was supposed to act both as a polymerase and as an exporter. In this process, membrane-embedded glycosyltransferase protein assisted simultaneous polymer formation and translocation across the inner membrane (Hubbard et al. 2012). It can be observed that in the above-mentioned three mechanisms, the precursor molecules were transformed into the cell by enzymes and yielded activated mono-saccharides or monosaccharides with a carboxyl group. On the other hand, accumulation of monosaccharides was possible by cleavage of di- or trisaccharides through extracellular production pathway.

EPS biosynthesis comprises three phases; during the first phase, assimilation of carbon substrates occurs, second phase comprises intracellular syntheses of the polysaccharides, and exudation of polysaccharides from the cells take place in the third or the final stage. The relationship between exopolysaccharide biosynthesis and glycolysis in *Lactococcus lactis* is illustrated in Fig. 3, which indicates that two metabolic targets and their corresponding genes were involved (Ramos et al. 2001). This can help to engineer the pathway of EPS-production process. Lavender et al. (2002) proved that a lipid carrier is always required for peptidoglycan as well as EPS biosynthesis. Modification of the gpsA and galU genes was reported to improve EPS production (Levander et al. 2002). The researcher cloned a gene which translated into UDP-glucose pyrophosphorylase enzyme in *Streptococcus thermophilus*, and the researchers also proposed a pathway to enhance exopolysaccharide production, thereby exploiting these bacteria by metabolic engineering approach (Fig. 4). Although the flux-controlling function of these enzymes, i.e. PGM, GalU, and GalE involved in enhanced EPS production was not established. The above-mentioned study reported the highest EPS yield, 0.36 g/mol of carbon, after knocking out the gene *pgm*A in Gal + strain.

Han et al. (2017) described the simplified biosynthesis pathways of exo-polysaccharide for a blue–green alga *Nostoc flagelliforme* (Fig. 5). They also reported that the essential pathways, along with the important enzymes (UDP-glucose pyrophosphorylase and UDP-glucose dehydrogenase), are involved in typical EPS biosynthesis process (Han et al. 2017).

Dextran is known as the primary contaminating EPS accumulated during sugarcane deterioration which is synthesised by dextransucrase enzyme (EC 2.4.1.5) using sucrose as a precursor molecule. This dextransucrase extracellular

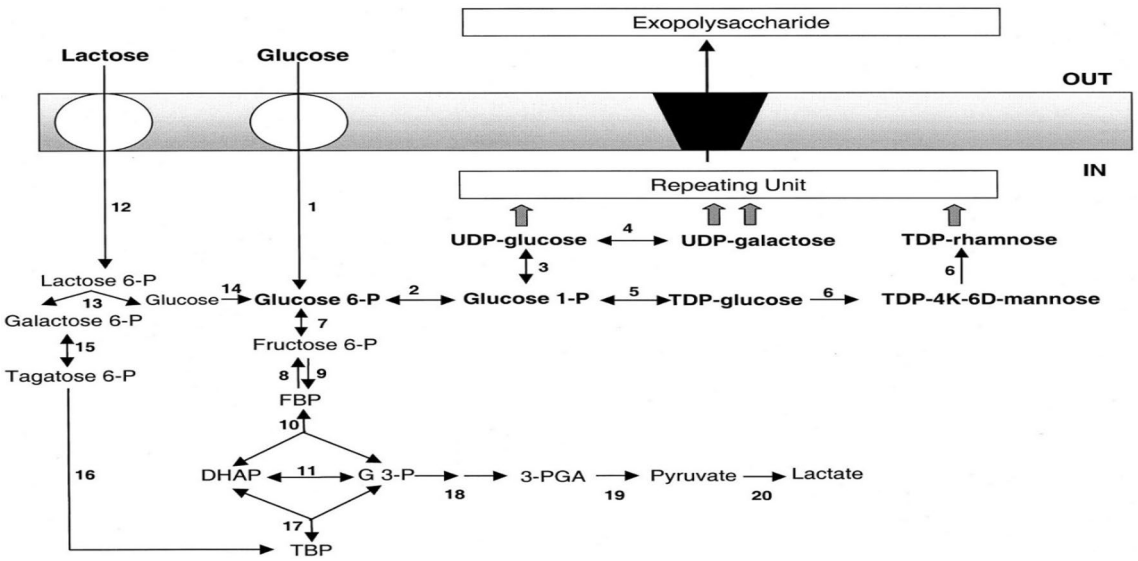

Fig. 3 Projected pathway of EPS biosynthesis in *L. lactis* (Ramos et al. 2001). The figure displays that ^{13}C label at position C-6 does not get incorporated in the EPS repeating units

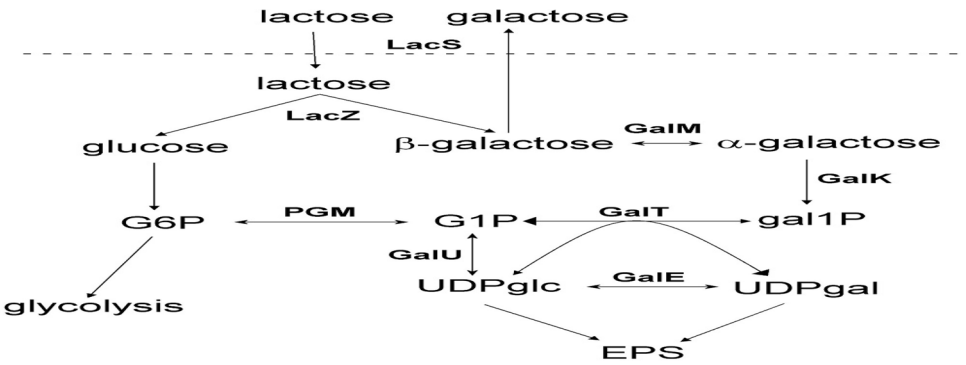

Fig. 4 Metabolic pathways for EPS production in *Streptococcus thermophiles*. *GalE* UDP glucose 4-epimerase; *GalK* galactokinase; *GalM* mutarotase; *gal1P* galactose 1-phosphate; *LacS* lactose transporter; *GalT* galactose 1-phosphate udridyltransferase; *GalU* LacZ, *β*-galactosidase; *PGM* *α*-phosphoglucomutase; UDP glucose pyrophosphorylase (Levander et al. 2002). *S. thermophiles* follows Leloir pathway, in which UDP galactose (UDPgal) and UDP glucose (UDPglc) may be produced either from the galactose or glucose moiety of lactose. Enzyme, phosphoglucomutase (PGM), connects the Leloir pathway with glycolysis, catalyses inter-conversion of molecules of glucose 1-phosphate (G1P) and glucose 6-phosphate (G6P)

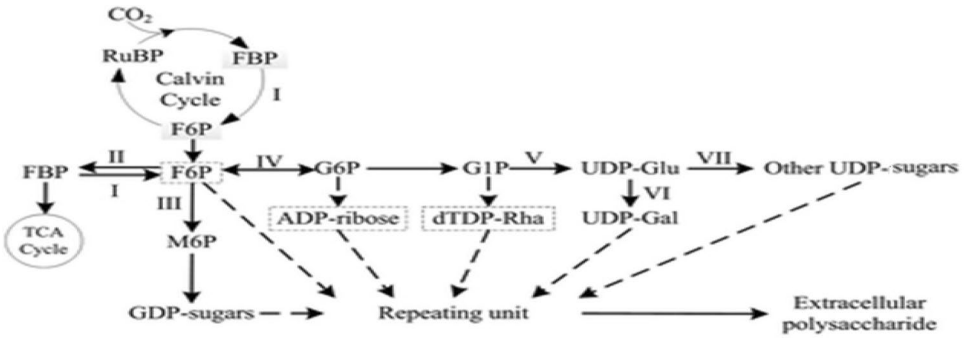

Fig. 5 Biosynthesis pathways of EPS from *N. flagelliforme*. The boxes and dashed arrows (shown in the figure) indicate precursors of EPS and putative biosynthesis pathways, respectively. *I* fructose-1,6-bisphosphatase (FBPase); *II* phosphofructokinase (PFK); *III* phosphomannose isomerase (PMI); *IV* phosphoglucose isomerase (PGI); *V* UDP-glucose pyrophosphorylase (UGPase); *VI* UDP-galactose-4-epimerase (UGE); *VII* UDP-glucose dehydrogenase (UGDH) (Han et al. 2017)

enzyme is usually produced by *Leuconostoc, Lactobacillus*, and *Weissella* spp.

The propagation of dextran chains is catalysed by dextransucrase. The enzyme facilitates the addition of a glucosyl radical derived from the sucrose molecule at the C6-hydroxyl of the non-reducing, terminal glucose unit of a growing dextran chain (Fig. 6). Fructose units accumulate in the medium along with dextran polymer. Formation of dextran polymer continues till the entire sucrose present in the medium exhausts (Hehre 1951).

Producer of EPS

Different types of microbial exopolysaccharides were studied in the last decade by various researchers, but a meagre amount of useful information exists regarding their large-scale production. Numerous factors can affect the EPS-production process, among which some of the important factors, such as the composition of fermentation medium (broth) and the fermentation conditions (aeration rate, temperature, pH, etc.), play a key role in increasing the yield of EPS. Several categories of optimisation have been undertaken to enhance the productivity (Table 1).

The molecular mass of EPS molecules is a crucial feature of a polysaccharide as this characteristic directly influences the rheological properties of the EPS solution (Ketabi et al. 2008). Knowledge about conditions of EPS production and cellular-regulation mechanisms are essential for the production of starting cultures and their processing into ready-to-use formulations, mainly when microorganisms produce high concentrations of EPS. Several strategies are being established by various researchers to arbitrate the entire process at the genomic level for the effective improvement of EPS productivity.

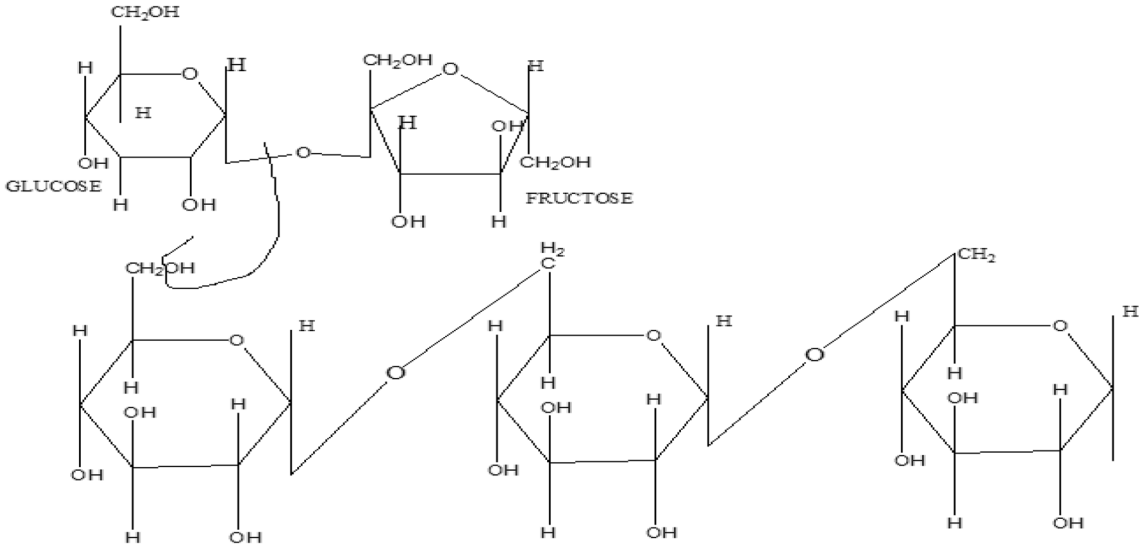

Fig. 6 Formation of dextran molecule from sucrose molecule. Degradation of sucrose by sucrose enzyme leads to formation of dextran and fructose which includes initial breakdown of substrate into fructose and glucosyl residue, ultimately links with glucose moiety at the non-reducing end of dextran chain

Table 1 Optimisation tools used by different researchers for enhancing EPS production

Name of microorganism	Optimisation technique used	Optimised media components	Product yield (gL^{-1})	References
Lentinus edodes	Box–Behnken design, Plackett–Burman design (PBD)	Glucose, yeast-powder and pH	0.751	Feng et al. (2010)
Cordyceps taii	central composite design, Plackett–Burman design (PBD)	Xylose $(NH_4)_2SO_4$, and vitamins A and D	43.87	Xiao et al. (2010)
Armillaria luteo-virens Sacc	Fractional factorial design (FFD) and central composite design (CCD)	glucose, yeast extract powder, K_2HPO_4, KH_2PO_4, $MgSO_4$, pH unadjusted	5.40	Jiao et al. (2008)
Agrocybe cylindracea	Orthogonal matrix method	Maltose Martone A, $MgSO_4$ and $CaCl_2$	3	Kim et al. (2005)
Fusarium solani	One variable at a time method and response surface methodology	Glucose, yeast extract, KCl, KH_2PO_4, with medium pH	2.276 ± 0.032	Mahapatra and Banerjee (2013)
Fomes fomentarius	Orthogonal matrix method	Glucose, silkworm chrysalis, yeast extract, $CaCl_2$, and $MgSO_4$	3.64	Chen et al. (2008)
Pseudomonas fluorescens	Plackett–Burman design (PBD), central composite design (CCD)	Rice bran peptone NaCl $MnCl_2$	4.62	Sirajunnisa et al. (2016)
Bacillus licheniformis	Response surface methodology	Yeast extract, sodium succinate and sucrose	48.57	Malick et al. (2017)
Agaricus brunnescens	Orthogonal methods	Wheat bran, Glucose and peptone	12.13	Ban et al. (2015)
Lactobacillus acidophilus	Plackett–Burman design (PBD) and central composite rotatory design (CCD)	Sucrose, Yeast extract, salts [KH_2PO_4 and $MgSO_4$ (1:1)], Sodium chloride	0.4	Deepak et al. (2016)

Bacteria

The EPS harvested are widely used in food, chemical industries as bio-flocculants, bio-absorbents, etc. Most researchers worked on *Alcaligenes, Leuconostoc, Xanthomonas, Sphingomonas* spp. and *Pseudomonas* genera, which synthesise EPS, such as curdlan. gellan, xanthan, dextran from various agricultural waste, etc. (Padmanaban et al. 2015). Maalej et al. examined the EPS-production parameters of *Pseudomonas stutzeri* in the submerged fermentation process (Maalej et al. 2015). Maximum EPS production of 10.2 gL^{-1} was reported after 24 h of incubation at 30 °C at 250 rpm stirring level with a pH of 8·0 and 10% inoculum size (Kho et al. 2016). EPS-producing LAB (Lactic acid bacteria) are industrially important microorganisms for manufacturing different functional food products and fermented food, such as cheese, dahi, yoghurt, and cereal-based product. Exopolysaccharide from *Lactobacillus brevis* was studied on the surfaces of selected food powders to evaluate adhesion behaviour. The results exhibited improved food powder and better adhesion when EPS was used (Karasu and Ermis 2019). EPS (glucomannan) from a food grade, *Lactobacillus plantarum*, was extracted having a molecular weight 2380 kDa and reported to have profound anti-diabetic, antioxidant and cholesterol-lowering properties (Sasikumar et al. 2017). Reuteran, a specific α-glucan, is another promising EPS produced by the species *Lactobacillus reuteri* and is generally associated with thickening of fermented milk products (Meng et al. 2015b).

Fungi

A number of yeasts from diverse ecological niches, higher basidiomycetes, and several filamentous fungi have been identified for their capability to produce EPS in the laboratory level. A majority of the published research articles illustrated about the liquid-submerged fermentation methods for EPS production. Several researchers apply statistical optimisation techniques including Box–Behnken design, central composite design, Plackett–Burman design, or fractional factorial design for the optimisation of various modes of fermentation route of the EPS-production process. Bae et al. (2001) reported the influence of different carbon resources on EPS production using *Paecilomyces japonica* and claimed that disaccharide maltose was the favourable source of carbon which yields EPS production approximately 30 gm/L, whereas the same microorganism at the same fermentation condition produced only 25 gm/L EPS while using sucrose (Bae et al. 2001). Most of the fungi which can produce EPS were either facultative anaerobic or aerobic and claimed that oxygen limitation in fermentation broth did not facilitate EPS production. Sodium nitrate was reported to be preferable for the production of epiglucan by *Epicoccum nigrum.* The yield of scleroglucan increased four times by *S. rolfsii* when grown in 150 gL^{-1} sucrose (Farina et al. 2001).

Yang et al. (2000) observed the consequences of supplementary fatty acids on the growth of *Ganoderma lucidum* and EPS production by the submerged and immobilized fermentation process. Their study reported that less than 0.25 gm/L oleic acid and palmetic acid in the suspension culture of *Ganoderma lucidum* supported the EPS production. However, linoleic acid had a reductive effect on the EPS production. Significant impact on EPS production in immobilized cultures was reported after supplementation with only palmitic acid (Yang et al. 2000).

Cyanobacteria

Isolation and optimisation of fermentation conditions, for exopolysaccharide production from various cyanobacteria, were studied by various researchers. Their study reported that cyanobacteria might produce a right amount of EPS, which comprises a substantial amount of organic carbon (Chen et al. 2014). Ciu et al. (2017) worked on the consequences of the exopolysaccharide on the growth rate and the morphogenesis of the *Nostoc flagelliforme* (a filamentous cyanobacterium). Their study indicated that the EPS matrix formed by *Nostoc flagelliforme* might serve as a basal barrier, which helps to bend trichomes throughout their elongation, although prolonged growth reported to be helpful to their straight elongation. These research findings can help to direct future production and application of *Nostoc flagelliforme (*Cui et al. 2017). Morris et al. (2001) reported aqueous solutions of the EPS produced by *Cyanospira capsulata* and *Aphanothece halophytica* exhibited xanthan-like physical properties (Morris et al. 2001). Mancuso et al. (2009) studied on 800 microalgal cultures from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Collection of Living Microalgae (CCLM) for exopolysaccharide production by examining the viscosity of supernatants from optimised media. Their study claimed that maximum viscosity of EPS (produced by a non-axenic isolate of *Microcystis aeruginosa f. flosaquae.*) observed was 6.55 cP which is equivalent to 1.16 gL^{-1} xanthan gum. This adhesive can tie wooden lap joints with a shear strength of 1.5 MPa, which is four times higher than the shear strength observed in the similar tests using polyvinyl acetate (PVA) glue (commercial grade) as a substrate (Mancuso Nichols et al. 2009). Leung et al. (2009) measured the viscosity of the liquid culture of EPS sample produced by the mycelia *Cordyceps sinensis.* The harvested samples (from each day) were dissolved in a distilled water, and the viscosity of the resultant solutions was measured using a viscometer at 25ºC in a temperature-controlled room. The observed intrinsic viscosity of the EPS displayed a noticeable increase with the

culture period from day 4 (800 mL/g) to day 5 (1150 mL/g) of fermentation and became relatively stable in the remaining period from day 5 to day 8 (Leung et al. 2009). Bellezza et al. (2006) isolated two heterocystous cyanobacteria, Scytonema *ocellatum* and *Fischerella maior*, and analysed the exopolysaccharides produced. This study reported neutral sugars, such as glucose, arabinose, and the charged galactouronic acid as the primary source of carbon. The spectrum of circular dichroism (CD) at varying pH and temperature revealed that these polymers did not represent a structured, organized conformation at an ambient temperature but followed a random coil model (Bellezza et al. 2006). A consolidated comparison of the yield using different microorganism, i.e. bacteria, yeast or algae is shown in Table 2.

EPS yields from bacteria are always higher when compared to yeast and algae, but the maintenance of culture and fermentation conditions is much easier in yeast or algal cultures.

Studies have indicated that with the use of appropriate technology, maximum extraction of EPS from microorganisms can be achieved. Tiwari et al. characterized 40 different types of cyanobacteria strains for EPS production that had a bio-flocculent property and suggested that EPS derived from both *Anabaena* sp. and *Nostoc* sp. can be used commercially as a substitute for synthetic and abiotic flocculants available in the market (Tiwari et al. 2015). Mohanpriya et al. (2014) worked on ten different cyanobacteria strains for exopolysaccharide production. Optimisation was done to increase the production of exopolysaccharide using different parameters like pH, temperature, carbon sources and time of incubation. It was reported that *Chroococcus* sp. can produce a high amount of exopolysaccharide (55 mg/mL) in a growth medium containing 1% sucrose, at a pH of 7.5 and incubation temperature of 25 °C in 9 days. Kiran et al. (2008) reported the potentiality of chromium adsorption using EPS produced by *Lyngbya putealis.* The effect of different variables on chromium adsorption using EPS with various parameters, such as pH of the medium (2–6), metal-ion concentration (10–100 mg/L), and temperature (25–45 °C), was investigated. Results revealed that a pH of 2 and a temperature of 45 °C of the medium were most optimal for biosorption of chromium by the exopolysaccharides secreted from cyanobacteria. Albeit the study reported that the adsorption capacity of EPS increased from 45 to 157 mg/g as the initial Cr(VI) concentration increased from 10 to 30 mgL^{-1} (Kiran and Kaushik 2008). Exopolysaccharide-producing *Bacillus licheniformis* was also studied to adsorb mercury, and more than 70% mercury was removed by 25 mg dried biomass of *Bacillus licheniformis* at pH 7.0 under optimum reaction condition (Upadhyay et al. 2017). Dayananda et al. (2007) worked on the growth of *Botryococcus braunii* for the production of exopolysaccharides using various autotrophic media, such as the modified Chu-13 medium, bold basal medium (BBM), blue–green medium (BG11), and bold basal with ammonium carbonate (BBMa). The study reported that BG11 was the best medium for biomass and EPS production. Homogeneous illumination and continuous stirring at 90 rpm had a maximum impact on EPS production (Dayananda et al. 2007). *Chlamydomonas reinhardtii* strain was isolated by Bafana (2013) based on its ability to secrete exopolysaccharide, which had a molecular weight of 2.25×10^5 Da, and showed a febrile structure with a sheet-like appearance on the surface with a significant antioxidant activity. The production medium (pH 7) consisted of $CaCl_2$ (74 mgL^{-1}), $NaNO_3$ (422 mgL^{-1}), K_2HPO_4 (10 mgL^{-1}) and $MgSO_4$ (200 mgL^{-1}). The maximum EPS production was reported to be 628 mgL^{-1} (Bafana 2013). Mishra et al. (2011) characterized the EPS produced by *Dunaliella salina* and found it to be a promising candidate for industrial exploitation (Mishra et al. 2011). Khattar et al. (2010)

Table 2 Comparison of EPS yield produced by different microorganisms

	Name	Substrate	EPS	Yield ($g\ L^{-1}$)	References
Bacteri a	*Xanthomonas campestris*	Glycerol	Xanthan	11	Wang et al. (2016)
	Pseudomonas sp	Sucrose	Curdlan	5.92	Yang et al. (2016)
	Bacillus licheniformis	Succinate/ Sucrose	Heteropolymer	48.57	Malick et al. (2017)
	Phyllobacterium sp.	Sucrose	Heteropolymer	21.9	Li et al. (2017)
	Bacillus velezensis	Molasses	Heteropolymer	7.88	Moghannem et al. (2018)
Yeast	*Candida guilliermondii*	Maltose	Heteropolymer	2.98	Gientka et al. (2016)
	Sporobolomyces salmonicolor	Sucrose	Similar to Manan	5.64	Poli et al. (2010)
	Aureobasidium pullulans	Sweet potato	pullulan	9.3	Padmanaban et al. (2015)
Algae	*Rhodella violacea*	Modified F/2 medium	sulphated and xylose	0.59	Villay et al. (2013)
	Microcoleus vaginatus	BG11	Heteropolymer	0.36	Ge et al. (2014)
	Neochloris oleoabundans	Lactose	Heteropolymer	5	Wu et al. (2011), Moghannem et al. (2018)

worked on batch fermentation (21 days) using filamentous cyanobacteria *Limnothrix redekei* and reported 304 µg EPS/mL culture. The study reported that maximum EPS production was observed during the initial days of growth, and the rate of production was about 313 µg EPS/mg protein/day. The production rate reduced to 140 µg EPS/mg protein/day after 21 days of growth (Khattar et al. 2010).

Recovery of extracellular microbial polysaccharides from the culture broth, i.e. downstream processing, usually involves the following steps: (1) harvesting the microbial cell, which includes dilution by addition of deionized water into the culture broth, followed by centrifugation, (2) precipitation of polymer (EPS) from the cell-free supernatant by the addition of a precipitating agent, such as ethanol, isopropanol, etc., in which the polymer is insoluble at low temperature; besides this, the addition of salt for salting out or protein precipitation with trichloroacetic acid and the addition of enzymes (Wang et al. 2007) are also practiced, (3) dialysis (for removing excess salt), and finally, (4) drying of the precipitated EPS using freeze-drying (laboratory scale) or drum-drying method (Bramhachari et al. 2007).

Properties of exopolysaccharide

Rheological property

The word rheology derived from the Greek word "*rheos*" which means "to flow". Therefore, rheology is the study of movement and deformation during the flow of material. The rheological properties validate the potential applicability of EPS in various fields of biotechnology. Depending on the flow behaviour observed in aqueous media or fermentation broth, the properties of the fluid also change accordingly, which can be broadly classified as linear and dynamic viscoelasticity and thermo-viscoelasticity property. Stability of viscoelastic properties of EPS under extreme conditions of pH, salinity, and temperature as well as emulsification ability is the desirable feature. Xanthan gum has high viscosity and pseudoplastic behaviour, thereby justifying its application as a stabilizer and thickening agent (Sajna et al. 2013). Liu et al. (2011) observed the viscosity of the crude EPS solution produced by *Zunongwangia profunda* (habitant of deep sea) at different shear rates (1, 2, 10, 15, 20, 40, 60 rpm) and reported that the viscosity of the EPS was directly proportional to the shear rate, which displayed non-Newtonian behaviour in solution. The aqueous dispersions of the EPS showed a shear-thinning behaviour with pseudoplastic properties within the shearing rate ranges mentioned above (Liu et al. 2011). Vasanthakumari et al. (2015) examined rheological characterisation of dextran-like high molecular weight polysaccharide (3037 kDa) from the culture broth of *Weissella cibaria.* The study revealed that the EPS produced by *Weissella cibaria* showed pseudoplastic behaviour. The flow index value of EPS solution was 0.677. With increasing shear stress, the apparent viscosity of EPS solution was reduced. These phenomena indicated the non-Newtonian behaviour of EPS solution. The results revealed that EPS can be applied in the food industry as a stabilizing, thickening and gelling agent (Vasanthakumari et al. 2015). Velasco et al. (2009) (Fig. 7) claimed in their study on *β*-glucan (EPS) from *Pediococcus parvulus* which exhibited a non-Newtonian pseudoplastic behaviour with a shear thinning zone at high shear rates. Though a linear plateau was observed at low shear rates which signified Newtonian behaviour of the fluid, entanglements were observed (Fig. 7) between β-glucan chains by shearing forces which reflected the reduction of viscosity. Low viscosity is always favourable in industrial operations, such as pumping and mixing. Low viscosity also enhances the sensory qualities (mouth fullness, flavour release, etc.) of the finished food products (Velasco et al. 2009).

Thermostability

Thermo gravimetric analysis (TGA) is carried out dynamically between temperature and weight loss for EPS, as done with all polymers, by subjecting to different temperatures. The primary events that occur with the initial increase in temperature are gelatinization and swelling, while further increase in temperature causes dehydration and pyrolysis of the exopolysaccharides. A significant characteristic

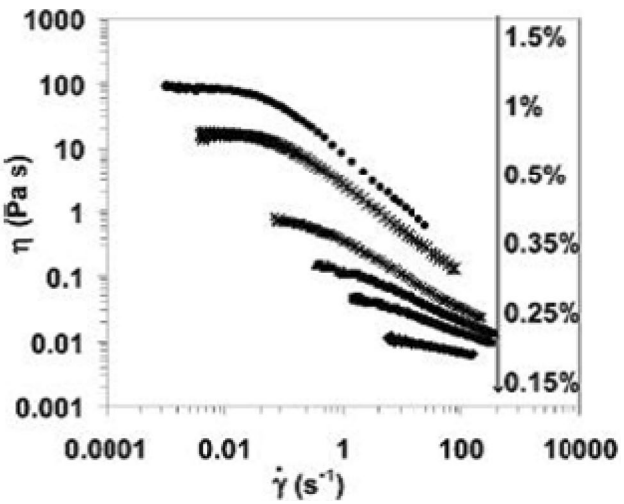

Fig. 7 The viscosity of *β*-glucan solutions in water (*η*) as a function of shear rate ($\dot{\gamma}$) at constant temperature 10 °C (Velasco et al. 2009). Viscosity enhancement with polysaccharide concentration can be observed. *β*-glucan (all concentrations) exhibited non-Newtonian pseudo plastic behaviour with a shear thinning zone at high shear rates. Newtonian or linear plateau can also be noticed at low shear rates, defines a shear rate independent viscosity

considered for industrial application of EPS is thermal stability, especially in the food industry, since most of the manufacturing and processing of the food and allied products are carried out usually at high temperatures (Sajna et al. 2013). Most of the researchers conduct TGA experiment in a nitrogen atmosphere to find out the thermostability of a polymer with a heating rate of 10° C/min. Most EPS types endure degradation in three different phases; Fig. 7 depicts the EPS degradation profile based on the work by Kanamarlapudi et al. (2017) using the EPS derived from *Streptococcus thermophiles* (Kanamarlapudi and Muddada 2017). The thermogram (Fig. 8) displayed an initial weight loss between 50 and 110 °C, which is due to the loss of moisture. Many reports advocated that the initial loss of moisture was due to the carboxyl groups that were present in high level and bound to water molecules. The dotted line in the figure showed the release of energy that represented an exothermic process with a maximum temperature of 355 °C, where the maximum mass loss was observed (Kanamarlapudi and Muddada 2017). In most cases, the first event, i.e. loss of mass, occurred between 25 and 102°C; this first loss of mass event was associated with the loss of moisture present in it. Thermostability of EPS secreted by the endophytic bacterium *Bacillus licheniformi* was found to be very high. Maximum loss (59.6%) at ≥ 330°C was observed (Ravindra Pal Singh 2011). EPS (levan) derived from *Leuconostoc mesenteroides* S81 (isolated from traditional sourdough) also showed a high level of thermalstability. The weight loss (20–25%) of levan was initiated between 200 and 250 °C temperature. The weight loss was reported to be around 40% when the temperature reached 300 °C, and 70% weight loss was reported at a temperature of 800 °C (Taylan et al. 2019). In general, heat treatment denatures the polysaccharide structure, but the thermostable EPS can be used in food processing industries where high temperature is required for the preparation of food items.

Applications of EPS

Research on exopolysaccharide is like a "Cinderella field" as it has an immense promising potential both from an industrialist and a scientist. Microbial EPS, owing to their significance in chelation of heavy metals, nutrient sequestration, detoxification of hazardous chemicals, and shielding effect against osmotic shock, opens up new possibilities of employment generation through industrialisation. The EPS matrix functions as a protective physical barrier and even if antimicrobials can penetrate, biofilm cells are usually in stationary phase and phenotypically well adapted to withstand stress. Interesting properties of microbial exopolysaccharides facilitated a broad spectrum of applications in the field, e.g., coagulation, stabilization, thickening, gelling, and water retention capabilities. Therefore, EPS may be useful in industries involved in the production of detergents, adhesives, paper, paint, food, textile, etc. Neutral polymer dextran having α-(1 → 4) and α-(1 → 6) glucopyranosyl linkages can be considered in confectionary products (Wang et al. 2015; Palma et al. 2015). Dextrans were reported to increase viscosity and moisture retention property and also inhibit sugar crystallization of the final product.

Palma et al. (2015) reported a coating material for iron oxide magnetic nanoparticles from fucose-containing exopolysaccharide known as Fucopol. These EPS molecules

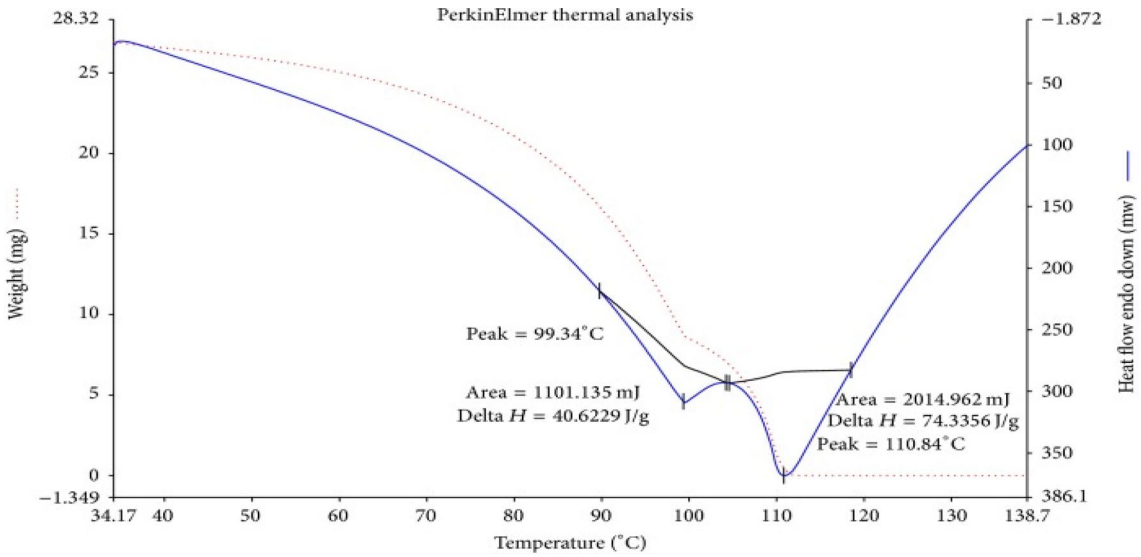

Fig. 8 Thermogram of EPS produced by *Streptococcus thermophiles* (Kanamarlapudi and Muddada 2017). During initial increase in temperature, EPS showed gelatinization and swelling after which increase in temperature caused dehydration

were produced from glycerol by the bacterium *Enterobacter* A47 DSM 23,139. Gellan is an anionic linear heteropolysaccharide with a recurring unit of α-rhamnose (Palma et al. 2015). Since 1990, FDA approved Gellan (two residues of *β*-d-glucuronate and *β*-d-glucose) as a thickener and a stabilizer in food. It was also used as a disintegration agent used for manufacturing tablets, and at higher concentrations as a matrix-forming excipient for slow release of the drug (Osmałek et al. 2014). Exopolysaccharide produced by lactic acid bacteria have similar characteristics, such as water retention properties, ability to form networks, etc. EPS contribute to the texture, taste perception, and stability of the final milk-based dairy products (Jung 2008).

Curdlan is an industrially important EPS derived from bacteria. The sulphate salt of curdlan is a promising vaccine adjuvant as it increases antigen-specific immunity in mice that are immunized with a human-recombinant hepatitis B protein (Li et al. 2014). Pullulan is a linear homopolysaccharide consisting of repeating α-(1 → 4)–maltotriosyl units (3-d-glucopyranosyl) and joined through α-(1 → 6). Gupta et al. (2004) reported a novel method for enhancing the delivery of nucleic acid molecules into the cells by encapsulating them inside the nanoparticles of hydrogel pullulan (Gupta and Gupta 2004). Hydrophobic poloxamer carboxymethyl pullulan microparticles were used for the controlled release of tetanus toxoid (Mocanu et al. 2011).

Xanthan is a branched anionic heteropolysaccharide. The building block molecules of xanthan are five-carbon sugar repeating units and different amounts of acetate and pyruvate. Initially, xanthan finds extensive use in oil refinery industries for enhancing oil recovery. Xanthan was also recognised as one of the permitted food additives in both the USA and Europe (1969). Beside, xanthan is also used as a stabilizer, emulsifier, and suspending and thickening agents in food industries. It is often used in combination with guar gum to enrich food quality. Anionic polymer alginate is composed of *β*-(1 → 4) mannuronic and *α*-(1 → 4) guluronic acid. It acts as an excellent disintegrating agent in tablets and antacid stomach protector in capsules. The sodium salt of alginate is widely used for cell microencapsulation. Apart from making suspensions, emulsions, and thickening and stabilizing agents, alginate is also used in microsphere vectors for drug delivery in pharmaceutical and drug industries, and as a matrix or delivery system for fertilizers, pesticides, and nutrients to crops in agriculture-related industry.

Fungal *β*-glucans show variable activity against diseases like mammary cancer, colon cancer, sarcomas, etc. (Laroche and Michaud 2007). Schizophyllan and scleroglucan both were reported as the promising EPS antitumor in diseases like carcinomas, sarcoma, fibro sarcoma, mammary carcinoma, etc.) and immune-boosting agent (Farina et al. 2001). These fungal EPS are made up of *β*-(1 → 3)-d-glucopyranose backbone, which is again branched with a single *β*-(1 → 6)-d-glucopyranose residue at every third glucose unit produced by *Schizophyllum commune* and *Sclerotium rolfsii* (Zhang et al. 2013). Botryosphaeran produced by *Botryosphaeria rhodina* is another fungal-secreted EPS which is composed of *β*-(1 → 3, 1 → 6)-glucan (1 → 3 backbone, 1 → 6 branched glucose and gentiobiose). This EPS exhibited anti-clastogenic activity in vivo in mice after cyclophosphamide, anti-diabetic and hypo-cholesterolemic activities (Miranda et al. 2008). *Pycnoporus sanguineus* fungus-derived heteropolysaccharide (Hetero EPS; composed of mannose, glucose, galactose, xylose) also exhibited in vitro antioxidant activity (Cao et al. 2014). *Lasiodiplodia theobromae*, a fungal plant pathogen produced Lasiodiplodan exopolysaccharide [*β*-(1 → 6)-d-glucan] which exhibits anti-proliferative activity in breast cancer MCF-7 cells, and its sulphonated derivative exhibits heparin-like anticoagulant and antithrombotic activities. Meng et al. (2015a, b) reported that EPS produced by *Hirsutella* sp. was neutral in nature and this EPS (containing mannose, glucose, galactose) exhibited excellent antioxidant property, and the importance of mannose in EPS structure was also explored. The study revealed that the EPS derived after submerged fermentation of *Inocutis tamaricis* too shows in vitro antioxidant and antitumor (Hep G2 cells) activities (Meng et al. 2015a). Inturri et al. (2017) reported that EPS synthesised by *Bifidobacterium longum* W11 has an antioxidant capability with an absence of toxicity on human fibroblasts (MRC-5) by MTT assay. It was further confirmed that the highest EPS-W11 concentration tested (2 mg mL^{-1}) did not exert a cytotoxic effect. To check the antioxidant properties of EPS-W11, the levels of intracellular ROS and RSH in MRC-5 cells were determined, and a significant ($p < 0.05$) rise in intracellular ROS level was recorded after treatment with 300 μM H_2O_2 (Inturri et al. 2017).

Exopolysaccharides from lactic acid bacteria were reported as non-Newtonian and nontoxic in nature which facilitated most of the processes used in fermentation and food industry. The addition of EPS in fermented food products reported to influence viscosity, firmness as well as sensory properties (Mende et al. 2016).

EPS-producing microorganisms along with EPS are also used in the remediation of environmental effluents or wastewater treatment process. Biofilm-mediated remediation process is reported as useful and harmless because microorganisms growing within a biofilm have more chances of adaptating to different environmental conditions and their subsequent survival. The potential role of EPS for removing the heavy metals from the wastewater is due to their property of flocculation and capability to chelate with metal ions in solution.

Researchable issues and future prospects

Although a majority of research on EPS focused on isolation, followed by characterisation from different microbial sources, the EPS molecules harvested from different microorganisms exhibited interesting and specific physicochemical and biological properties which need more effective commercial exploitation. Exploiting several structural features of polysaccharides, after synthesis through different EPS biosynthetic pathways, is an appealing area for a synthetic biologist. The primary complications which need to be solved include substrate specificities and facilitating non-hindrance secretion of polysaccharides from microorganisms using tools of metabolic engineering of different EPS-originating pathways. Based on the present state of research, nucleotide sugars appear to be feasible precursors for EPS biosynthesis. A deep knowledge of structural insights, during the interactions of glycosyltransferase enzymes with the substrate and other molecules of the biosynthetic complex, is essential for choosing the most promising molecules to function in novel pathways.

A substantial growing interest is observed in the isolation and characterisation of microbial EPS owing to their importance in various commercial applications, such as nutrient sequestration, osmotic shock-preventing additives, chelation of heavy metals, and detoxification of toxic compounds. Among the EPS-producing microorganisms, bacteria and fungi are most prolific. Production of EPS is directly proportional to the growth of microorganism, which has relevant physiological and ecological functions. Information on the aspects related to genomics and proteomics is very scanty, which can definitely make the EPS production more economically feasible. Another untapped area is the biotechnological potential of EPS from marine and estuarine environment that can yield unique and useful types of EPS. The present review intends to provide a new framework for understanding the research of EPS conducted over the past few years worldwide, and pave new paths of thinking towards addressing problems of economic and sustainable development of the world today.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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