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Bacterial Exopolysaccharides: sources, production and applications

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Review Article

ABSTRACT

Exopolysaccharides (EPSs) are secreted by both eukaryotes (fungi, phytoplankton, and algae) and prokaryotes (archaeobacteria and eubacteria). EPSs produced by bacteria include a broad range of chemical structures and may either be heteropolymeric or homopolymeric. Their molecular weights vary from 10 to 1000 kDa. Exopolysaccharides have multifarious applications in different fields of life. These EPSs are used as anticoagulant, immunomodulator, emulsifiers, anticancer and bioflocculants. They are widely used in food industry and bioremediation. This review article describes sources, synthesis, fermentation, extraction and applications of exopolysaccharides.

Key words: Rheology, humic, biofilm, Neosorb™, xenobiotics, sclerogluca

INTRODUCTION

Microbial polysaccharides are generally classified into three categories according to their functions; capsular polysaccharides such as K30 O-Antigen, intracellular storage polysaccharides such as glycogen, and exopolysaccharides such as dextran, xanthan, cellulose etc. (Schmid *et al.,* 2015). Prokaryotes (archaebacteria and eubacteria) and eukaryotes (phytoplankton, fungi and algae) can produce exopolysaccharides (Kumar *et al.,* 2007). Exoploysaccharides (EPSs) synthesizing enzymes are located in cell wall (Huassain *et al*., 2017).

Bacterial exopolysaccharides include dextran, alginate, xanthan, curdlan, cellulose, succinoglycan, glucuronan, colanic acid etc. Bacteria producing exopolysaccharides are *Acetobacter* spp., *Rhizobium meliloti*, *Pseudomonas aeruginosa*, *Lactobacillus helveticus, Lactobacillus rhamnosus, Xanthomonas* spp., *Shigella* spp., *Escherichia coli*, *Salmonella* spp., *Enterobacter* spp. etc (Ahmad *et al.,* 2015). Several bacteria such as *Geobacillus thermodenitrificans, Bacillus thermantarcticus* etc isolated from intense environments: deep-sea hydrothermal vents, geothermal springs, saline lakes and Antarctic ecosystems have been studied as possible sources of Exopolysaccharides (Freitas *et al.,* 2011).

EPSs have high molecular weights varying from 10 to 1000 kDa and they may be either homopolymeric or heteropolymeric in composition (Nwodo *et al.,* 2012). Mostly EPSs consist of carbohydrates and proteins, but nucleotide, humic substances and lipids are also included (Fleming, 2000).

The characteristics of Bacterial EPSs are identified by their molecular structure, chemical composition, average molecular weight, conformation of single molecules and their assemblies in case of aggregation process and gel systems (Morris *et al*., 2011). In case of concentrated solutions, viscosity and viscoelasticity are determined by rheometry (Iijima *et al.,* 2007; Simsek *et al*., 2009).

EPSs producing microbes are present in diverse ecological niches. High C/N ratio mediums e.g effluents from the paper, sugar or food industries and wastewater treatment plants are rich sources of exopolysaccharides producing microorganisms (Morin, 1998).

Currently mechanisms for the bacterial EPSs production are classified into four types: (1) ATP-binding cassette transporter-dependent pathway (2) Wzx/Wzy-dependent pathway (3) extracellular synthesis (4) synthase-dependent pathway (Donlan & Costerton, 2002; Schmid *et al*., 2015).

A cassette of genes regulates the synthesis of EPSs. Usually, a gene sequence of 12–17 kb is mandatory. After polymerization, the repeating units are excreted extracellularly via the carrier lipids

Author's Contribution: **M.Z. & S.S.I.B**., Designed study concept; **M.H.A**., Did acquisition of data; **M.Z. & S.J**., Analyzed and did interpretation of data; **M.I.,** Did drafting of manuscript

(Sutherland, 2001a). In some bacteria e.g *L. casei subsp. cremoris* instead of chromosomes megaplasmids regulate the EPSs synthesis (Vanhooren & Vandamme, 1998).

Kumar *et al*. (2007) separated EPS producing bacteria from complex or chemically defined (synthetic) media. In case of fermentation of EPSs, no single set of culture conditions assures high EPSs yields, since organisms vary in their C-N source utilization, mineral necessities, pH and temperature (Fazenda *et al*., 2008). Environmental and nutritional conditions control the yield and quality of EPSs. Production of EPS might be improved by controlling the conditions of culture. Physiological properties of medium regulate the degree of EPSs branching, pattern, the relative molecular mass and residues quantity (Kumar *et al*., 2007).

EPSs help in bacterial colony formation. They are either secreted into the medium or remain bound to the outer surface of the cell. They help in biofilm formation (a thin, slimy film of bacteria that adheres to a surface). Total organic matter of biofilm contains 50% to 90% EPSs (Donlan, 2002; Donlan &Costerton, 2002). They outline a defensive layer around the cells and supply as energy and carbon reserves in severe environmental conditions. They also protect against toxins, antibiotics, pathogenesis and symbiosis (Whitfield & Valvano, 1993; Roberts, 1996).

EPSs are economically important because they have multifarious applications in the food, health, environment (remediation, flocculation etc) and industry (Nwodo *et al.,* 2012). They have antiviral, antitumor, immunostimulatory, antioxidant, antiulcer and lowering blood cholesterol properties (Madhuri & Rabhakar, 2014a).

Table I: Characteristics and functional properties of some bacterial EPSs (Nwodo *et al*., 2012).

Bacterial Exopoly-saccharide	**Chemical Composition**	**Molecular Weight (Da)**	**Properties**	**Uses**	**Bacteria strains**
Alginate	Guluronic acid and mannuronic acid	0.3×10^6–1.3×10^6	Film forming, Gelling capacity	Medicine (wound management, surgical dressings etc), Food hydrocolloid	*A. vinelandii* and *P. aeruginosa*
Cellulose	Glucose	~10^6	High tensile strength and Insoluble in most solvents	Foods ,Biomedical-wound healing etc, foods	*Acetobacters spp.*
Curdlan	Glucose	2×10^6-5×10^4	Water insoluble , edible, gel-forming capacity, edible	Heavy metal removal, foods and medicines	*A. radiobacter and R. meliloti*
Dextran	Glucose	10^6–10^9	Newtonian fluid property, high stability, Non-ionic	Chromatographic media, foods, medicines	*L.mesenteriodes*
Colanic acid	Glucose, fucose, galactose and glucoronate	2×10^4–6×10^5	Good gelling capacity	Individual care products and Cosmetics	*Salmonella spp., E. coli*, *Enterobacter* spp. and *Shigella* spp.

Glucuronan	Glucuronic acid	6×10^4–10^5	Thickening and gelling Capacity	Cosmetics products and foods	*G. hansenii S.* and *meliloti* M5N1CS
Xanthan	Mannose, glucuronic acid and glucose	2.0×10^6–50×10^6	Stable at variable range of pH, salt concentrations temperature, high viscosity	Foods, pharmaceuticals, petroleum industry, individual care products and cosmetics	*Xanthomonas* spp.
Succinoglycan	Glucose and galactose	1×10^6–5×10^3	High acid stability and viscosity and	Oil recovery and food	*A. faecalis* var. *myxogenes* 10C3

Sources of EPSs

EPSs producing microbes are present in a variety of ecological niches. High C/N ratio medium contain these microbes such as effluents from the industries of sugar, paper or food and sewage treatment plants (Morin, 1998). They are also present in terrestrial and marine hot springs. There are many microorganisms that produce EPSs as isolated slime in the nearby environment or as attached capsular material (Bajaj *et al*., 2007).

EPSs producing microorganisms include various genera of algae, fungi and bacteria that may be thermophilic, mesophilic, and halophilic. Famous mesophilic bacteria include Lactic Acid Bacteria (LAB). Other mesophilic bacteria are *Escherichia* spp., *Bacillus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Acetobacter* spp., *Aureobasidium* spp., *Escherichia* spp., and *Lactobacillus* spp. Thermophilic archaebacteria are *Sulfolobus, Thermococcus* and *Archaeoglobus fulgidus* (Nicolaus, *et al.,* 1993; Rinker & Kelly, 1996; Lapaglia & Hartzell, 1997). Numerous thermophilic bacteria act as excellent EPSs producers such as *Geobacillus thermodenitrificans, Bacillus thermantarcticus* and *Bacillus licheniformis*. *Methanococcus jannaschii, Thermotoga maritima* and *Geobacillus tepidamans* V264 co-cultures produce a huge amount of EPSs (Kambourova *et al.,* 2009). Many halophilic Archaea such as *Haloferax, Halococcus, Natronococcus, Haloarcula* and *Halobacterium* also produce EPSs (Antón *et al*., 1988; Nicolaus *et al*., 1999; Paramonov, 1998).

Isolation of EPSs producing bacteria

A chemically-defined (synthetic) medium or a complex (undefined) medium is used for bacterial isolation. Watery or mucoid surfaced colonies of these bacteria can be detected macroscopically (Vescovo *et al.,* 1989).

Slime producing bacteria can be detected by Congo red staining (Neu, 2000). Capsule staining using an aqueous solution of 20% $CuSO_4$ and crystal violet can be used to identify the occurrence of capsular EPS (Cain, 2009). India ink can also be used for capsular staining (Cheryl *et al*., 2010).

Characteristic features of EPSs

The evaluation of biopolymers (EPSs) is done by their physicochemical and biological properties which help in understanding their behavior in various environments.

Chemical composition

Mostly EPSs consist of carbohydrates and proteins but also contain DNA, lipids, humic substances, non-carbohydrate substituents (pyruvate, phosphate, acetate and succinate), amino sugars such as N-acetylamino sugars, neutral sugars (rhamnose etc) and some uronic acids (mainly galacturonic and glucuronic acids). Most bacterial EPSs consist of repeating units of different sizes and degrees of ramification. Some EPSs have irregular structures such as bacterial alginates. Their variability is enhanced by diversified configurations and glycosyl linkages (Ullrich, 2009). Bacterial EPSs might contain numerous pyruvate ketals and ester-linked substituents. These acyl groups give anionic properties to EPSs, increase their lipophilicity and also affect their capacity to act together with cations and other polysaccharides (Satyawali & Balakrishnan, 2009).

EPS chemical composition deals with the detection of sugar residues, constituents of chain groups such as phosphate and acyl groups and sugar based repeating units. Anion- exchange chromatography at high pressure along with

electrochemical or amperometric detection is currently used (Panagiotopoulos *et al*., 2001). Capillary electrophoresis for characterization of single carbohydrates can also be used (Gao *et al*., 2010).

Chemical structure

EPSs have intricate chemical structures ranging from linear to highly branched heteropolysaccharides because of numerous combinations of sugar-based monomeric units. In the most recent method assessment of monomeric bonding pattern is done by conversion of EPSs into incompletely methylated alditol acetates that can be examined by GC-MS (Gas chromatography - mass spectrometry). The polysaccharides can be selectively fragmented by Smith degradation. Using linkage specific enzymes and nonspecific fractional acid hydrolysis, branching and molecular weight of EPSs can be decreased. Low molecular weight fragments are detected by GC-MS (Gas chromatography –mass spectrometry) and NMR (Nuclear magnetic resonance) spectroscopy (Hallack, *et al*., 2010). MALDI-TOF-MS (Matrix-assisted laser desorption/ionization-time of flight mass spectrometry) also be used (Gauri *et al*., 2009).

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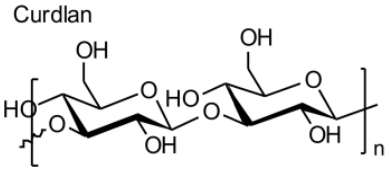

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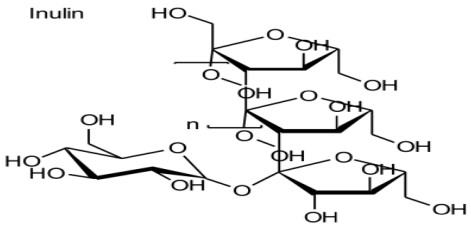

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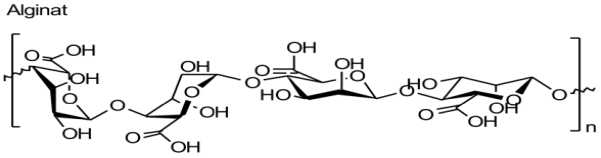

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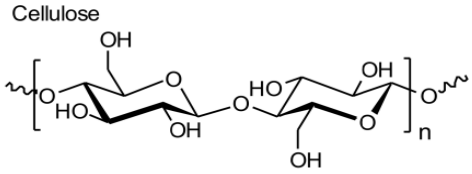

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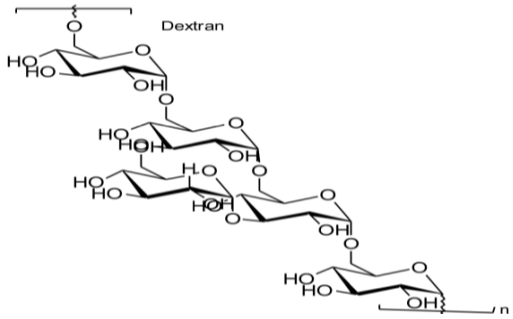

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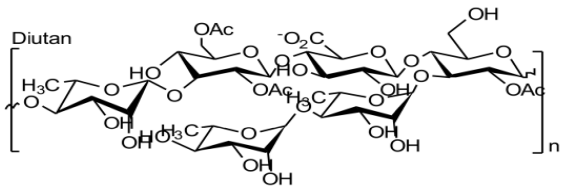

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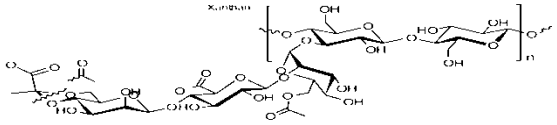

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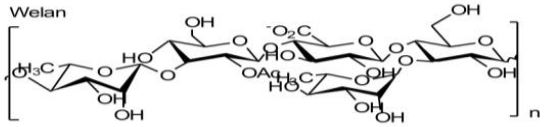

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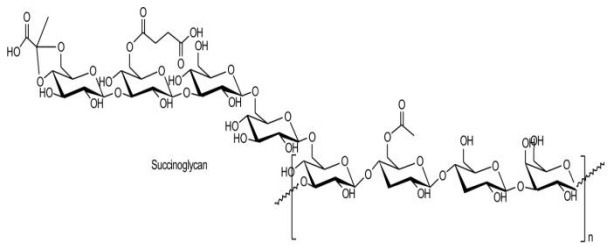

Fig. 1: Structures of some most important EPSs A. Curdlan; B. Inulin ; C. Alginate; D. Cellulose; E. Dextran; F. Diutan; G. Xanthan; H. Welan ; I. Succinog (Schmid *et al*., 2015)

Table II: Important bacterial exopoly-saccharides (Schmid *et al*., 2015)

EPS	Substi-tuents	Compo-nents	Biosynthesis Mechanism	Uses
Welan	Ace	Man, Rha, Glc, GlcA,	Wzx/Wzy dependent Pathway	Chemistry Construction
Levan		Glc, Fru	Etracellular synthesis	Medicines, Industry, Glue, Feed, Food
Succino-glycan	Pyr , Ace, Suc	Gal, Glc,	Wzx / Wzy dependent Pathway	Cosmetics , Oil industry
Cellulose		Glc	Synthase dependent Pathway	Medicine, Cosmetics , Acoustics
Hyaluronic acid		GlcNAc , GlcA	Synthase dependent Pathway	Cosmetics , Medicine
Diutan	Ace	Rha, Glc, GlcA,	Wzx/Wzy dependent Pathway	Chemistry, Construction
Alginate	Ace	ManA ,GulA	Synthase dependent Pathway	Food, Research Medicine, Feed
Xanthan	Pyr, Ace	Man, Glc, GluA	Wzx/Wzy dependent Pathway	Feed, Oil drilling, Food, Technical applications
Curdlan		Glc	Synthase dependent Pathway	Food, Medicine, Cosmetics , Chemistry, Construction
Gellan	Gly, Ace	Rha, Glc, GlcA	Wzx/Wzy dependent Pathway	Chemistry, Food, Construction, Feed
Dextran		Glc	Extracellular synthesis, Dextran-sucrase	Chromato g-raphy, Medicine

Average molecular weight

Various techniques are used to determine average molecular weight and polydispersity index. High performance size exclusion chromatography along with multi-angle laser light scatter detection, is a highly efficient method for the assessment of absolute molecular weight of polysaccharide (Boukari *et al*., 2009).

Properties in solution

In aqueous solution, EPSs behave as polyelectrolytes because of the presence of ionizable groups. These ionizable groups greatly affect the molecular conformation, flexibility, intrinsic viscosity, intra/intermolecular interactions and critical overlap concentration. To determine viscosity of EPS, dilute solution viscometry is used (Hilliou *et al*., 2009; Alves *et al*., 2010). In order to evaluate the complicated and integral 3D molecular structure of EPSs, AFM (Atomic force microscopy) can also be used (Morris *et al*., 2011).

Mechanisms of biosynthesis of EPSs in bacteria

At present four mechanisms have been recognized for bacterial EPSs' production: (i) The synthase-dependent pathway (ii) The extracellular synthesis (iii) Wzx/Wzy-dependent pathway (iv) The ABC (ATP-binding cassette) transporter-dependent pathway (Donlan and Costerton, 2002 and Schmid *et al*., 2015).

Synthase dependent pathway

In this mechanism, complete polymer strand is secreted transversely to the cell membrane and the cell wall. After polymerization, synthase proteins translocate EPSs extracellularly. It is used for the production of homopolymers (Rehm, 2010).

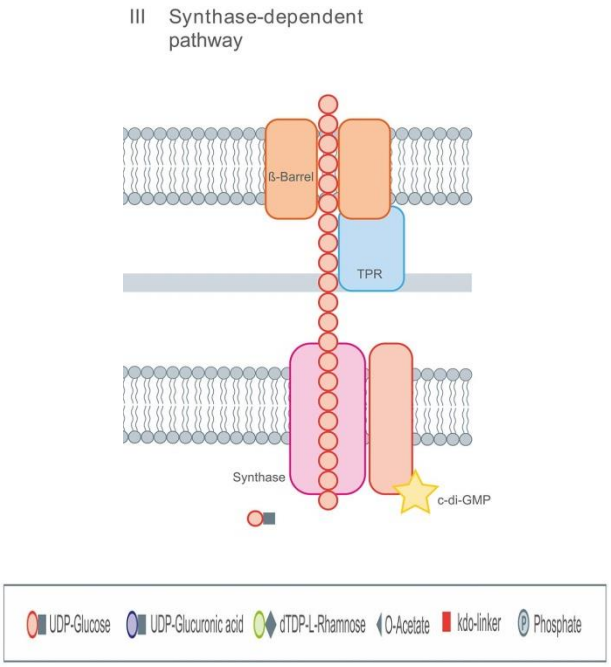

Fig. 2: Synthase dependent pathway (Schmid *et al*., 2015)

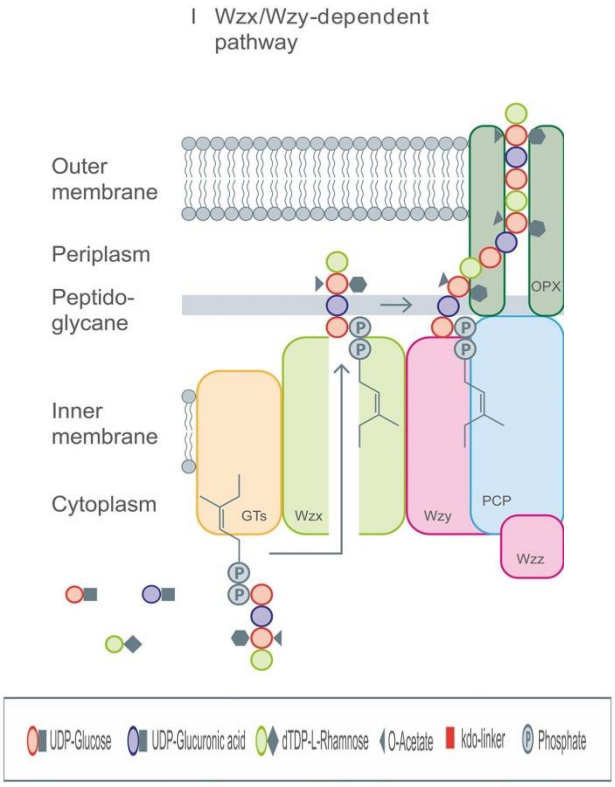

Fig. 3: Wzx/Wzy dependent pathway (Schmid *et al*., 2015)

The extracellular synthesis

This process is governed by glycosyltransferases, covalently attached to the surface of the cell and EPSs are secreted outside the cell. EPS, such as dextran or levan is synthesized (Czaczyk & Myszka, 2007).

Wzx/Wzy dependent pathway

In this pathway at the inner membrane, individual repeating units are attached to an undecaprenol diphosphate anchor (C55), arranged by sever46 glycosyltransferases and translocate across the cell membrane by a Wzx protein (flippase). At the periplasmic space, polymerization is done by flippase (Islam & Lam, 2014). The PCP (Polysaccharide co-polymerase) and OPX (Outer membrane polysaccharide exporters); formerly OMA (Outer membrane auxiliary) families help in translocation. Heteropolymers (e.g. xanthan) are synthesized by this pathway (Morona *et al*., 2009).

ABC transporter-dependent pathway

Capsular polysaccharide (CPS) is prepared by this pathway. CPS is attached to the cell surface. CPS is arranged at the inner membrane with the help of glycosyltransferases. Both heteropolymers and homopolymers are produced depending upon number of operons (GT–containing) involved (Whitney & Howell, 2013). A pump like composite (tripartite efflux) composed of PCP and OPX (periplasmatic proteins) and ABC-transporters help in translocation (Cuthbertson *et al*., 2009), closely related to secretion process of the Wzx/Wzy pathway. CPS formed has a glycolipid (poly-2-keto-3-deoxyoctulosonic acid as a linker and phosphatidylglycerol at the reducing end). (Willis *et al*., 2013; Willis & Whitfield, 2013).

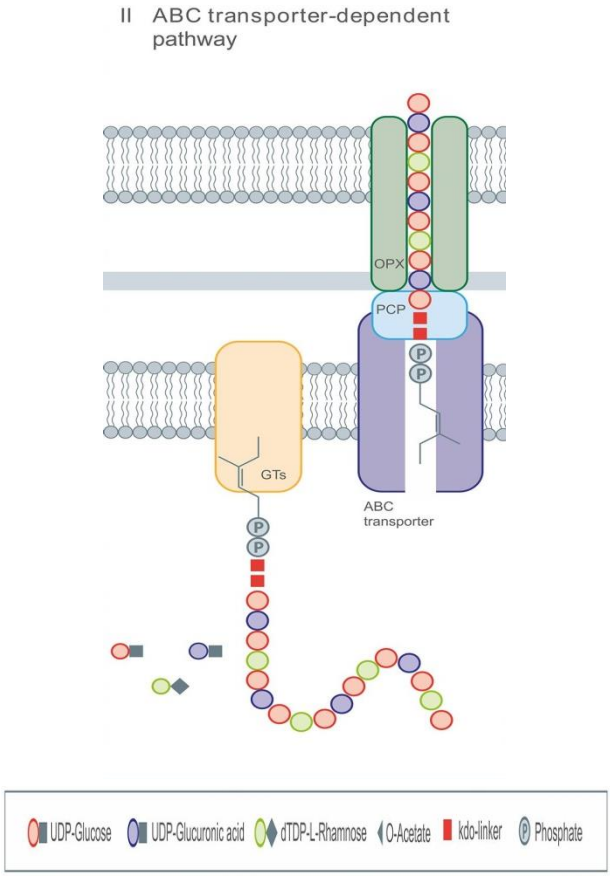

Fig. 4: ABC transporter-dependent pathway (Schmid *et al*., 2015)

Inheritance and maintenance of exopolysaccharide formation

Formation of EPSs occurs intracellularly which involves nucleoside diphosphate sugars. It is regulated by group of genes whose products cause acylation and the sugar addition to specific isoprenoid lipid acceptors. After polymerization, the repeating units are released extracellularly. With respect to the polysaccharides complexity, normally a gene sequence of 12–17 kb could be obligatory. Various polysaccharide-synthesizing processes produce almost similar gene products (Prombutara, 2015).

In case of *Xanthomonas campestris*, enzymes required for the precursors are individually controlled. For xanthan synthesis, gene products are present in the linked set of genes:

gumB	gumC	gumD	gumE	gumF	gumH	gumI	gumJ	gumK	gumL	gumM

The sequence of gum C, gum M, gum H and gum I characterizes the sequence of 5monosaccharide transferases forming the repeating unit (pentasaccharide). Acetylase is the product of gum F while gum L product is ketalase. The product of gum E is polymerase. Translocation is controlled by other genes. In *P. aeruginosa,* an intricated sequence of regulatory system involving algA, algD, algE, algC and algK genes, controls the formation of alginate. The most inclusive research has been done in *Rhizobium* strains. They synthesize succinoglycan. The genes and also the enzymes concerned with succinoglycan formation have been completely identified (Sutherland, 2001b).

EPSs formation is regulated all the way through megaplasmids. The EPSs genes are present on plasmids of nearly all mesophilic lactic acid bacteria, such as in *Lactobacillus casei* subsp. *cremoris* (Vanhooren & Vandamme, 1998).

Fermentative production of Exopolysaccharides

In order to attain intended economic target, a good fermentation process depends upon concentration of the product, yield and productivity. The activity of microbes greatly is affected by their environment relating to the design of equipment operated. No particular set of culture conditions of culture ensures high EPSs production. Microbes vary in their source consumption (carbon and nitrogen), mineral supplies, temperature and pH (Sutherland, 1972).

Carbon source

The production and size of EPSs are associated with the nature of source of carbon used. For example, alginate produced from glucose and fructose has (relative molecular mass) 276 kDa and 500 kDa respectively after 24 hours (Beavo *et al*., 1994). *Lactobacillus delbruckii* produces different EPSs when it is grown on fructose or glucose (Grobben *et al*., 2000). West & Strohfus (1998) observed gellan production by *Sphingomonas paucimobilis* showing the effects of different source used. According to them, gellan can be produced from glucose and corn syrup. However, it is not necessary that higher cell weight produces higher yield of gellan.

Nitrogen source

Even in trace amounts nitrogen sources cause increased growth rate of microorganisms and EPSs production (Farres *et al*., 1997). In some

cases, nitrogen source may contain some carbon, which may act as a raw material for EPS synthesis (De Souza & Sutherland, 1994). According to Vergas-Garcia *et al*., (2001), biomass levels amplified with higher concentration of nitrogen in the medium. EPS formation showed a reverse behavior in growth under these circumstances. EPS synthesis was elevated at lower concentration of nitrogen. Small amounts of combined nitrogen in media initiates EPS yield (Vermani *et al*., 1997). Gorret *et al*. (2001) verified that addition of yeast extract in the medium enhanced the growth of *Propionibacterium acidi-propoinici* and EPS production.

Carbon: Nitrogen ratio

High carbon:nitrogen ratio enhances the EPS synthesis. Highest EPS synthesis occurs at C:N ratio of . 10:1 respectively. Loss of nitrogen from the medium might indicate EPS production such as in scleroglucan and pullulan (Morin, 1998).

Ions

Some factors such as limitations of ions such as that of nitrogen, oxygen, phosphate and carbon influence the changing of carbon source into polysaccharide. In case of *Pseudomonas mendocina,* nitrogen, carbon and oxygen restrictions exaggerated the alteration of glucose into alginate and mannuronate to glucuronate ratio. EPS production by *Klebsiella* spp decreases in the existence of phosphate ion (Farres *et al*., 1997). Clementi *et al*. (1995) observed that Phosphate-limited environment increased the alginate production up to about 7g/l. In case of *L. bulgaricus,* exclusion of ions of zinc, ammonium and iron has no influence on the growth and EPS yield (Grobben *et al*., 2000). Only manganese or its amalgamation with sulfate, citrate and calcium strongly initiate EPS synthesis by *Lactobacillus casei* (Douglas & Klaenhammes, 2010).

Aeration rate

EPS yield increases with high aeration rate (Lee *et al*., 2001). Yang & Liau (1998) observed that higher agitation and aeration rate enhance the formation of EPS by *Ganoderma lucidum*. Oxygen is involved in metabolism and oxidation of pyridine nucleotides and sugar. In the presence of good supply of nutrients and aeration (Bayer *et al*., 1991; Dassy *et al*., 1991). Oxygen uptake rate increases in the presence of detergents. However, in *Fusarium solani* opposite effects were observed in case of elevated oxygen-transfer rates (Rau *et al.,* 1990).

Dilution rate

Sutherland (1977) observed that EPS production increases with low dilution rate and decreases with high dilution in *Klebsiella spp*. Lower dilution rate increases the residence time of microbes which in turn gets more time to use source completely.

Incubation temperature

Greater production of EPS occurs at the sub-optimal growth temperature (Cerning *et al*., 1992; Gancel & Novel, 1994). At low temperature, high EPS formation was given by *Propionibacterium acidipropionici* (Gorret *et al*., 2001). However, Garcia-Garibay & Marshall (1991) observed different results that high temperature caused increased EPS synthesis in *Lactobacillus delbrueckii* subsp*. bulgaricus*.

Incubation pH

Media buffered at neutral pH is used for numerous exopolysaccharide producing microorganisms. Culture media buffered at 5.2−6.5 pH containing 2−4% NaCl was used for EPS production by some meat starters (Hwu *et al*., 1997). Gorret *et al*., 2001 observed that pH 5.3–6.5 is required for *Propionibacterium acidi-propionici* to produce EPSs.

Age of the exopolysaccharide producing cells and growth

Bergmaier *et al*. (2002) observed that age of *Lactobacillus rhamnosus* RW-9595M is directly proportional to lactic acid concentration. Petry *et al*. (2000) described that bulk of the EPS synthesis occurs in the stationary phase and it was experimentally observed in two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Osmotic concentration

Solute concentration is important in EPSs production. Two different water-soluble EPS, namely succinoglycan and galactoglucan are produced by fast-growing *Rhizobium melilotii*, the ratio of production is affected by the culture medium osmolarity. Medium without NaCl contained both the EPSs in the ratio of 1:1. The proportion of succinoglycan increased to 85% by addition of 0.6 M NaCl in the medium (Stredansky *et al*., 1998).

Detergents

A rise of 1.45 fold was observed at 0.1 g l^{-1} concentration of Triton X-100. Addition of detergent

after 24h of growth reduces severe foaming. These detergents could affect elements regulating the rheological feature of xanthan. They could increase the polymerization of xanthan by reacting with the membrane of *Xanthomonas campestris* (Barreras *et al.*, 2004).

Upgrading of EPSs

It includes concentration, separation and purification of EPSs thus constitute their total production cost. Its most important aims are: i) concentration of fermentation broth to enable microbiological strength, complete solubility, easy handling, transfer and storage. ii) EPSs purification to eliminate impurities such as salts, cells and unwanted contaminating enzymes (Smith & Pace, 1982).

Isolation of EPSs

EPSs when present as slime can be secluded from microbes by centrifugation. The centrifugation time and speed differ with the thickness and nature of the EPS. By means of ultra-centrifugation, the bulk of the cells and cell debris are removed. EPS when present as a capsule is first separated from the cells. The interaction of EPS and cells specifies the separation method. Weakly associated capsular EPSs can be separated by centrifugation. In case of strong association, alkaline treatment by NaOH is used before centrifugation and precipitation of EPS by using alcohol (Subair *et al*., 2015). In case of thermally stable EPS, the isolation of microbes from the broth can be enhanced by heat treatment to some extent cells death and inactivation of some of the broth enzymes occur by pasteurization and decreasing viscosity (Sutherland, 1990).

Precipitation of EPSs

Solvent precipitation is generally used for recovery of EPSs from the culture broth. The addition of polar organic solvent (lower alcohols or acetone) can be used for precipitation of EPS in the supernatant. The proportion of solvent used is variable; mostly two volumes of the culture broth are used. Lowering of EPS solubility in water due to organic solvents enhances separation. During solvent precipitation, EPS may be precipitated along with proteins and salts of the medium. In order to get pure EPS, deproteination and desalting treatments may be used (Morin, 1998). Then EPS is harvested following centrifugation, filtration, settling or pressing. EPS is dried under vacuum or with inert gas and finally milled to the required mesh size. Depending on purity, the final EPS products are off-white to white. Other methods such as reverse osmosis and ultrafiltration for reducing the water content of EPS preparations are available (Sutherland, 1990).

Purification of EPSs

EPSs can be purified by desalting method and deproteination. In case of *Klebsiella* spp in the presence of 11.32 M HCl at 4-5 pH, rhamnose was separated as slime and capsule from the cells before autoclaving. 40% of the protein having EPS in the supernatant was concentrated in this way, thus increasing the rhamnose recovery. At present nitron and CTAB are also used. 75% of EPS can be precipitated using three volumes of propanol (Morin, 1998). Then EPS is harvested by centrifugation, filtration, pressing and settling. The EPSs can be dried through vacuum or by inert gas and finally crushed to the required size. EPS may be white off- to white in colour (Sutherland, 1990).

Application of EPSs

Exopolysaccharides in bacterial biofilm

EPSs are important in the formation of biofilm and attachment of cells to substrates. EPS is 50%-90% of entire organic matter of biofilm (Fleming, 2000). Biofilms are called symbolically dubbed microbial cities (Daegelen *et al.*, 2009). According to Nwodo (2012)**,** the roles of exopolysaccharides in biofilms are:

Attachment

EPSs help in the attachment to the surfaces (abiotic and biotic).

Aggregation of bacterial cells

EPSs join cells, thus temporarily immobilize the bacterial population.

Maintenance of water

Being hydrophilic, EPSs maintain a hydrated environment in the region of biofilm and help bacteria in water lacking environments.

Source of nutrients

In biofilms, EPSs are huge sources of compounds containing carbon, phosphorus and nitrogen.

Protective shield

EPSs produce resistance against host (non- specific and specific) during infection. They produce resistance to different antimicrobial agents, give protection against phagocytic protozoa and protect cyanobacterial nitrogenase against the detrimental effects of oxygen.

Sorption of inorganic ions and organic Compounds

Charged and hydrophobic EPSs regulate the sorption of xenobiotics and accretion of the nutrients of the environment. They help in environmental detoxification by supporting SDS (Sodium Dodecyl Sulphate) resulting in the formation of minerals, accretion of noxious metal ions and ion exchange.

Table III: Some human diseases associated with bacterial biofilms. (Nwodo, 2012)

Diseases	Biofilm Bacteria
Dental caries	Acidogenic Gram positive cocci and *Streptococcus* spp.
Periodontitis	Gram negative anaerobic oral bacteria
Cystic fibrosis	*P. aeruginosa,* pneumonia, *Burkholderia cepacia*
Musculoskeletal infections	*Staphylococci* and other Gram-positive cocci
Otitis media	*Haemophilus influenza*
Biliary tract infection	*E. coli* enteric bacteria (*E. coli)*
Urinary catheter cystitis	Gram-negative rods (*E. coli)*
Bacterial prostatitis	Gram-negative bacteria (*E. coli)*

Commercial uses

BioFill, a derived cellulose material from *Acetobacter xylinum* can be used as an implantable material in plastic and general surgery. It is used in wound dressings in cases of burns and chronic skin ulcers. *Rhizobium and Agrobacterium* species produce curdlan, which is widely used in food industry. *Xanthomonas campestris* produces *Xanthan*, a major commercial biopolymer (Sutherland, 1998). EPSs are added into foods to alter the rheological properties of the water present (Welman & Maddox, 2003).

Food industry

EPSs play a major role in the quality, stability and taste of the dairy products. These are widely used in synthesis of dairy products (fermented) in Asia, Eastern and Northern Europe. Bacteria producing EPSs are widely used in fermented milk production (Ruas-Madiedo, 2002 and 2003). A wide range of LAB strains are used in fermentation. The most important are *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus.* Viili is fermented milk used in Finland. Kefir is used in Eastern Europe slightly alcoholic fermented milk (Brodbent *et al*., 2003).

Dairy appetizer cultures having LAB strains (slime-forming) are commercially accessible in Europe and USA. In yoghurt making, ropy LAB appetizer cultures are mostly used in several countries of Europe (De Vuyst & Degeest , 1999). *S. thermophilus* produces HePS which is used in mozzarella cheese for increasing moisture (Perry *et al*., 1997 & 1998; Low *et al*., 1998). They are used as texturizer in various food products like yogurt (Toba *et al*., 1990).

Table V: Commercial applications of EPSs (Madhuri & Rabhakar, 2014a)

EPSs	Source	Application
Gelrite or Kelcogel	*Sphingomonas paucimobilis*	Used in foods as stabilizing, suspending and gelling agent
Xanthan	*Xanthomonas compestris*	Used in oil recovery and various foods as viscosifying agent
Emulsan	*Pseudomonas fluorescence*	Used in various food as an emulsifying agent
Dextran	*Leuconostoc mesenteroides, Streptococcus mutans*	Used to purify different molecules such as Sephadex
Curdlan	*Agrobacterium* and *Rhizobium* spp	Used in biomedical applications such as antithrombotic activity etc
BioFill	*Acetobacter xylinum*	Used in general plastic surgery as Implantable material

Health benefits of EPS

Table IV: Exopolysaccharides of bacteria and their health benefits (Madhuri and Rabhakar, 2014b)

Sr. No	Source of EPS	Health benefit
1.	*Lactobacillus casei*	Activated mouse acrophages
2.	*Bifidobacterium bifidum*	Antiulcer activity
3.	*Bacillus licheniformis*	Antiviral and immunostimulatory activities
4.	*Lactobacillus kefiranofaciens*	Increased gut mucosal immunity
5.	*Lactobacillus plantarum*	Antimutagenic activity
6.	*Bacillus coagulans* RK–02	Antioxidant and Antihyperglycemic activities

EPSs provide important health benefits like antioxidant (Kodali & Sen, 2008), cholesterol lowering (Welman & Maddox, 2003), antiviral and immunomodulatory activities (Arena *et al*., 2006), antitumor (Hosono *et al.*, 1997). The reactive oxygen species (ROS) like nitric oxide ($NO^{\cdot}$)hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot-}$), etc. cause serious diseases including atherosclerosis, Parkinson's disease, rheumatoid arthritis and cancer. It has been proved that EPSs have antioxidant and free radical scavenging characteristics. The EPS, free radical scavenging property can also be used to inhibit vegetable oils oxidation. Probiotic bacteria produce EPSs, that show anti-ulcer activity. Nagaoka *et al.* (1994) observed the anti-ulcer property of lactobacilli, *Bifidobacteria* and *Streptococci* using the ethanol-induced erosion models and acetic acid-induced gastric ulcer in rats. EPSs containing rhamnose more than 60% were more efficient in the curing of gastric ulcers. It has been reported that the EPSs stimulate the immune system and also show anticancer effects.

Bioremediation and wastewater treatment

EPSs producing bacteria (Enterobacter and Pseudomonas species) are widely used in bioremediation and wastewater treatment. They are used in the cleaning of effluents formed because of mining. They are also used to treat hydrocarbons, heavy metals and enormous volumes of industrial and public wastewater. They are also used in anaerobic breakdown of different organic pollutants (Kumar *et al*., 2007).

REFERENCES

Ahmad, N.H., Mustafa, S. and Che Man, Y.B., 2015. Microbial polysaccharides and their modification approaches: a review. *Int. J. Food.Prop*.18 (2):332-347

Alves, V. D., Freitas, F., Costa, N., Carvalheira, M., Oliveira, R., Gonçalves, M. P. and Reis, M. A., 2010. Effect of temperature on the dynamic and steady-shear rheology of a new microbial extracellular polysaccharide produced from glycerol byproduct. *Carbohydr. Polymer*. 79(4): 981-988.

Antón, J., Meseguer, I. and Rodriguez-Valera, F., 1988. Production of an extracellular polysaccharide by *Haloferax mediterranei*. *Appl. Environ. Microbiol.* 54(10): 2381-2386.

Arena, A., Maugeri, T. L., Pavone, B., Iannello, D., Gugliandolo, C. and Bisignano, G., 2006. Antiviral and immunoregulatory effect of a novel exopolysaccharide from a marine thermotolerant *Bacillus licheniformis*. *Int. J. immunopharmacol.* 6(1): 8-13.

Bajaj, I. B., Survase, S. A., Saudagar, P. S. and Singhal, R. S., 2007. Gellan gum: fermentative production, downstream processing and applications. *Food Technol. Biotech*. 45(4): 341.

Barreras, M., Abdian, P. L., and Ielpi, L. 2004. Functional characterization of GumK, a membrane-associated β-glucuronosyltransferase from *Xanthomonas campestris* required for xanthan polysaccharide synthesis. *Glycobiology.* 14(3): 233-241.

Bayer, A. S., Speert, D. P., Park, S., Tu, J., Witt, M., Nast, C. C. and Norman, D. C., 1991. Functional role of mucoid exopolysaccharide (alginate) in antibiotic-induced and polymorphonuclear leukocyte-mediated killing of *Pseudomonas aeruginosa*. *Infect. Immun*. 59(1): 302-308.

Beavo, J. A., Conti, M., and Heaslip, R. J. 1994. Multiple cyclic nucleotide

phosphodiesterases. *Mol. Pharmacol.* 46(3): 399-405.

Bergmaier, D., Lacroix, C. and Champagne, C. P., 2002. Exopolysaccharide production during chemostat cultures with free and immobilized *Lactobacillus rhamnosus* RW-9595M. *Biotechnol. Prog. soumis pour publication*.

Boukari, I., Putaux, J. L., Cathala, B., Barakat, A.,Saake, B., Rémond, C. and Chabbert, B., 2009. In vitro model assemblies to study the impact of lignin− carbohydrate interactions on the enzymatic conversion of xylan. *Biomacromol.* 10(9): 2489-2498.

Broadbent, J. R., McMahon, D. J., Welker, D. L., Oberg, C. J. and Moineau, S., 2003. Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: a review. *J. Dairy. Sci*., 86 (2): 407-423.

Cain, D., Hanks, H., Weis, M., Bottoms, C. and Lawson, J., 2009. Microbiology laboratory manual. *Collin County Community College District, McKinney, TX*.

Cerning, J., Bouillanne, C., Landon, M. and Desmazeaud, M., 1992. Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. *J. Dairy. Sci*. 75(3): 692-699.

Cheryl, D., Chun, H., and Madhani, D., 2010. Ctr2 Links Copper Homeostasis to Polysaccharide Capsule Formation and Phagocytosis Inhibition in the Human Fungal Pathogen *Cryptococcus neoformans*. *Plos One.* 5(9): 12503-12530.

Clementi, F., Fantozzi, P., Mancini, F. and Moresi, M., 1995. Optimal conditions for alginate production by *Azotobacter vinelandii*. *Enzyme Microbial. Technol*. 17(11): 983-988.

Cuthbertson, L., Mainprize, I.L., Naismith, J.H. and Whitfield, C., 2009. Pivotal roles of the outer membrane polysaccharide export and polysaccharide copolymerase protein families in export of extracellular polysaccharides in gram-negative bacteria. *Microbiol. Mol. Biol. Rev.* 73: 155-177.

Czaczyk, K. and Myszka, K., 2007. Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial bioflm formation. *Polish J. Environ. Stud.* 16(6): 799-806.

Daegelen, P., Studier, F. W., Lenski, R. E., Cure, S. and Kim, J. F., 2009. Tracing ancestors and relatives of Escherichia coli B, and the derivation of B strains REL606 and BL21 (DE3). *J. Mol. Biol.* 394(4): 634-643.

Dassy, B., Stringfellow, W. T., Lieb, M. and Fournier, J. M., 1991. Production of type 5 capsular polysaccharide by *Staphylococcus aureus* grown in a semi-synthetic medium. *J. Gen. Microbiol*. 137(5): 1155-1162.

De Souza, A. M. and Sutherland, I. W., 1994. Exopolysaccharide and storage polymer production in *Enterobacter aerogenes* type 8 strains. *J. Appl. Bacterial*. 76(5): 463-468.

De Vuyst, L. and Degeest, B., 1999. Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol. Rev*. 23(2): 153-177.

Donlan, R. M., and Costerton, J. W. 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev*. 15(2):167-193.

Donlan, R. M., 2002. Biofilms: microbial life on surfaces. *Emerg. Infect. Dis*. 89.

Douglas, G.C., and Klaenhammer, T.R., 2010. Genomic evolution of domesticated microorganisms. *Annu. Rev. Food Sci. Technol*. 1:397-414

Farres, J., Caminal, G. and Lopez-Santin, J., 1997. Influence of phosphate on rhamnose-containing exopolysaccharide rheology and production by *Klebsiella* I-714. *Appl. Microbiol. Biotechnol*. 48(4): 522-527.

Fazenda, M.L., Sviour, R., Mc Neil, B. and Harvey, L.M., 2008.Submerged culture fermentation of “higher fungi ’’ the macrofungi. *Adv. Appl. Microbiol*. 63:33-103

Fleming, K. G. 2000. Riding the wave: structural and energetic principles of helical membrane proteins. *Curr. Opin. Chem. Biol.* 11(1):67-71.

Freitas, F., Alves, V. D. and Reis, M. A., 2011. Advances in bacterial exopolysaccharides from production to biotechnological applications. *Trends biotechnol*. 29(8): 388-398.

Gancel, F., and Novel, G. 1994. Exopolysaccharide production by *Streptococcus salivarius* subsp. *thermophilus* cultures. 1. Conditions of production. *J. Dairy. Sci.* 77(3): 685-688.

Gao, Q., Araia, M., Leck, C. and Emmer. A. 2010. Characterization of exopolysaccharides in marine colloids by capillary electrophoresis with indirect UV detection. *Analytica. Chimica. Acta.* 662(2): 193-199.

Garcia-Garibay, M., and Marshall, V. M. E. 1991. Polymer production by Lactobacillus delbrueckii subsp. bulgaricus. *J. Appl. Bacteriol.* 70(4) :325-328.

Gauri, S. S., Mandal, S. M., Mondal, K. C., Dey, S. and Pati, B. R., 2009. Enhanced production

and partial characterization of an extracellular polysaccharide from newly isolated *Azotobacter* sp. SSB81. *Bioresour. Technol*. 100(18): 4240-4243.

Gorret, N., Maubois, J. L., Engasser, J. M. and Ghoul, M., 2001. Study of the effects of temperature, pH and yeast extract on growth and exopolysaccharides production by *Propionibacterium acidi-propionici* on milk microfiltrate using a response surface methodology. *J. Appl. Microbiol*. 90(5): 788-796.

Grobben, G. J., Boels, I. C., Sikkema, J., Smith, M. R. and De Bont, J. A., 2000. Influence of ions on growth and production of exopolysaccharides by *Lactobacillus delbrueckii* subsp*. bulgaricus* NCFB 2772. *J. Dairy. Res*. 67(1):131-135.

Hallack, L. F., Passos, D. S., Mattos, K. A., Agrellos, O. A., Jones, C., Mendonça-Previato, L. and Todeschini, A. R., 2010. Structural elucidation of the repeat unit in highly branched acidic exopolysaccharides produced by nitrogen fixing *Burkholderia*. *Glycobiology*. 20(3): 338-347.

Hilliou, L., Freitas, F., Oliveira, R., Reis, M. A., Lespineux, D., Grandfils, C. and Alves, V. D., 2009. Solution properties of an exopolysaccharide from a *Pseudomonas* strain obtained using glycerol as sole carbon source. *Carbohydr. Polymers*.78(3): 526-532.

Hosono, A., Lee, J., Ametani, A., Natsume, M., Hirayama, M., Adachi, T., and Kaminogawa, S. 1997. Characterization of a water-soluble polysaccharide fraction with immunopotentiating activity from Bifidobacterium adolescentis M101–4. *Biosci. Biotechnol. Biochem*. 61(2): 312-316.

Hussain, A., Zia, K. M., Tabasum, S., Noreen, A., Ali, M., Iqbal, R., and Zuber, M. 2017. Blends and composites of exopolysaccharides; properties and applications: A review*. Int. J. Biol. Macromolec*. 94, 10-27.

Hwu, C. S., Van Beek, B., Van Lier, J. B., & Lettinga, G. 1997. Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: effect of washed out biomass recirculation. *Biotechnol. Lett*. 19(5): 453-456.

Iijima, M., Shinozaki, M., Hatakeyama, T., Takahashi, M. and Hatakeyama, H., 2007. AFM studies on gelation mechanism of xanthan gum hydrogels. *Carbohydr. Polymers*. 68(4): 701-707.

Islam, S.T. and Lam, J.S., 2014. Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. *Can. J. Microbiol.* 60: 697-716.

Kambourova, M., Mandeva, R., Dimova, D., Poli, A., Nicolaus, B. and Tommonaro, G., 2009. Production and characterization of a microbial glucan, synthesized by *Geobacillus tepidamans* V264 isolated from Bulgarian hot spring. *Carbohydr. Polymers*. 77(2): 338-343.

Kodali, V. P., and Sen, R. 2008. Antioxidant and free radical scavenging activities of an exopolysaccharide from a probiotic bacterium. *Biotechnol. J.* 3(2): 245-251.

Kumar, S., Anita, Mody, K. and Jha, B., 2007. Bacterial exopolysaccharides–a perception. *J. Basic Microbiol.* 47(2):103-117.

Lapaglia, C. and Hartzell, P. L., 1997. Stress-Induced Production of Biofilm in the hyperthermophile *Archaeoglobus fulgidus*. *Appl. Environ. Microbial*. 63(8): 3158-3163.

Lee, H. S., Park, S. H., Lee, J. H. and Lee, H. K., 2001. Effect of aeration rates on production of extracellular polysaccharide, EPS-R, by marine bacterium *Hahella chejuensis*. *Biotechol. Bioprocess. Eng.* 6(5): 359-362.

Low, D., Ahlgren, J. A., Horne, D., McMahon, D. J., Oberg, C. J. and Broadbent, J. R., 1998. Role of *Streptococcus thermophilus* MR-1C capsular exopolysaccharide in cheese moisture retention. *Appl. Environ. Microbiol.* 64(6): 2147-2151.

Madhuri, K. V. and Rabhakar, K. V., 2014a. Microbial Exopolysaccharides: Biosynthesis and Potential Applications. *Oriental. J. Chem.* 30(3): 1401-1410.

Madhuri, K.V. and Rabhakar K.V. 2014b. Recent trends in the characterization of microbial exopolysaccharides. *Orient*. *J. Chem*. 30(2):895-904

Morin, A., 1998. Screening of polysaccharide-producing microorganisms, factors influencing the production and recovery of microbial polysaccharides. *Polysaccharides–Structural Diversity and Functional Versatility. New York: Marcel Dekker Inc*, 275-296.

Morona, R., Purins, L., Tocilj, A., Matte, A. and Cygler, M., 2009. Sequence-structure relationships in polysaccharide co-polymerase (PCP) proteins. *Trends Biochem. Sci.* 34: 78-84.

Morris, V. J., Gromer, A., Kirby, A. R., Bongaerts, R. J. and Gunning, A. P., 2011. Using AFM and force spectroscopy to determine pectin structure and (bio) functionality. *Food hydrocoll.* 25(2): 230-237.

Nagaoka, M., Hashimoto, S., Watanabe, T., Yokokura, T. and Mori, Y., 1994. Anti-ulcer effects of lactic acid bacteria and their cell wall polysaccharides. *Biol. Pharm. Bull.* 17(8): 1012-1017.

Neu, T. R., 2000. In situ cell and glycoconjugate distribution in river snow studied by confocal laser scanning microscopy. *Aquat. Microb. Ecol*. 21(1): 85-95.

Nicolaus, B., Manca, M. C., Romano, I., & Lama, L. 1993. Production of an exopolysaccharide from two thermophilic archaea belonging to the genus Sulfolobus. *FEMS. Microbiol. Lett.* 109(2-3): 203-206.

Nicolaus, B., Lama, L., Esposito, E., Manca, M. C., Improta, R., Bellitti, M. R. and Gambacorta, A., 1999. *Haloarcula* spp. able to biosynthesize exo-and endopolymers. *J. Ind. Microbiol Biotechnol.* 23(6): 489-496.

Nwodo, U., Green, E. and Okoh, A. I., 2012. Bacterial exopolysaccharides: functionality and prospects. *Int. J. Mol. Sci.* 13(11): 14002-14015.

Panagiotopoulos, C., Sempere, R., Lafont, R. and Kerhervé, P., 2001. Sub-ambient temperature effects on the separation of monosaccharides by high-performance anion-exchange chromatography with pulse amperometric detection: Application to marine chemistry. *J. Chromatogr. A*. 920(1): 13-22.

Paramonov, N. A., Parolis, L. A., Parolis, H., Boán, I. F., Anton, J. and Rodríguez-Valera, F., 1998. The structure of the exocellular polysaccharide produced by the Archaeon *Haloferax gibbonsii* ATCC 33959. *Carbohydr. Res.* 309(1): 89-94.

Perry, D.B., McMahon, D.J. and Oberg, C.J., 1997. Effect of exopolysaccharide-producing cultures on moisture retention in low-fat Mozzarella cheese. *J. Dairy Sci.* 80: 799-805.

Perry, D. B., McMahon, D. J. and Oberg, C. J., 1998. Manufacture of low fat Mozzarella cheese using exopolysaccharide-producing starter cultures. *J. Dairy Sci.* 81(2): 563-566. *Appl. Environm. Microbial.* 66(8): 3427-3431.

Petry, S., Furlan, S., Crepeau, M. J., Cerning, J. and Desmazeaud, M., 2000. Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii* subsp. bulgaricus grown in a chemically defined medium. *Appl. Environm. Microbial.,* 66(8): 3427-3431.

Prombutara, P., 2015. Identification of genes involved in flocculation by whole genome sequencing of *Thauera aminoaromatica* strain MZ1T floc-defective mutans. University of North Texas.

Rau, U., Muller, R. J., Cordes, K. and Klein, J., 1990. Process and molecular data of branched 1, 3-β-D-glucans in comparison with Xanthan. *Bioprocess. Eng.* 5(2): 89-93.

Rehm, B.H., 2010. Bacterial polymers: biosynthesis, modifications and applications. *Nat. Rev. Microbiol.* 8: 578-592.

Rinker, K. D. and Kelly, R. M., 1996. Growth physiology of the hyperthermophilic archaeon *Thermococcus litoralis*: Development of a sulfur-free defined medium, characterization of an exopolysaccharide, and evidence of biofilm formation. *Appl. environ. microbiol*, 62(12): 4478-4485.

Roberts, I. S., 1996. The biochemistry and genetics of capsular polysaccharide production in bacteria. *Ann. Rev. Microbiol*. 50(1): 285-315.

Ruas-Madiedo, P. and Zoon, P., 2003. Effect of exopolysaccharide-producing *Lactococcus lactis* strains and temperature on the permeability of skim milk gels. *Colloid Surf.* 213(2): 245-253.

Ruas-Madiedo, P., Hugenholtz, J. and Zoon, P., 2002. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *Int. Dairy J.* 12(2): 163-171.

Satyawali, Y., and Balakrishnan, M., 2009. Effect of PAC addition on sludge properties in an MBR treating high strength wastewater. *Water. Res.* 43(6): 1577-1588.

Schmid, J., Sieber, V. and Rehm, B., 2015. Bacterial exopolysaccharides: biosynthesis pathways and engineering strategies. *Front. Microbiol*. 6: 496.

Simsek, S., Mert, B., Campanella, O. H. and Reuhs, B., 2009. Chemical and rheologic al properties of bacterial succinoglycan with distinct structural characteristics. *Carbohydr. Polymers*. 76(2): 320-324.

Smith, I. H., and Pace, G. W. 1982. Recovery of microbial polysaccharides. *J. Chem. Technol. Biotechnol*. 32(1):119-129.

Stredansky, M., Conti, E., Bertocchi, C., Matulova, M., & Zanetti, F. 1998. Succinoglycan production by Agrobacterium

tumefaciens. *J. Ferment. Bioeng*. 85(4): 398-403.

Subair, H. 2015. Isolation and Screening Bacterial Exopolysaccharide (EPS) from Potato Rhizosphere in Highland and the Potential as a Producer Indole Acetic Acid (IAA). *Procedia. Food. Sci.* 3: 74-81.

Sutherland, I.W. 1972. Bacterial exopolysaccharides. *Adv. Microb. Physiol*. 8: 143-213. Academic Press.

Sutherland, I.W., 1977. Microbial exopolysaccharide synthesis. Extracellular Microbial Polysaccharides ACS Symposium Series 45. Sanford, P. A. and Laskin, A. Eds.: American Chemical Society, Washington, D.C: 40-57.

Sutherland, I. W., 1990. Biotechnology of microbial exopolysaccharides. 9.Cambridge University Press.

Sutherland, I. W. 1998. Novel and established applications of microbial polysaccharides. *Trends. biotechnol.* 16(1):41-46.

Sutherland, I. W., 2001a. Biofilm exopolysaccharides: a strong and sticky framework. *Microbiol.* 147(1): 3-9.

Sutherland, I. W., 2001b. Microbial polysaccharides from Gram-negative bacteria. *Int. Dairy J.* 11(9): 663-674.

Toba, T., Nakajima, H., Tobitani, A. and Adachi, S., 1990. Scanning electron microscopic and texture studies on characteristic consistency of Nordic ropy sour milk. *Int. J. food Microbiol.* 11(3): 313-320.

Ullrich, M.Ed., 2009. Bacterial polysaccharides: current innovations and future trends. Horizon Scientific Press.

Vanhooren, P., and Vandamme, E. J., 1998. Biosynthesis, physiological role, use and fermentation process characteristics of bacterial exopolysaccharides. *Recent Res. Dev. Ferment. Bioeng*. 253-300.

Vargas-Garcia, M. C., Lopez, M. J., Elorrieta, M. A., Suárez, F. and Moreno, J., 2001. Influence of nutritional and environmental factors on polysaccharide production by *Azotobacter vinelandii* cultured on 4-hydroxybenzoic acid. *J. Ind. Microbiol. Biotechnol.* 27(1): 5-10.

Vermani, M.V., Kelkar, S.M. and Kamat, Y., 1997. Novel exopolysaccharide production by *Azotobacter vinelandii* MTCC 2459, a plant rhizosphere isolate. *Lett. Appl. Bacteriol*. 24: 379–383.

Vescovo, M., Scolari, G. L., and Bottazzi, V. 1989. Plasmid-encoded ropiness production in *Lactobacillus casei* subsp*. casei*. *Biotechnol. lett.* 11(10):709-712.

Welman, A. D., and Maddox, I. S. 2003. Exopolysaccharides from lactic acid bacteria:

perspectives and challenges. *Trends. biotechnol.* 21(6): 269-274.

West, T.P. and Strohfus, B. 1998. Effect of carbon source on exopolysaccharide production by Sphingomonas paucimobilis ATCC 31461. *Res. Microbiol*. 153: 327–329.

Whitfield, C. and Valvano, M. A. 1993. Biosynthesis and expression of cell-surface polysaccharides in gram-negative bacteria. *Adv. Microb. Physiol*. 35: 136-136.

Whitney, J.C. and Howell, P.L. 2013. Synthase-dependent exopolysaccharide secretion in Gram-negative bacteria. *Trends Microbiol.* 21: 63-72.

Willis, L.M. and Whitfield, C. 2013. Structure, biosynthesis and function of bacterial capsular polysaccharides synthesized by ABC transporter-dependent pathways. *Carbohydr. Res.* 378: 35-44.

Willis, L.M., Stupak, J., Richards, M.R., Lowary, T.L., Li, J. and Whitfield, C. 2013. Conserved glycolipid termini in capsular polysaccharides synthesized by ATP-binding cassette transporter-dependent pathways in Gram-negative pathogens. *PNAS.* 110: 7868-7873.

Yang, F. C. and Liau, C. B. 1998. The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. *Process Biochem*. 33(5): 547-553.