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An overview of the exopolymer produced by bacteria and its biomedical application

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Abstract

The nature harboured assorted group of organisms represent immense and precious source of natural products. Exclusively, the bacteria secrete several novel metabolites. The bacteria produces various bioactive compounds. Most bacteria produce exopolymeric substances with massive function; it maintains the structural and functional stability of the cells. It supports the bacteria to strive in adverse conditions like osmotic shock, predation, and drought. Technological development has led to the discovery of valuable secondary metabolites from various organisms. Exopolymeric substances are ubiquitous, and their chemical and physical properties vary from one to another organism. The non-toxic, biocompatible, and biodegradable nature of bacterial exopolymeric substances meets an essential requirement. All these advantages make the bacterial exopolymeric substances unique, and their properties to launch in a range of fields.

Keywords: Bacteria, Biomedical, Bioactive

Introduction

The assorted group of bacteria produces various bioactive compounds having antitumor, antimicrobial, immunomodulatory, antiviral activity and anti-coagulant properties. Most bacteria produce exopolymeric substances with massive function; it maintains the structural and functional stability of the cells. It supports the bacteria to strive in adverse conditions like osmotic shock, predation, and drought. Although, the exopolymer synthesis was not an essential character for all the bacterial cells. In general, some of the bacteria produce the exopolymer during exponential stage of bacterial growth. Numerous studies have argued that in marine environment, the bacterial cells consume the organic matter as carbon sources to form exopolymeric substances.

Bacterial Exopolysaccharides

Bacteria is a unicellular organism and ubiquitous in the earth. Exopolymeric substances are the secondary metabolites synthesized by bacterial strains of a broad range of families. The term exopolysaccharide was coined by Sutherland in 1972 (Sutherland, 1972). The abbreviation "EPS" has been used for "extracellular polymeric substances", "extracellular polysaccharides", "exopolymers", and "exopolysaccharides". These substances might attach to the cell surface in the form of capsular polysaccharides (CPS) or completely separated from the cell in the form of exopolymeric substances (EPS) (Sutherland, 1972). The bacteria produce exopolymeric substance as a strategy for growth, adhered to solid surfaces, and to survive in adverse conditions (Wrangstadh *et al*., 1986; Decho, 1990).

Exopolymer synthesis is an energy-dependent process, and it differs from one genus to another. A significant carbon investment and noticeable energy cost (up to 70 %) required for the exopolymer production by the bacterial cell (Wolfaardt *et al*., 1999). The amount of exopolymer production depends on the medium and cultural conditions used for the growth of microbes. High carbon and low nitrogen substrate ratio favoured for the EPS production (Kimmel *et al*., 1998). The exopolymer produced by marine bacteria consist of heteropolysaccharides (Decho, 1990), amino or uronic acids, organic or inorganic substituents such as sulphate, phosphate, acetic acid, succinic acid and pyruvic acid (Kenne and Lindberg 1983).

Most bacteria produce exopolymeric substances with massive function; it maintains the structural and functional stability of the cells. It supports the bacteria to strive in adverse conditions like osmotic shock, predation, and drought (Decho, 1990). Although, the exopolymer synthesis was not an essential character for all the bacterial cells. In general, some of the bacteria produce the exopolymer during exponential stage of bacterial growth. Numerous studies have argued that in marine environment, the bacterial cells consume the organic matter as carbon sources to form exopolymeric substances (Heissenberger and Herndl, 1994).

The exopolymeric substances protects and enables the bacterial cells to survive in extreme environmental conditions (Sutherland, 2001). The chemical and physical properties of bacterial exopolymer entice to focus on the exploitation of valuable exopolymeric substances, and make it biotechnologically significant. The exopolymer possess antiviral, immunostimulatory (Arena *et al*., 2009), and anticancer (Matsuda *et al*., 2003),

The exopolymer producing bacterial strains were primarily screened based on the presence of viscous texture on solid media. However, there has been limited literature has been published

on the mucoid texture are associated with exopolymer production in bacterial strains. The exopolymer producing *Halomonas* sp. showed mucoid growth in solid medium (Bejar *et al*., 1998). Knoshaug *et al*., (2000) also described that exopolymer producing *Lactobacillus* sp. are mucoid and slimy appearance. Chen *et al*., (2013) stated that the sticky characteristic of *Bacillus amyloliquefaciens* was able to produce exopolymer.

Bacterial exopolymers are diverged in chemical properties. Some of these exopolymers serve the same chemical structure and function, whereas others are specific for individual taxa and serve distinct biological functions (Anderson *et al*., 1990; Rehm and Valla, 1997). The overwhelming diversity of bacterial exopolymeric substances allows for categorization based on chemical structure, functionality, molecular weight and linkage bonds. The bacterial exopolymeric substances are composed of sugars or sugar derivatives, non-sugar components like proteins, uronic acids, sulphate residues, etc. (Sutherland, 1977; Ruas-Madiedo *et al*., 2002).

The exopolysaccharide yields depend on several critical factors like carbon and nitrogen source utilization, mineral requirements, temperature, optimal pH, etc. The limited nutrient (such as nitrogen, phosphorus, sulphur and potassium) favoured the excessive exopolymer production in marine bacteria stated by Sutherland (Sutherland, 1982). Several studies have revealed that the suitable carbon and nitrogen sources for the growth and exopolymer production varied within the bacterial strains. Hence, selection of most appropriate carbon source for the growth medium composition represents the right effort to optimize the polymer production.

A considerable amount of literature has been published on the impact of various carbon sources on exopolymer production. Linton, (1990) reported that the amount of EPS production by organisms was influenced by the carbon sources. Rodrigues and Bhosle (1991) have reported that among various carbon sources such as glucose, fructose, mannitol and glycerol employed in the medium, glucose supported maximum EPS production.

Bacillus polymyxa produced maximum amount (38 g/L) of EPS in the presence of sucrose (Lee *et al*., 1997). *Vibrio* sp. isolated from marine source produced the highest amount of exopolymer for sucrose and ammonium sulphate (0.006 %) as a nitrogen source (Majumdar *et al*., 1999).

Yun and Park (2003) reported that the *Bacillus* sp. CP912 produced 10 g/L of the extracellular polysaccharide by consuming 11.5 g glucose. Moriello *et al*., (2003) reported that sucrose was the best carbon source for maximal EPS production, compared to glucose, fructose, galactose, trehalose, mannose, and cellobiose by *Geobacillus* sp. However, *Bacillus*

thermantarcticus produce a maximum of EPS for mannose (Nicolaus *et al*., 2004). Wang *et al*., (2011) observed a high level of EPS production (10.45 g/L) in *B. thuringiensis* by making use of maltose as a carbon source and peptone as a nitrogen source. Bragadeeswaran *et al*., (2011) reported that sucrose favours the growth and exopolysaccharide (EPS) production for *Bacillus cereus* GU812900.

B. lichniformis SVD1 produced a high amount of EPS for 4 % sucrose and 0.5 % peptone in the medium (Dyk *et al*., 2012). Razack *et al*., (2013) reported sucrose (2 %) was the best carbon source for high yield (2.6 g/L) of exopolymer in *Bacillus subtilus.*

As far as nitrogen sources are concerned organic nitrogen sources were absorbed by the cells easier than the inorganic ones stated by Gandhi *et al*., in 1997. Inorganic ions affected the exopolymer production combined with the enzyme. The organic nitrogen source promotes both the growth rate and the exopolymer production (Quesada *et al.*, 2004), even if there is some evidence showing that exopolymer production was higher at a lower nitrogen concentration (Gorret *et al*., 2001).

Vreeland *et al*., (1980) reported that *Halomonas elongata* exhibited growth over the range of 5 to 25 % NaCl and grows over the pH range of 5 to 9. Rodriguez-Valera *et al*., (1980) reported that *Halomonas* sp. produced EPS in a MY medium supplemented with 1 % glucose and 7.5 % marine salts.

In MY medium supplemented with 7.5 % sea-salts, favour for EPS production by *Halomonas maura* (Bouchotroch *et al*., 2000). *Halomonas maura* produced a high number of EPS (4.28 g/L) with a medium containing 5 % salt (Arias *et al*., 2003). A moderate halophilic bacteria *Halomonas koreensis* SS20T grows at an optimum pH of 7-8 and the salinity range from 10-12 % and the optimum temperature were 35˚C (Lim *et al*., 2004). A marine bacterium named *Idiomarina* species showed best bacterial growth and polysaccharide production (1.45 g/L) at 32˚C and 7.5 % sea-salt concentrations (Mata *et al*., 2008).

The optimum temperature and pH for the EPS production by *B. thuringiensis* was observed at 28˚C, pH 7.0 (14.96 g/L) (Wang *et al*., 2011). The exopolymer production in *Pseudoalteromonas* sp. increased when NaCl concentration rose from 10 to 30 g/L (Al-nahas *et al*., 2011). *Zunongwangia profunda* SM-A87, produced a maximum of 6.47 g/L when lactose was added to the basic marine medium (Liu *et al*., 2011).

Llamas *et al*., (2012) found *H. almeriensis* M8T which produced a maximum of exopolymer when a medium containing 1 % glucose as a carbon source and 7.5 % of the salt concentration at 32ºC. The optimal temperature, pH, and salt concentration for growth and

exopolymer production observed for *Bacillus licheniformis* at 50°C, pH 8, and 5 % salt concentration (Spano *et al*., 2013).

Characterization of bacterial exopolymeric substance

FT-IR spectroscopy is a most extensively used tool for the structural determination of polymer structure and the analysis of functional groups (Zbinden, 1964; Koenig and Kendall, 1966; Nyquist, 1961). Titus *et al*., (1995) reported that the FT-IR spectrum of EPS produced by *P. alcaligenes* displayed strong peak for O-H, C=O and C-O stretching, indicating the presence of these functional groups in the exopolymer. FT-IR spectrum of EPS produced by *Alteromonas macleodii* exhibited COOH absorption at 1630 cm^{-1} to 1730 cm^{-1} (Raguenes *et al*., 1996). Anton *et al*., (1988) illustrated a strong absorption band at 830 cm^{-1} and 1240 cm^{-1} in the exopolymer of *Haloferax mediterranei* indicated the presence of S=O. The absorption peak approximately at 616 cm^{-1} (690–515 cm^{-1}range) in the exopolymer produced by *Vibrio parahaemolyticus* corresponded to stretching of alkyl-halides (Kavita *et al*., 2011). The absorption at 2988.4 cm^{-1} was due to the C–H stretching vibration in the exopolymer of *Bifidobacterium animalis* RH (Shang *et al*., 2013).

The monosaccharide composition in the exopolymer was analysed by HPLC, and the relative proportion of the peak areas were calculated to estimate the monomer composition (De Vuyst *et al*., 1998). The mono sugar composition in the exopolymer produced by *Bacillus subtilis* has been analysed by HPLC by Vijayabaskar *et al*., (2011). Yilmaz *et al*., (2012) observed the monosaccharide composition in the EPS *Bacillus sphaericus* 7055 by HPLC. Muhammadi and Afzal, (2014), performed HPLC analysis for the determination of monosaccharide composition in the exopolymer produced by *Bacillus* sp.

Applications of Bacterial exopolymeric substances

For the past few decades, there is an increased demand for natural polymers in food, cosmetics, pharmaceutical and other industries where they are used as thickening, stabilizing, emulsifying, gelling agent and water binding agent. Hence, it has led to a remarkable interest in polymer produced by microorganisms. At present, microbial exopolymeric substances are focused well, since they exhibit high growth rate, growth conditions, and high yield. The versatile applicability of some bacterial polysaccharides urged to a synthesize of huge level and are used as raw materials for food processing, in medicine and in industrial preparations. The unique properties of bacterial exopolymeric substance, makes advances in the commercial production like xanthan (*Xanthomonas campestris*), acetan (*Acetobacter*

xylinum) and gellan (*Sphingomonas paucimobilis*) and dextran (*leuconostoc mesenteroides*) (Sutherland, 1998).

Anticancer activity

The ideology of using bacterial exopolymer in the anticancer study was introduced by Shear in 1943 by injecting the exopolysaccharide derived from *Serratia marcescens* to mice, later called as shears polysaccharide. Acetoxane, a polysaccharide complex from *Acetobacter xylinum* was effective against solid Sarcoma 180 (Braude, 1962).

Zhao *et al*., (2010) reported that the exopolysaccharide from *Rhizobium* sp. N613 had strong antitumor activity in mice bearing Sarcoma 180, Hepatoma 22, and Ehrlich ascites carcinoma tumour. The Induction of apoptosis in human T leukaemia cells observed in the exopolymer of *Halomonas stenophila* (Ruiz-Ruiz *et al*., 2011). A sulphated exopolymer produced by *Halomonas stenophila* strain B100 exerted antitumor activity on T cell lines derived from acute lymphoblastic leukaemia (ALL). Only tumour cells were susceptible to apoptosis induced by the sulphated EPS (B100S) while primary T cells were resistant (Ruiz-Ruiz *et al*., 2011). The EPS of *B. licheniformis* has displayed cytotoxic activity (Spano *et al*., 2013) and antioxidant activity (Fang *et al*., 2013). The exopolysaccharide produced by *Bacillus amyloliquefaciens* sp. showed antitumor activity against gastric carcinoma cell lines reported by Chen *et al*., in 2013. The polysaccharides produced by *Bacillus* sp. and *Pseudomonas* sp. showed anticancer activities against Human breast cancer cell lines and Colon cancer cell lines (Vidhyalakshmi and Vallinachiyar, 2013).

Immunomodulatory and antiviral activity

Pseudomonas sp. WAK-1 strain produces extracellular polysaccharide, which showed inhibitory activity on Herpes Simplex Virus-1 in RPMI 8226 cells (Matsuda *et al*., 1999). Exopolysaccharide V2-7 produced by *Halomonas eurihalina* has a strong immunomodulatory activity, which enhanced the unspecific proliferation of human lymphocytes in response to the presence of anti-CD3 monoclonal antibody (Perez-Fernandez *et al*., 2000). The exopolymer produced by *B. licheniformis* strain B3-15, showed antiviral activities (Maugeri *et al*., 2002 and Arena *et al*., 2006). An extracellular polysaccharide (EPS-2) produced by *Geobacillus thermodenitrificans* showed strong immunomodulatory activity by inducing the secretion of IFNα, IL-12, IFNγ, TNFα, IL-18 (Arena *et al*., 2009). The exopolysaccharides of *Lactobacillus* sp. displayed potent antioxidant and immune stimulant activity (Liu *et al*., 2011).

Antioxidant activity

Yasser *et al*., (2007) reported the DPPH radical scavenging activity of the exopolysaccharides of *Rhizobium meliloti*, *Agrobacterium* sp. (curdlan), and *Xanthomonas campestris* (xanthan). Liu *et al*., (2010) reported the antioxidant potential of exopolysaccharide of *Paenibacillus polymyxa* EJS-3 that showed strong scavenging activities on superoxide and hydroxyl radicals. The exopolymer of *Bacillus licheniformis* KS-17, KS-20 has antioxidant activity (DPPH radical scavenging activity) (Song *et al*., 2011). Radha Krishna *et al*., (2011) have demonstrated the antioxidant potential of culture extracts of marine *Bacillus subtilis*. Liu *et al*., (2011) reported that exopolysaccharide of *Lactobacillus* sp. has antioxidant and immunomodulation activity.

The EPS produced by *Paenibacillus polymyxa* SQR-21 showed good superoxide scavenging, flocculating and metal chelating activities while moderate inhibition of lipid peroxidation and reducing activities also takes place (Raza *et al*., 2011). The extracts of *Bacillus subtilis* NRC1aza shows a strong antioxidant, and antitumor activity against human Hepato carcinoma cell line (HepG2) (Aza M Abdel-Fattah *et al*., 2012). Fang *et al*., (2013) studied the antioxidant potential of exopolysaccharide produced by *Bacillus licheniformis*. The crude extract of *Bacillus* sp. JS showed DPPH scavenging activity (Abdel-Wahab *et al*., 2013).

Several *in vitro* assays revealed the antioxidant potential of the EPS produced by marine *Pseudomonas* sp.PF-6 showed scavenging actions on DPPH˙˙OH and O_2^- (Ye *et al*., 2012). The EPS produced by *Labrenzia* sp. showed DPPH and superoxide radicals scavenging activity. It displayed a linear dose-dependent increase in total antioxidant capacity and ferric reducing power activities (Priyanka *et al*., 2014).

Other medicinal properties

A considerable amount of literature has been published on potential application of bacterial exopolymeric substance in medicinal field. Bacterial cellulose, produced by *Acetobacter xylinum* used in the wound dressing for patients with burns, chronic ulcers or extensive tissue loss (Sutherland, 1998). Dextran, produced by *Leuconostoc mesenteriodes*, has been used as plasma substitutes and for the treatment of shock and the blood loss (Silver *et al*., 1998). Glycosaminoglycan produced by *Escherichia coli* K5, *E.coli* K4, and *Pasteurella multocida* is the drug of choice in the prevention and treatment of thromboembolic disorders (Esko and Lindah 2001). Matou *et al*., (2005) reported angiogenic activity of the EPS secreted by *Alteromonas infernus*.

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