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Microbial exopolysaccharides and their potential applications.

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ABSTRACT

Exopolysaccharides (EPS) are organic macromolecules produced by various microorganisms throughout the fermentation process from diverse carbon sources and released beyond the cell wall as slime or into the extracellular media as a jelly-like substance. Polymerization of simple or identical building components, which may be organized as repeating units within polymer molecules, produces EPS. EPSs are hypothesized to protect cells against desiccation, poisonous chemicals, bacteriophages, osmotic stress, allow attachment to solid surfaces, and aid in the production of biofilms. The rising need for natural polymers for industrial uses has drawn a lot of attention to EPS in recent years. Exopolysaccharides' material features, which include a plethora of functional uses and possibilities, have transformed the industrial and medicinal industries. Pharmacological, nutraceutical, functional food, cosmeceutical, herbicides, and insecticides are only a few of the applications of microbial exopolysaccharides, with anticoagulant, antithrombotic, immunomodulation, anticancer, and bioflocculant applications on the horizon.

Keywords : EPS, macromolecules, natural polymers, pharmacological, nutraceutical, immunomodulation..

INTRODUCTION

Exopolysaccharides (EPS) are produced by a variety of bacteria, algae, fungi, and yeasts. Microorganisms' ability to manufacture EPS is a simple and rational response to stressors in the natural world. Exopolysaccharides have been examined in bacteria from hydrothermal vents, halobacteria, methanogens, autotrophic organisms, acidophiles, and microbes from ground water and sewage sludge (Dave et al., 2016). Several microorganisms isolated from harsh environments, such as deep-sea hydrothermal vents, Antarctic ecosystems, salty lakes, and geothermal springs, are being investigated as possible sources of valuable biopolymers, such as EPSs (Nicolaus et al., 2010, Poli et al., 2011). Physical features of microbial exopolysaccharide, such as rheology in solution and capacity to form gel at low concentration, are critical for commercial success. The utility of bacterial biopolymers to humans has been discovered thanks to technological improvement (Nwodo et al., 2012). In nature, microbial polysaccharides are biodegradable and less hazardous to the environment than manmade polymers. This adds to their environmental friendliness in industrial applications, sewage disposal, and other environmental applications

EPSs: Characteristics and Physiological Roles

Microbial EPSs are vital in cellular interactions, nutrition, and micro- and macro environments. Organisms that produce EPS survive better in oligotrophic environments and live below the nutrient concentration threshold that is necessary (Wingender et al., 2012). Microorganism's adherence to surfaces is aided by extracellular polymeric products, biofilms are formed as a result of this. EPS may potentially have a function in maintaining the biofilm's structure and stability. The majority of biofilm is made up of EPS. Microbial EPS promotes cell adhesion to solid surfaces and aids in the creation of aggregates, suggesting that it may play an indirect role in the exchange of genetic materials. The role of the EPS, as seen in various ecological niches, is determined by the microorganism's natural habitat. In general, microbial EPSs are assumed to protect microbial cells in their natural environment against conditions such as desiccation, osmotic stress, antibiotics, or poisonous substances (e.g., toxic metal ions, sulphur dioxide, and ethanol), protozoan predation, phagocytosis, and phage assault. A microorganism's capacity to envelope itself with a highly hydrated exopolysaccharide coating might protect it from desiccation and protozoan predation. In addition, the existence of a gelled polysaccharide layer around the cell may have a significant impact on the diffusion characteristics of the cell. Production of the polysaccharide was linked to dramatically improved protection under heat stress (60-fold), acid stress (20-fold), and simulated gastric juice stress in heat stress tests (15-fold). In pathogenic bacteria, the creation of EPS in the form of capsules is common, and the pathogenicity of an organism is determined by the rate of synthesis and the volume of EPS generated. Capsules containing the pathogen enable phagocytosis to be avoided. One interesting finding is that all capsular polysaccharides do not stimulate the immune system, possibly because their chemical structures are similar to those on the host cell surface. The lectins, which are polysaccharide-binding proteins released by plants (e.g., Trifolin A), are essential for the formation of the symbiotic relationship between *Rhizobium spp*. and leguminous plants. EPS may aid bacterial cell adherence to solid surfaces and the production of biofilms, as well as cellular recognition. Capsular polysaccharide may help bacteria cling to biological surfaces, making it easier for germs to colonize different ecological niches. The EPS was discovered in adherent biofilms (Tsuda et al., 2008); the EPS might serve as both initial and persistent adhesion chemicals (Whitfield, 1988). Furthermore, EPS may act as adhesive factors and encourage plant-bacteria interactions, such as levan synthesis by the sugar cane root invading *G. diazotrophicus* (Allison and Sutherland, 1987). Oral streptocci produce homopolysaccharides (glucans and fructans), which have a significant impact on the production of dental plaque. They have a role in bacterial adhesion to one other and to the tooth surface, as well as influencing material transport via plaque and functioning as extracellular energy stores (Hernández et al., 1995). EPS generated by LAB has been linked to cellular recognition, adhesion, and the development of biofilms in several studies (Russell, 1990 and De Palencia, 2009).

Applications of EPS

They're used in the food, textile, detergent, and beverage sectors, as well as the pharmaceutical, biotechnological, agricultural, paper, paint, and petroleum industries, as well as drug delivery and cancer therapy, and culture media formulation (Quesada et al., 1993).

Food and Beverages

Lactic acid bacteria (LAB) generate exopolysaccharides (EPS), which are used to make fermented dairy products like yoghurt, drinkable yoghurt, cheese, fermented cream, and milk-based sweets (Duboc and Mollet, 2001). EPS can function as both texturizers and stabilisers, increasing the viscosity of a finished product and binding water and reacting with it (Tabibloghmany and Ehsandoost E, 2014). As a result, EPS can reduce the production of hazardous by-products and enhance the product's stability. Furthermore, it has been suggested that EPS can have a favourable impact on gut health (Broadbent et al., 2003). Exopolysaccharides increase the quality of foods that have been processed with its aid. LAB produced EPS is commonly used to enhance the body and texture of yoghurt and other fermented milk products such as dahi (Whistler and Daniel JR, 1990). Different EPS generating cultures of *L. Lactis subsp. Lactis PM23, S. thermophilus ST,* and *L. Lactis* are used to make low fat dahi. Kefir is an Eastern European traditional self-carbonated, somewhat alcoholic fermented milk (Kulicke and Heinze, 2005, Becker, 1998). Kefir grains, which are made up of homofermentative and heterofermentative LAB, yeasts, and acetic acid bacteria, are used to make kefir. Dextran derived from *Leuconostoc mesenteroides* is used in commercial baking improvers. *Weissella* strains that produce EPS in sourdoughs enhanced the textural qualities and bread quality (Yun, 1996 and Yamamoto et al., 1990). Lactobacilli-derived polymers are thus predicted to improve one or more of the following technical aspects of dough and bread such as: (i) dough absorption, (ii) dough rheology and machinability, (iii) dough stability during frozen storage, (iv) loaf volume, and (v) bread staling. Fructose-oligosaccharides (FOS) are attractive for culinary applications because they have lower sweetness than sucrose, are calorie-free, and are noncariogenic (Daba and Ezeronye, 2003). Inulin and FOS are used in food primarily for their prebiotic characteristics. Surprisingly, fructose-based polymers may be digested by gut bacteria, resulting in improved intestinal flora and improved mineral absorption (Hidalgo et al., 2012, Maiden et al., 2013).

Prebiotic effect

Korakli et al. (Crescenzi, 1995) effectively proved the ability of fructan-type EPS generated by one strain of *L. sanfranciscensis* to behave as prebiotic substrates. Salazar et al. (Xu et al., 2006) demonstrated that EPS produced by intestinal *Bifidobacteria* serves as a fermentable substrate for microorganisms in the human gut environment, altering interactions between intestinal populations.

Pharmaceuticals

The intrinsic biocompatibility and apparent non-toxic character of several of these bacterial exopolysaccharides has led to their usage as scaffolds or matrices in tissue engineering, drug delivery, and wound dressing, making them more appealing than polysaccharides derived from plants and microalgae. Antitumor and immune-stimulating polysaccharides have been found in polysaccharides of *Basidiomycetes mustran*. They also have antiviral properties. Exopolysaccharides have been shown to have health-promoting properties such as cholesterol reduction, immunological modulation, and prebiotic benefits.

Antigastritis, antiulcer and cholesterol lowering effects

Purified EPS from *S. thermophilus* CRL 1190 has been shown to help prevent chronic gastritis (Faber et al., 2001). Nagaoka et al. (Shah and Prajapati, 2013) found that EPS generated by bifidobacteria, lactobacilli, and streptococci strains have antiulcer properties. The EPS-producing strain *Lc. Lactis ssp. cremoris SBT0495* is used to make fermented milk which has cholesterol-lowering effects; however, the mechanism is uncertain (Tamime et al., 2005, Galle et al., 2010).

Antitumour properties

L. helveticus ssp. jugurti generated an antitumor EPS, according to Oda et al. (Kitazawa et al., 1992). The scientists came to the conclusion that the EPS's anticancer effect may be due to its host-mediated activities. EPS has also been shown to have immunomodulatory and anticancer properties in a few studies (Colliec-Jouault et al., 2001, Vanhooren and Vandamme, 2000, Martin et al., 1984). *B. adolescentis* slime was shown to have immunomodulatory effects on mice splenocytes (Baruah et al., 2016). The slime-forming *Lc. Lactis ssp. cremoris KVS20* demonstrated anticancer action, according to Kitazawa et al. (Arena et al., 2009), and the slime included significant B-cell dependent mitogenic chemicals. Bacterial EPSs would be linked to anti-tumor, anti-viral, anti-inflammation, inducer of interferon production, platelet aggregation inhibition, and colony stimulating factor actions, among others (CSF). EPSs generated by marine *Vibrio* and *Pseudomonas sp.,* for example, exhibit anticancer, antiviral, and immunostimulatory properties.

Some polysaccharides are used in vaccinations generally combined with appropriate protein. Meningitis vaccines and multivalent polysaccharide vaccines against *Streptococcus pneumoniae* and *Klebsiella* have been developed in this manner.

Anti-mutagenic properties

Mutagens such as heterocyclic amines were bound to EPS-attached cells of strain *L. plantarum*, and the mutagens were inactivated by attaching to EPS (Sutherland IW, 1998, Otero and Vincenzini M, 2003).

Cosmetics:

Pharmaceutical cream formulations and barium sulphate preparations both benefit from the excellent suspension stability of EPS. The cosmetic sector, for example, uses this high cream stability advantage in toothpaste technology, where the toothpaste holds its components (high viscosity) and then brushes easily onto and off the teeth (high shear thinning).

Bioremediation

Bacterial polysaccharides have been demonstrated to have the capacity to bind cations and have ion absorption capabilities, bolstering its effectiveness in bioremediation procedures. The degree of acetylation influences the selectivity of certain EPS for metal binding sites (Rehm, 2010). These characteristics might be extremely useful in sewage treatment, particularly for the elimination of harmful heavy metal contaminants (Sutherland, 1983). They have a higher metal complexing capability; therefore, they may be used as a complement or potential replacement for conventional metal removal methods in mining and industrial waste. In the presence of an EPS layer, mineral solubilization is observed to have a considerable impact. *Acidithiobacillus ferrooxidans* also produces an EPS layer, which aids in the extraction of metals from sulphidic minerals (Choi and Yun, 2006). Exopolysaccharide-producing bacteria have also been employed as a bioinoculant to increase rhizosphere soil aggregation and water retention as a function of soil water content.

Cryopreservation

The most acceptable approach for the long-term preservation of microbial cultures is lyophilization with cryoprotectants (one or more chemicals that protect cell membranes against the effects of exposure to low temperature) (Yu et al., 2011).

Table 1. Applications of different microbial EPSs along with their sources in food and beverage industry (Galle et al., 2012)

Fermented foods and beverages	**EPS-producing microorganisms**	**Applications**
Fermented beverages		
Kefir Yogurt Mexican Pulque	*L. kefiranofaciens* *S. thermophilus; L. delbrueckii subsp. Bulgaricus* *Leuc. mesenteroides*	To minimize the quantity of additional milk solids, increase end product viscosity, texture, stability, and mouthfeel, and eliminate syneresis (whey separation) during fermentation or storage (Rühmkorf et al., 2012, Wolter et al., 2014a and 2014b, Palomba et al., 2012).
Fermented breads		
Gluten-free breads	*W. cibaria; W. confusa; Leuc. mesenteroides; L. sanfranciscensis*	Improved texture and quality (Galle et al., 2011, Galle et al., 2014, Di Cagno et al., 2014, Wang et al., 2012, Hassan et al., 2004, Kumar et al., 2007).
Wheat	*Leuc. lactis, L. curvatus*	Enhanced viscoelasticity and quality (Nagaoka et al., 1994)
Gluten-free sorghum	*L. casei FUA3185 and FUA3186, L. buchneri FUA3154*	Enhanced rheology of sorghum sourdoughs (Escalante et al., 2008)
Cheeses		
Mexican Chihuahua	EPS-producing starter culture	Enhanced cheese yield and texture (van Kranenburg et al., 1999)
Low-fat Italian Cacciotta type	*S. thermophilus*	Pleasant to taste and chew, better flavor, overall acceptability (Cosa et al., 2011)
Reduced-fat	*S. thermophilus TM11*	Enhanced moisture content and high yield (Vanhooren and Vandamme, 1998)
Egyptian Karish		Improved acceptability, spreadability, and creaminess (Mabinya et al., 2012)

Table 2. Microbial biopolymers and their applications in other industries (Aparna et al., Nakajima et al., 1992)

Source Organism	**Biopolymer**	**Applications**
Bacillus, Streptococcus, Pseudomonas, Zymomonas	Levan	As a viscosifer and stabilizer in the preparation of sweet confectionery and ice cream
Leuconostoc mesenteroides	Dextran	Used for thickening and gelation of syrups (van de Guchte et al., 2002), gelling agent in gum and jelly sweets. To increase moisture retention, viscosity, and sugar crystallization resistance in confectionery items, used in ice creams and puddings to give the desired body, texture and mouth feel (de Valdez et al., 1995). Dextran has been employed as a component of various chromatographic stationary phases, in separation technology and aqueous two-phase systems (Dilna et al., 2015) and as a blood plasma extender (Kumar and Mody, 2009), blood flow improvement agent, and cholesterol reducing agent in veterinary and human medicine, and as a micro-carrier in tissue/cell culture.
Pseudomonas aeruginosa and Azotobacter vinelandii	Alginate	As a viable cell and enzyme immobilization matrix, a covering for seedlings and plants' roots to avoid desiccation, a microencapsulation matrix for fertilizers, insecticides, and nutrients, and hypoallergic wound-healing tissue
Xanthomonas campestris	Xanthan	Used in both food and non-food applications (De Vuyst L and Degeest, 1999), suspending agent in food industries, such as dairy products, beverages, confectionary, dressing, bakery products, syrups, and pet foods, as well as the oil, pharmaceutical, cosmetic, paper, paint, and textile industries. Also used in secondary and tertiary crude oil recovery, paints, pesticide and detergent formulations, pharmaceuticals, cosmetics, and printing inks, as a viscosifier, stabilizer, emulsifier, and food as a thickening and stabilizing agent, often used in combination with guar gum. This biopolymer, according to Becker et al. (Wang et al., 2015), has a high viscosity at low concentrations in solution, significant pseudoplasticity, and is stable throughout a wide pH, temperature, and ionic strength range.
Acetobacter spp.	Cellulose	In human medicine, as temporary artificial skin to cure burns or surgical wounds, in nutrition, as natural non-digestible fibers (that can be impregnated with amino acids, vitamins, and minerals), and in separation technology, as acoustic membranes in audio-visual equipment.
Streptococcus equii and Streptococcus zooepidemicus	Hyaluronic acid	N-acetyl glucosamine, glucuronic acid used in ocular surgery, as a replacement for eye fluid, in artificial tear-liquid, as a synovial fluid replica, in wound healing, and in the beauty sector (lotions, moisturizing agent)
Sphingomonas paucimobilis	Gellan	As a food stabilizer and suspending agent. As a gelling agent for hardening culture medium, particularly for research on marine microorganisms.
Sinorhizobium meliloti M5N1CS, Gluconacetobacter hansenii	Glucuronan	Food and cosmetics products
Rhizobium meliloti and Agrobacterium	Curdlan	Curdlan, in combination with zidovudine (AZT), acts as a gelling agent and has significant antiretroviral activity (anti-AIDS-drug)

radiobacter		
Alcaligenes faecalis var. myxogenes	Succinoglycan	As a gelling agent and other functions are same as for curdlan
E. coli, Shigella spp., Salmonella spp., Enterobacter spp.	Colanic acid	Cosmetics and personal care products
Acetobacter xylinum	Acetan	As a food viscosifier and gelling agent. It is used to make sweet confectionery and vinegar.
Acinetobacter calcoaceticus	Emulsan	Crude-oil recovery and other applications are similar as for alginate

Cryoprotectants such as HoPS (homopolysaccharides) and dextran have been employed to prepare bacterial suspensions before drying (Patel and Prajapati, 2013). HePS can also be utilized to protect cells against freezing. HePS from LAB has also been shown to have antioxidant action in the food and fermentation sectors. HePS exhibits antioxidant effects that are equivalent to those of the powerful antioxidant ascorbic acid (Di Cagno et al., 2006). HePS from *Lactobacillus plantarum RJF4* has been proven to scavenge DPPH free radicals (measurement of antioxidant activity). The rheological characteristics of EPS allow the generation of viscous solutions at low concentrations (0.05–1.0 percent) and stability throughout a wide temperature, pH, and ionic strength range (Kumar et al., 2007). In the food and pharmaceutical industries, EPS with non-Newtonian and pseudo-plastic behavior can be employed as a viscosifier (Dudman, 1977, Korakli et al., 2002). Although various HePS from LAB have been recorded, HoPS from LAB are widely employed as commercial viscofiers.

Miscellaneous applications

Alteromonas infernus, collected from deep-sea hydrothermal vents, was shown to produce a low molecular weight heparin-like EPS with anticoagulant properties (Rodríguez et al., 2008). Clavan, a polysaccharide containing L-fructose, has potential use in limiting tumor cell colonization of the lung, regulating white blood cell formation, treating rheumatoid arthritis, synthesis of antigens for antibody generation, and in cosmeceuticals as a skin moisturizing agent (Salazar et al., 2008). In vitro 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, chelation of ferrous ions, prevention of linoleic acid peroxidation, and reducing power were all exhibited in the EPS of *L. paracasei subsp. paracasei NTU 101* and *L. plantarum NTU 102.* According to Martin et al. (Schwab et al., 2008), certain bacterial EPS alone or in conjugates can operate as a highly effective somnogen, so sleep induction using a natural product with minimal adverse effects will eliminate the need for xenobiotics.

Future Prospects for Bacterial EPS

Although bacterial EPS has a wide range of applications in industry (textiles, dairy, cosmetics, etc.), health (medicine and pharmaceuticals), and the environment (remediation, flocculation, etc.), its use in the flocculation process will be a significant milestone for health promotion and environmentally friendly use, particularly in municipal and wastewater treatment processes. Microbial EPS might be used as a safe flocculant alternative. Biopolymers generated by non-lactic bacteria such as *VirgiBacillus spp., Bacillus spp., and Artrobacter spp.* have been shown to have a high flocculation effectiveness (Torino et al. De Vuyst and Degeest, 1999). These findings suggest that bacterial EPS successfully induces flocculation and can thus be used in large-scale industrial operations, specifically in the treatment of water and wastewater. EPS may potentially have a role in the absorption of metal ions and the supply of lower oxygen tension (Vaningelgem et al., 2004, Wang et al., 2008). Many metal ions, including Fe^{2+}, Zn^{2+}, Cu^{2+}, and Co^{2+}, might be bound by EPS (Qin et al., 2007). It could also combine colloidal and suspended particles and performed as an excellent flocculating agent.

CONCLUSION:

Microorganisms have evolved a variety of strategies for surviving in harsh environments, particularly in soils. EPS generation is a crucial approach for maintaining a moist environment, capturing nutrients, promoting chemical processes, and protecting cells from environmental factors, antibiotics, and predator assault. Extracellular polymers from bacteria have a wide range of activities that are dependent on their composition and structure. Extracellular polymeric compounds have long piqued researchers' attention due to their biodegradability, biocompatibility, and ability to thicken, gel, and emulsify. To attain high yields, the polymers and their synthesis may be modified, but this requires the characterisation and physiological investigation of EPS-producing bacteria. Understanding the underlying processes and regulatory pathways is necessary for improving polymer synthesis. Novel EPS and polymers generated by less characterized microbial strains are still underexplored, in contrast to the considerable study focused on enhancing EPS output and changing the features of well-known polymers. Understanding the structure and characteristics of EPS is critical to comprehending how they interact with soil. The integration of multiple domains and the combining of traditional microbiological techniques with new high-throughput approaches are critical for expanding knowledge of EPS composition, structure, function, and applications.

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