Biopolymers Research

Review Article Open Access

Exploring and Utilization of Some Bacterial Exopolysaccharide

Tereza Cristina Luque Castellane[1,2*], Manoel Victor Franco Lemos[2] and Eliana Gertrudes de Macedo Lemosa[1]

[1]Departamento de Tecnologia, UNESP - Univ Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Rod. Prof. Paulo Donato Castellane km 5, Jaboticabal, SP, Brazil

[2]Departamento de Biologia Aplicada à Agropecuária, UNESP - Univ Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Rod. Prof. Paulo Donato Castellane km 5, Jaboticabal, SP, Brazil

Abstract

In the mid of the 19th century, the first remarkable example of a Microbial Exopolysaccharide (EPS) used in pharmaceutical applications was discovered in wine, a neutral polymer with α-(1→6) and α-(1→4) glucopyranosyl linkages, called dextran. Other than this, a vast number of extracellular carbohydrate polymers were described over the recent decades, with only a few of them emerging as industrially important macromolecules. Since an impressive large volume of publications has been dedicated to the potential novel applications of EPS, we believe that several novel EPS with significant commercial value will certainly spark interest soon. This review aims to achieving the status of the recent potential candidate EPS to future commercial applications.

Keywords: Biological applications; Bioflocculants; Extracellular carbohydrate polymers; Heavy metals; Rhizobium

Introduction

In recent years, increasing attention has been paid to the exploration and discovery of recent advances in exopolysaccharides (EPS) and their potential biotechnology and biological applications opportunities. Therefore, stimulating a strong interest towards the finding of new types of EPS molecules and the new techniques for optimizing their production for product quality and properties improvement [1-9]. EPS are produced by microorganisms, particularly bacteria and fungi via intracellular or extracellular pathways Independent of their origin, EPS are secreted on the outside the microorganism's cell surface. These biopolymers are synthesized via different biosynthesis pathways and these processes can be divided into three main steps. First, the recognition of a carbon substrate must occur through the cell and then afterwards, intracellular synthesis of polysaccharides takes place. Last, the produced EPS are transported to the outside of the cell [10], except for levans, alternans and dextrans, which are synthesized by an extracellular process [4,11]. Direct precursors for EPS's biosynthesis are formed intracellularly using intermediates of the central carbon metabolism [1]. There is important information on the genome of the EPS producer microorganisms that will enable them to develop additional strategies to the successfully enhance EPS's production rate and to engineer their properties by modifying monomers composition, functional groups, and chain length [12-16]. EPS are long-chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives and no-carbohydrate components [3,17-19]. Depending on the species and cultivation conditions the EPS production by microorganisms using glucose and sucrose may range between 0.29 and 65.27 g/L [20]. Utilization of different carbon and nitrogen sources for the growth and EPS production by bacteria were reported earlier [4,21,22].

The unique biological, physicochemical and rheological properties of bacterial EPS, such as bioadhesives, gelling agents, stabilizing, and thickeners, makes them interesting candidate for many industrial applications. EPS can be used as bioflocculants [23-25], bioabsorbants of heavy metals [5,7,8,26,27], drug delivery [27], anticoagulant, antioxidant [26], anti-inflammatory, antibiofilm, antithrombotic, immunomodulatory, immunostimulatory, antiulcer, and anticancer agents [28,29] and parasitological and entomological properties [30]. The properties of some microbial exopolysaccharides have been summarized (Table 1).

Information on properties of certain EPS like xanthan, a biopolymer synthesized by Xanthomonas campestris pv. campestris, as well levan, dextran and pullulan are abundants, whereas industrial application data for other EPS (i.e. rhizobial EPS) is still limited.

Recent researches revealed that cell motility can be enhanced by EPS in some bacteria [31] and its accumulation can protect the cells from antibiotics, phagocytosis, bacteriophage attack, toxic metal diffusion and unfavorable environmental factors, i.e. high salt stress, desiccation, and oxidative stress conditions [32-34]. Furthermore, EPS offer a potential use in agriculture due to it adhesive properties and it ability to form gels as summarized by Bomfeti et al. [19].

Generally, a majority of the exopolysaccharides have excellent biocompatibility, low toxicity, antimicrobial, hypoallergic, and biodegradable properties [35,36]. For these reasons, EPS has emerged as a biopolymer more promising owing to the advantages and the ample applicability. For example, pure EPS mediated metal remediation are environmentally safe, and them can be recycled after desorption of adsorbed metal ions that resulting cost effective adsorbent [5]. Compared to synthetic polymers, low EPS efficiency of biosorption in real industrial samples has been a limiting factor in the non-industrial applicability of several prospective exopolysaccharides.

Although we believe that several novel bacterial exopolysaccharides with significant commercial value may spark interest soon. This review aims to achieving the status of the recent potential candidate EPS to future commercial applications. The presented data highlight such new EPS applications instead of officially acknowledge its worldwide accepted documents curated by diverse authorities.

Composition of EPS from some species

Cellulose, dextran, mutan, alternan, pullulan, levan, and curdlan

***Corresponding author:** MTereza Cristina Luque Castellane, Departamento de Tecnologia, Laboratório de Bioquímica de Microrganismos e Plantas, UNESP - Univ Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Rod. Prof. Paulo Donato Castellane km 5, CEP 14884-900, Jaboticabal, SP, Brazil, Tel: +55 16 997962875; Fax: +55 16 32092675; E-mail: teluque@yahoo.com.br

Received March 30, 2018; **Accepted** April 27, 2018; **Published** May 02, 2018

Citation: Castellane TCL, Lemos MVF, Lemosa EGdeM (2018) Exploring and Utilization of Some Bacterial Exopolysaccharide. Biopolymers Res 2: 106..

Microorganism	EPS	Monomer units	Main use	Reference
Lactobacillus sp. *Leuconostoc* sp. *Streptococcus* sp	Dextran	Glc	Non-ionic, good stability Newtonian, fluid behavior. EPS can be used in confectionary to improve moisture retention, viscosity and inhibit sugar crystallization.	[35]
Azotobacter vinelandii Pseudomonas aeruginosa	Alginate	GulA, ManA	It is biocompatibility, biodegradability, low toxicity, hypoallergic with gelling capacity, film forming, and binding agents. This type of polymers can also be used as an immobilization matrix for viable cells and enzymes	[36-38]
Xanthomonas spp.	Xanthan	Glc, Man, GlcA	It has good water-solubility, increase viscosity, thickening, stabilizer, emulsifier and suspending agent in food industries, which excellent biocompatibility.	[39-41]
Alcaligenes xylosoxidans	Welan	Glc, Glc, GlcA, Man	Construction chemistry.	[42]
Pseudomonas elodea	Gellan	Glc, Rha, GlcA	Construction chemistry, food, feed.	[43]
Rhizobium meliloti; Agrobacterium radiobacter	Curdlan	Glc	Gel-forming ability, water insolubility, edible and non-toxic has biological activity on heavy metal removal. As a concrete additive and immobilization matrix.	[44]
Acetobacter spp.	Cellulose	Glc	Not soluble in most solvents. Foods (indigestible fiber), biomedical (wound healing, tissue engineered blood vessels) and audio speaker diaphragms	[44]
Alcaligenes faecalis var. Myxogenes and *Agrobacterium* spp.	Succinoglycan	Glc, Gal	High viscosity and acid stability. Applications Food and oil recovery	[45]
Sinorhizobium meliloti M5N1CS and *Gluconacetobacter hansenii*	Glucuronan	GluA polymer	Gelling and thickening capacity for the food and cosmetics products	[44]
Sphingomonas spp.	Sphingans	Glc, GlcA, Man, Rha	As gelling, stabilizing and suspending agent in foods, bioemulsifying activity	[46-48]
Acinetobacter spp.	Emulsan	Sugar and fatty acid	It forms and stabilizes oil, emulsion stabilization features, such as the bioremediation of heavy metals and crude oil and other applications are similar as for alginate	[49]
Enterobacter A47	FucoPol	Fuc, Gal, Glc, GlcA	Viscous aqueous solutions with shear thinning behaviour, film-forming capacity, flocculating activity, and emulsion forming and stabilizing capacity.	[10,50-52]
Aureobasidium pullulans	Pullulan	Glc	High viscosity	[53]
Rhizobium undicola strain N37	EPS of N37	Gal, Man	Good stability Newtonian, fluid behavior	[54]
Rhizobium sp. LBMP-C04	EPS of LBMP-C04	Rha, Glc, Gal, Man, GlcA,	It is a good water-solubility, viscosifier, emulsifier agent	[55]
R. tropici Semia 4077	EPS of 4077	Glc, Gal, GlcA, GalA, Man, Rha	It is a good water-solubility, viscous aqueous solutions with shear thinning behaviour, film-forming capacity, and emulsifier agent.	[3,56]

Table 1: Properties of some microbial exopolysaccharides.

are examples of homopolysaccharides which contain repeating units of only one type of monosaccharide [29,37]. With respect to the heteropolyaccharide, species such as, Acidobacteria [6], *Alteromonas infernus* and *Vibrio diabolicu* [38], *Klebsiella* [39], *Alteromonas* [9], *Rhizobium* [3], *Salipiger* [40], and *Sphingomonas* [41], *Lactococcus* [42], *Bifidobacterium* [43], *Cordycepsgracilis* (Grev.) [44], *Bacillus* sp. [8] and *Pseudomonas* [45] were reported to produce EPS.

Furthermore, there is a growing number of recent reports on other discovered EPS produced by Gram-negative soil bacteria belonging to Rhizobiaceae, a family of Rhizobiales order into Alphaproteobacteria class, that have distinct properties. EPS from various rhizobial species have been studied and characterized [3,21,46-55]. Additionally, it had also been shown that the rhizobia have others polysaccharides such as cyclic β-(1,2) glucans, outer membrane-localized lipopolysaccharide (LPS), capsule polysaccharide (CPS), gel-forming polysaccharide (GPS), K-antigen polysaccharide (KPS), and high-molecular-weight neutral polysaccharide (NP or glucomannan) [51].

Generally, the rhizobial EPS's contain D-glucose, D-galactose, moreover very few EPS's also contain several rare sugars such as D-Mannose, L-rhamnose, D-Xylose, or uronic acids. These data are similar to the findings reported by Castellane et al. [55], who found that EPS obtained from *Mesorhizobium huakuii* $LMG_1$4107, M. loti $LMG_6$125, *M. plurifarium* $LMG_1$1892, *Rhizobium giardinibv*. Giardini $H_1$52T, *R. mongolense* $LMG_1$9141 and *Sinorhizobium* (=Ensifer) kostiense $LMG_1$9227 were primarily composed of glucose and galactose. In contrast, Rossi & De Philippis [56] reported that hexoses, glucose, galactose, pentose, ribose, mannose, fructose, xylose, arabinose, acidic hexoses, deoxy hexose, fructose, rhamnose, methyl rhamnose, glucuronic acid, galacturonic acid are the main building blockers usually noticed in cyanobacterial EPS. Hexoses and pentoses are usually found in the cyanobacterial EPS along with non-sugar moieties like uronic acids. EPS from cyanobacteria are heteropolymers having high molecular weight heteropolymers which is made of monosaccharides, lipids, proteins and DNA [57].

Various studies have found that single repeating unit is decorated by different non-carbohydrate such as hydroxyl, carbonyl, carboxyl, thioether, and sulfonate functional groups, thus revealed the complex nature of EPS. Priyanka et al. [53] studied a type of EPS producing *Rhizobium* sp. (PRIM-18) isolated from the root nodule of a sand dune legume *Canavalia trifolia* collected from Someshwara coast, India. These authors reported that this isolate, named PRIM-18, produced highly mucoid colonies on yeast mannitol agar. EPS from PRIM-18, with molecular weight of 9.33×10^6 Da, showed a characteristic peak of polysaccharide with broad and intense band representing O-H stretching of hydroxyls (3400 cm^{-1}) and peak at 879 cm^{-1} assigned to β-glycosidic linkage and a peak at 1253 cm^{-1} and 1729 cm^{-1} corresponding to acetyl group and the presence of succinyl groups, respectively [53].

Recently, Borah et al. [25], Identified the EPS of the *Cyanobacterium, Cyanothece epiphytica* AUS-JR/DB/NT-021, which contains glucose (42.49%), galactose (20.47%), mannose (11.74%), arabinose (6.50%), xylose (14.84%) and fucose (3.97%) as the carbohydrate component.

This EPS was also composed of 18.83% of sulphate and a low amount of acidic hexose, uronic acid (5.38%) including protein moieties (8.39%).

According to a study by Ruiz et al. [58], succinoglycan, a symbiotically important type of EPS of Rhizobium sp. is also produced by microorganisms as *Agrobacterium* and *Pseudomonas* sp. However, *Agrobacterium radiobacter* NBRC 12665 isolates when immobilized on a loof sponge produce succinoglycan from the substrates lactose and sugar cane molasses was 9.3 g/L and 14.1 g/L, respectively [58]. In addition, these EPS exhibited weight-average molecular weights of 2.734×10^6 g/mol and 2.326×10^6 g/mol, respectively, and the presence of carbohydrates residues, carboxylic groups exhibiting non-Newtonian and shear thinning behavior [58].

Several factors can influence the rheological behavior of succinoglycans, such as non-saccharide substituents. Compared to bacteria of the genera *Acetobacter, Lactobacillus, Streptococcus*, and *Xanthomonas*, the synthesis of rhizobial EPS is believed to be similar and their extraction is much simpler [54] and apparently non-toxic. Various authors reported that the xanthan gum is a heteropolysaccharide composed of a glucose backbone with trisaccharide side chains consisting of two mannose units alternating with glucuronic acid. Approximately 50% of the terminal mannose residues are pyruvated and the non-terminal residues usually carry an acetyl group at C-6 [29,59,60]. Wellan, an anionic polysaccharide from Alcaligenes, carries only one acetyl group as substituent and the presence of glucose, galactose, rhamnose and glucouronic acid in a molar ratio of nearly 1.0:1.6:0.4:2.3, respectively [61]. While, considered the same comparison, the carbohydrate portion of sphingans contained about 13% glucuronic acid and some neutral sugars including mannose, glucose and rhamnose in the molar ratio of 1:2.28:2.12 [62,63]. Xanthan gum is still an important biopolymer for commercial purposes. However, other bacterial EPS such as sphingans and wellan with unique properties are currently used on new commercial applications [10,29].

A large diversity in EPS's chemical structure can be found within the diverse Bacteria kingdom, considering the monosaccharide composition and linkage in the single subunit, the repeating unit size, and the degree of polymerization, as well as non-carbohydrate decoration. Roca et al. [22] suggested that the EPS containing rare sugars (i.e. L-rhamnose or L-fucose), might have the improved biological properties compared to the EPS's containning only common monosaccharides (D-glucose, D-mannose, D-galactose, D-glucuronic and D-galacturonic acids).

Antimicrobial activity

The unique physicochemical characteristic of microbial exopolysaccharides makes them suitable candidates for many biological applications e.g. anticoagulant, antioxidant, anti-inflammatory, antimicrobial and, antibiofilm applications. In relation to the antimicrobial activity, many new isolates, mainly Lactic Acid Bacteria (LAB) belongs to the genera *Streptococcus, Lactobacillus, Lactococcus, Leuconostoc*, and *Pediococcus* were found to have LPSs exhibiting antibacterial or antioxidant activities [43,64]. However, so far there are a few studies, which have focused on the antioxidative and antibacterial effects of the EPSs produced by LAB [31,65].

Ouwehand and Salminen [66] reported that ability of LAB to produce antimicrobial compounds that might constitute a crucial characteristic for the effective elimination of intestinal pathogens and expression of probiotic effect for the host. EPS produced by the LAB have been associated with a number of healthy beneficial functions [43].

Li et al. [43] investigated the antioxidant and antibacterial activities of EPS from *Bifidobacterium bifidum* WBIN03 (B-EPS) and *Lactobacillus plantarum* $R_3$15 (L-EPS). The same authors, found that both EPS produced by them, had equal antimicrobial activities against *Listeria monocytogenes* $CMCC_5$4007, *S. aureus* $CGMCC_2$6003, *Bacillus cereus* $ATCC_1$4579, and *S. typhimurium* $ATCC_1$3311, whose inhibition zones were lower than those with 50 µg/mL ampicillin [43].

Similarly, Liu et al. [65] found that purified EPS from *Lactobacillus plantarum* $WLPL_0$4 could significantly inhibit the adhesion of *Escherichia coli* $O_1$57:H_7 to HT-29 cells with an inhibition rate of 20.24 ± 2.23, 29.71 ± 1.21, and 30.57 ± 1.73%, respectively. Furthermore, the EPS exhibited strong inhibition against biofilm formation by pathogenic bacteria, including *P. aeruginosa* $CMCC_1$0104, *E. coli* $O_1$57:H_7, *Salmonella typhimurium* $ATCC_1$3311, and *S. aureus* $CMCC_2$6003

Exopolysaccharides synthesized by the four LAB strains from Tunisia demonstrated inhibition properties against *L. monocytogenes, P. pentosaceus*-DPS, showing the most effective inhibition against *E. coli* (zone diameter of 0.9 ± 0.05 cm) [80]. However, these studies did not describe mechanistically the antibacterial properties of these EPS. Yet the literature reports that there are protocols from various studies, which might lead to new discoveries that could explain the antibacterial activity of exopolysaccharides produced by other species, such as *Lactobacillus* and yet belonged to the species fermentum and plantarum [47].

However, few studies have examined the EPS from strains belonging to Rhizobiaceae which had strong antimicrobial activity against several pathogens in vitro. Moreover, the production of EPS by the rhizobia is considered to have more important physiological implications and its critical constituents seemed to act only during the invasion process leading to the determination of nodule types [2,69]. These bacteria exhibit great morphological, physio-logical, genetic, and phylogenetic diversity and can be a valuable source for the screening of strains with specific properties [52].

Mechanism of EPS metal sorption

The treatment regimes for remediation of various heavy metals ions (e.g. coagulation, chemical precipitation, evaporative recovery, floatation, flocculation, ion exchange, nanofiltration and ultrafiltration) generate amounts of toxic sludges and byproducts, which significant environmental pollution [5,70]. Some environmentally safe strategies are been increasingly adopted for more sustainable practices.

Bacteria cells have the ability cover their peripheral surface with a shield of EPS to protect themselves in an attempt to reduce the infiltration of toxic heavy metal ions [7]. Exopolysaccharides are a good example of a green product, with innovations on their use and in doing so their hold significant importance as compared to the synthetic products [5,7-9,26].

The strategies to achieve the goal of remediation through exopolysaccharide use must be focused on utilizing the negatively charged EPS and other non-neutral molecules to be incorporated as a surface-active agent [5]. Recent studies have indicated that hydroxyl (O-H) and carbonyl (C=O) groups of the polymer are involved in metal chelation [26] since the EPS have the presence of negatively charged ionizable phosphate, carboxylate, acetate, amine, and rarely sulfate groups and also often polyanionic residues, due to association of these functional groups with the polysaccharide backbone, as Pal and Paul suggested [71]. It is for this reason that heavy metals ions are biosorbed or complexed by these functional groups.

Depend on the source of the EPS there are different strategies to microbial EPS's ability to orchestrate different heavy metal biosorption. Gupta and Diwan [5] described four mode based on the type of the EPS: homogeneous consortial EPS, usually from pure cultures, or heterogeneous consortium, dead or immobilized cells (immobilized EPS). Examples of metal biosorption following homogeneous consortial EPS are reported in the case of *Methylobacterium organophilum* [72], *Agrobacterium* species, *R. tropici* [73], and *Sinorhizobium meliloti* [74]. An EPS extracted and purified from *Klebsiella* sp. J_1 showed greater proportion of copper ion removal over other metal ions by bacterial EPS [39]. Even though EPS have different affinities for three classes of heavy metals (toxic metals, precious metals, and radionuclides), the environmental modifications could be observed due to the EPS involvement [75].

Emulsifying potential of the EPS

Many bacteria like *Acinetobacter, Halomonas, Pseudomonas, Rhizobium, Rhodococcus*, and *Actinomycetes*, and other biofilm forming bacteria have been reported to produce bioemulsifiers and biosurfactants. Studies by Alvarez et al. [76] and Castellane et al. [3] indicate that many rhizobacterial species could represent promising candidates to be considered as producers of bioemulsifiers. Alvarez et al. [76] tested the potential of the bioemulsifier produced by *Ensifer adhaerens* JHT_2 as a potential food additive by assessing the emulsification of olive, palm, corn, soy and canola oils, commonly used in food formulations, and observed after 96 h of cultivation, strain JHT_2 completely emulsified the palm oil (EI = 100%). The same authors reported that the monosaccharide composition of the bioemulsifier produced by strain JHT_2 contains different glycosidic linkages, e.g. non-reducing end units of mannopyranose (11.6%), galactopyranose (9.3%) and glucopyranose (8.9%).

In the study undertaken by Castellane et al. [3] the wild-type strain of *Rhizobium tropici* SEMIA 4080 and the mutant strain ($MUTZC_3$) were found to produce an extracellular and cells with specific activities, that EPS were used as emulsifying agent. The bioemulsifier produced by the cell-free medium from these bacterial cell cultures cultivated in the presence hexane and paraffin liquid oil, with low values as of 50%, exhibited considerable substrate specificity. Similar results have been obtained with the EPS from other bacterial species, such as Emulsan, which does not emulsify pure aliphatic, aromatic or cyclic hydrocarbons; however, mixtures of those compounds can be efficiently emulsified [77].

Furthermore, a study found EPS producing bacteria isolated from petroleum-contaminated sites. Sonawdekar and Gupte [78] showed that a total of 19 hydrocarbon-utilizing microorganisms have a good potential for oil emulsify capabilities. Peele et al. [79] selected one isolate based on its ability to produce high amounts of EPS (biofilm). This isolates showed good surface active properties which could be developed into a promising molecule for industrial and environmental applications. Polymers from *Halomonas eurihalina* species have emulsifying activity and jellifying properties at acidic pH [80].

Llamas et al. [40] described for the first time a halophilic Alphaproteobacteria that has been found to produce an EPS. This particular EPS is very stable and is composed of small, uniform droplets, resulting in a fine, smooth consistency [40]. Other best known exopolysaccharides, such as xanthan, are capable of producing stable emulsions, but they tend to be thicker and more viscous. This is not very desirable for some of the applications for which these emulsifiers are intended to be used [81]. The safety property of xanthan gum for industrial applications, which includes dairy products, drinks, bakery products, syrups, and pet foods, as well as the oil, pharmaceutical and cosmetic industries have been researched [82, 83].

Bioflocculating property of EPS

As previously mentioned, EPS had excellent bioflocculating properties. For the production of bioflocculants, the bacteria can utilize unusual carbon sources as nutrient along with the production of other microbial metabolites [84]. Medhi and Thakur [24] focus on the bioflocculating properties of EPS isolated from basal. The results indicated that EPS samples had good bioflocculating properties, after 60 min of EPS incubation, demonstrating a bioflocculating efficiency of 35% and 44% for the basal EPS and influent media EPS, respectively. The flocculating efficiency of influent media EPS was found to be higher than the basal EPS making it a suitable option to be used as flocculating agent, as Medhi and Thakur suggested [24]. Borah et al. [25] found an EPS from *Cyanothece epiphytica* with flocculation activity gradually increased from 42.76% at 0.02 to 98% at 0.6% EPS concentration. In addition, at 1% concentration of EPS produced by *C. epiphytica*, flocculating activity was close to 84%, a decrease of around 14% from maximum (98%) [25].

Insecticidal activity of EPS

According to some reported, some high-level of resistance after intensive chemical treatment has developed, such as control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm [85-90]. Remarkably, EPS showed insecticidal activity against the mosquito larvae. Therefore, in recent years, there has been an increasing demand for the isolation and identification of new microbial polysaccharides that can have insecticidal activity. *Pseudomonas chlororaphis* PCL_1391 and *Pseudomonas protegens* strains CHA_0 produce microbial metabolites that show to have insecticidal activity [86-95]. EPS is an example of microbial metabolites that show have larvicidal activity. Recently, a novel and effective approach was performed to synthesize ZnO nanoparticles using EPS from *Bacillus licheniformis* $Dahb_1$ [30]. These authors found one type of EPS achieved 100% mortality activity against third instars mosquito larvae of *Anopheles stephensi* and *Aedes aegypti* at very low doses. Moreover, damaged cells and tissues in the mid-gut of treated mosquito larvae were confirmed by histological assays [30]. This is another reason why we are interested in divulgating the potential of EPS's novel applications. We strongly believe that several novel EPS have the potential of future commercial interest.

Conclusion

Exopolysaccharide, an eco-friendly bio product, has been a great interest for a long time because of their potential biotechnology and biological applications opportunities. However, the search for bacteria with yield high exopolysaccharides and unique biological, physicochemical and rheological properties as well as the exploration of novel bacteria for their production are suggested tools for improving the chances of potential candidate for a future commercial scale production and novel field application of EPS, and these are the subject of important research groups around the world.

Acknowledgment

The authors would like to thank the National Council for Scientific and Technological Development (CNPq) for providing the Scholarships and Financial Support (Proceeding No. 401886/2016-1). We thank Fernanda Laroza Paganelli, University Medical Center Utrecht, Utrecht (the Netherlands) for carefully reading the manuscript.

References

1. Ates O (2015) Systems biology of microbial exopolysaccharides production. Fronti in Bioengin Biotec 3: 200.
2. Ghosh PK, Maiti TK (2016) Structure of extracellular polysaccharides (EPS) produced by rhizobia and their functions in legume-bacteria symbiosis. Achiev Life Sci 10: 136-143.
3. Castellane TCL, Campanharo JC, Colnago LA, Coutinho ID, Lopes ÉM, et al. (2017) Characterization of new exopolysaccharide production by *Rhizobium tropici* during growth on hydrocarbon substrate. Internati J Biolo Macrom 96: 361-369.
4. Freitas F, Torres CAV, Reis MAM (2017) Engineering aspects of microbial exopolysaccharide production. Bioresour Technol 245: 1674-1683.
5. Gupta P, Diwan B (2017) Bacterial exopolysaccharide mediated heavy metal removal: a review on biosynthesis, mechanism and remediation strategies. Biotechnol Rep 13: 58-71.
6. Kielak AM, Castellane TCL, Campanharo JC, Colnago LA, Costa OYA, et al (2017) Characterization of novel *Acidobacteria* exopolysaccharides with potential industrial and ecological applications. Sci Rep 7: 41193.
7. Muthu M, Wu HF, Gopal J, Sivanesan I, Chun S (2017) Exploiting Microbial Polysaccharides for Biosorption of Trace Elements in Aqueous Environments—Scope for Expansion via Nanomaterial Intervention. Polymers 9: 721.
8. Upadhyay KH, Vaishnav AM, Tipre DR (2017) Kinetics and mechanisms of mercury biosorption by an exopolysaccharide producing marine isolate *Bacillus licheniformis*, 3 Biotech 7: 313.
9. Zhang Z, Cai R, Zhang W, Fu Y, Jiao N (2017) A novel exopolysaccharide with metal adsorption capacity produced by a marine bacterium *Alteromonas* sp. JL2810. Marine Drugs 15(6): 175.
10. Freitas F, Alves VD, Reis MA (2011) Advances in bacterial exopolysaccharides: From production to biotechnological applications. Trends in Biotechnology 29: 388-398.
11. Donot F, Fontana A, Baccou JC, Schorr GS (2012) Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. Carbohy Poly 87: 951-962.
12. Sogutcu E, Emrence Z, Arikan M, Cakiris A, Abaci N, et al. (2012) Draft genome sequence of *Halomonas smyrnensis* AAD6T. J Bacteriol 194: 5690-5691.
13. Simeonidis E, Price ND (2015) Genome-scale modeling for metabolic engineering. J Ind Microbiol Biotechnol 42: 327-338.
14. Wibberg D, Alkhateeb RS, Winkler A, Albersmeier A, Schatschneider S, et al. (2015) Draft genome of the xanthan producer *Xanthomonas campestris* NRRL B-1459 (ATCC 13951). J Biotechnol 204: 45-46.
15. Yasar YSY, Radchenkova N, Arga KY, Kambourova M, Oner ET (2015) Genomic analysis of *Brevibacillus thermoruber* 423 reveals its biotechnological and industrial potential. Appl Microbiol Biotechnol 99: 2277-2289.
16. Zeidan A, Poulsen V, Janzen T, Buldo P, Derkx P, et al. (2017) Polysaccharide production by lactic acid bacteria: From genes to industrial applications. FEMS Microbiol Rev 41: 168-200.
17. Whitfield C (1988) Bacterial extracellular polysaccharides. Can J Microbiol 34: 415-420.
18. Sutherland IW (2000) The biofilm matrix-an immobilized but dynamic microbial environment. Trends Microbiol 9: 222-227.
19. Bomfeti CA, Florentino AF, Guimarães AP, Cardoso PG, Guerreiro MC, et al. (2011) Exopolysaccharides produced by the symbiotic nitrogen-fixing bacteria of leguminosae. Brazil Soil Scie 35: 657-671.
20. He X, He F, Hang J (2018) Bioprocess Biosyst Eng doi: 10.1007/s00449-018-1915-2.
21. Staudt AK, Wolfe LG, Shrout JD (2012) Variations in exopolysaccharide production by *Rhizobium tropici*. Arch Microbiol 194: 197-206.
22. Roca C, Alves VD, Freitas F, Reis MAM (2015) Exopolysaccharides enriched in rare sugars: bacterial sources, production, and applications. Front Microbiol 6: 288.
23. Okaiyeto K, Nwodo UU, Mabinya LV, Okoli AS, Okoh AI (2015) Characterization of a Bioflocculant (MBF-UFH) produced by *Bacillus* sp. AEMREG7. Int J Mol Sci 16: 12986-13003.
24. Medhi K, Thakur IS (2018) Bioremoval of nutrients from wastewater by a denitrifier *Paracoccus denitrificans* ISTOD1. Bioresource Techno Rep 1: 56-60.
25. Borah D, Nainamalai S, Gopalakrishnan S, Rout J, Alharbi N, et al. (2018) Biolubricant potential of exopolysaccharides from the cyanobacterium *Cyanothece epiphytica*. Appl Microbiol Biotechnol.
26. Shuhong Y, Meiping Z, Hong Y, Han W, Shan X, et al. (2014) Biosorption of Cu^{2+}, Pb^{2+} and Cr^{6+} by a novel exopolysaccharide from *Arthrobacter* ps-5. Carbohyd Polyme 101: 50-56
27. Raveendran S, Poulose AC, Yoshida Y, Maekawa T, Kumar DS (2013) Bacterial exopolysaccharide based nanoparticles for sustained drug delivery, cancer chemotherapy and bioimaging. Carbohydr Polym 91: 22-32.
28. Zhang DY, Wan Y, Xu JY, Wu GH, Li L, et al. (2016) Ultrasound extraction of polysaccharides from mulberry leaves and their effect on enhancing antioxidant activity, Carbohydr. Polym. 137: 473-479.
29. Moscovici M (2015) Present and future medical applications of microbial exopolysaccharides. Front Microbiol 6: 1012.
30. Abinaya M, Vaseeharan B, Divya M, Sharmili A, Govindarajan M, et al. (2018) Bacterial exopolysaccharide (EPS)-coated ZnO nanoparticles showed high antibiofilm activity and larvicidal toxicity against malaria and Zika virus vectors. J Trace Elem Med Biol 45: 93-103.
31. Liu X, Luo Y, Li Z, Wang J, Wei G (2017) Role of exopolysaccharide in salt stress resistance and cell motility of *Mesorhizobium alhagi* CCNWXJ12–2^T. Appl Microbiol Biotechnol 101: 2967-2978.
32. Jaszek M, Janczarek M, Kuczynski K, Piersiak T, Grzywnowicz K (2014) The response of the *Rhizobium leguminosarum* bv. *trifolii* wild-type and exopolysaccharide-deficient mutants to oxidative stress. Plant Soil 376: 75-94.
33. Lehman AP, Long SR (2013) Exopolysaccharides from *Sinorhizobium meliloti* can protect against H_2O_2-dependent damage. J Bacteriol 195: 5362-5369.
34. Navarini L, Cesaro A, Rossmurphy SB (1992) Exopolysaccharides from *Rhizobium meliloti* ye-2 grown under different Osmolarity conditions - viscoelastic properties. Carbohyd Res 223: 227-234.
35. Garcıa AC, Averous L (2018) Synthesis and evaluation of functional alginate hydrogels based on click chemistry for drug delivery applications. Carbohy Polydoi 190: 271-280.
36. Cimini D, De Rosa M, Schiraldi C (2012) Production of glucuronic acid-based polysaccharides by microbial fermentation for biomedical applications. Biotechnol J 7: 237-250.
37. Monsan P, Bozonnet S, Albenne C, Joucla G, Willemot RM, et al. (2001) Homopolysaccharides from lactic acid bacteria. Intern Dair J 11: 675-685.
38. Delbarre LC, Leyva SM, Sinquin C, Zykwinska A, Colliec JS (2017) Bioprospecting for Exopolysaccharides from Deep-Sea Hydrothermal Vent Bacteria: Relationship between Bacterial Diversity and Chemical Diversity. Microorganisms 5: 63.
39. Yang J, Wei W, Pi S, Ma F, Li A, Wu D, Xing J (2015) Competitive adsorption of heavy metals by extracellular polymeric substances extracted from *Klebsiella* sp. J1. Bioresour Technol 196: 533-539.
40. Llamas, Inmaculada (2010) Characterization of the Exopolysaccharide Produced by *Salipiger Mucosus* $A3^T$, a Halophilic Species Belonging to the *Alphaproteobacteria*, Isolated on the Spanish Mediterranean Seaboard. Marine Drugs 8.8: 2240-2251.
41. Meneghine AK, Moretto C, Castellane TCL (2017) Production, characterization and bioemulsifying activity of an exopolysaccharide produced by *Sphingomonas* sp. Isolated from freshwater. J Polym Environ 25: 1080-1086.
42. Song YR, Jeong DY, Cha YS, Baik SH (2013) Exopolysaccharide producedby *Pediococcus acidilactici* M76 isolated from the Korean traditional rice wine, Makgeolli. J Microbiol Biotechnol 23: 681-688.
43. Li S, Huang R, Shah NP, Tao X, Xiong Y, Wei H (2014) Antioxidant and antibacterial activities of exopolysaccharides from *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315. J Dairy Sci 97: 7334-7343.
44. Sharma SK, Gautam N, Arti NS (2015) Optimization, Composition, and Antioxidant Activities of Exo- and Intracellular Polysaccharides in Submerged Culture of *Cordyceps gracilis* (Grev.) Durieu & Mont. Evid Based Complement Alternat Med 2015:462864.

45. Benit N, Roslin AS (2018) Isolation and Characterization of Larvicidal Extracellular Polysaccharide (EPS) from Pseudomonas aeruginosa B01. Int J Curr Microbiol App Sci 7: 109-120

46. Moretto C, Castellane TCL, Lopes EM, Omori WP, Sacco LP, et al. (2015) Chemical and rheological properties of exopolysaccharides produced by four isolates of rhizobia. Int J Biol Macromol 81: 291-298.

47. Reinhold BB, Chan SY, Reuber TL, Marra A, Walker GC, et al. (1994) Detailed structural characterization of succinoglycan, the major exopolysaccharide of *Rhizobium meliloti* Rm 1021. J Bacteriol 176: 1997-2002.

48. Laus MC, Van BAAN, Kijne JW (2005) Role of cellulose fibrils and exopolysaccharides of *Rhizobium leguminosarum* in attachment to and infection of *Vicia sativa* root hairs. Mol Plant-Microbe Interact 18: 533-538.

49. Skorupska A, Janczarek M, Marczak M, Krol AMJ (2006) Rhizobial exopolysaccharides: Genetic control and symbiotic functions. Microb Cell Factories 5: 7.

50. Downie JA (2010) The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. FEMS Microbiol Rev 34: 150-170.

51. Janczarek M (2011) Environmental signals and regulatory pathways that influence exopolysaccharide production in Rhizobia. Int J Mol Sci 12: 7898-7933.

52. Castellane TCL, Lemos MVF, Lemos EGM (2014) Evaluation of the biotechnological potential of *Rhizobium tropici* strains for exopolysaccharide production. Carbohydr Polym 111: 191-197.

53. Priyanka P, Arun AB, Ashwini P, Rekha PD (2015) Versatile properties of an exopolysaccharide R-PS18 produced by *Rhizobium sp*. PRIM-18. Carbohyd Polyme 126: 215-221.

54. Ribeiro VA, Burkert CAV (2016) Exopolysaccharides Produced by *Rhizobium*: Production, Composition and Rheological Properties. J Polym Biopol Phys Chemis 4: 1-6.

55. Castellane TCL, Otoboni AMMB, Lemos EGM (2015) Characterization of exopolysaccharides produced by Rhizobia species. Brazil J Soi Sci 39: 1566-1575.

56. Rossi F, Philippis R (2016) Exocellular Polysaccharides in Microalgae and Cyanobacteria: Chemical Features, Role and Enzymes and Genes Involved in Their Biosynthesis. In: Borowitzka M, Beardall J, Raven J (eds) The Physiology of Microalgae. Developm Applie Phycol 6 Springer.

57. Bhunia B, Prasad UUS, Oinam G, Mondal A, Bandyopadhyay TK, et al (2018) Characterization, genetic regulation and production of cyanobacterial exopolysaccharides and its applicability for heavy metal removal. Carbohydr Polym 179: 228-243.

58. Ruiz SP, Martinez CO, Noce AS, Sampaio AR, Baesso ML, Matioli G (2015) Biosynthesis of succinoglycan by *Agrobacterium radiobacter* NBRC 12665 immobilized on loofa sponge and cultivated in sugar cane molasses. Structural and rheological characterization of biopolymer. J Mole Catal B: Enzym 122: 15-28.

59. Palaniraj A, Jayaraman V (2011) Production, recovery and applications of xanthan gum by *Xanthomonas campestris*. J Food Eng 106: 1-12.

60. Khan T, Park JK, Kwon JH (2007) Functional biopolymers produced by biochemical technology considering applications in food engineering. Korean J Chem Eng 24: 816-826.

61. Hassan AI, Ghoneim MAM, Mahmoud MG, Asker MMS, Mohamed SS (2016) Efficacy of polysaccharide from *Alcaligenes xylosoxidans* MSA3 administration as protection against γ-radiation in female rats. J Radiat Res 57: 189-200.

62. Li H, Jiao X, Sun Y, Sun S, Feng Z, et al (2016) The preparation and characterization of a novel sphingan WL from marine *Sphingomonas* sp. WG. Scientific Reports 6: 37899.

63. Pollock TJ (2005) Sphingan group of exopolysaccharides (EPS). Biopolym Online 5.

64. Li S, Zhao Y, Zhang L, Zhang X, Huang L, et al. (2012) Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. Food Chem 135: 1914-1919.

65. Liu Z, Zhang Z, Qiu L, Zhang F, Xu X, et al (2017) Characterization and bioactivities of the exopolysaccharide from a probiotic strain of *Lactobacillus plantarum* WLPL04. J Dair Scien 100: 6895-6905.

66. Ouwehand AC, Salminen SJ (1998) The health effects of cultured milk products with viable and non-viable bacteria. Int Dairy J 8: 749-758.

67. Caggianiello G, Kleerebezem M, Spano G (2016) Exopolysaccharides produced by lactic acid bacteria: From health-promoting benefits to stress tolerance mechanisms. Appli Microbio Biotech 100: 3877-3886. doi:10.1007/s00253-016-7471-2

68. Abid Y, Casillo A, Gharsallah H, Joulak I, Lanzetta R, et al (2018) Production and structural characterization of exopolysaccharides from newly isolated probiotic lactic acid bacteria. Int J Biol Macromol 108:719-728.

69. Ghosh PK, Ganguly J, Maji P, Maiti TK (2015) Production and composition of extracellular polysaccharide synthesized by *Rhizobium undicola* isolated from aquatic legume, *Neptunia oleracea* Lour. Proc Natl Acad Sci India B Biol Sci 85: 581-590.

70. Volesky B (1990) Biosorption of Heavy Metals; CRC Press: Boca Raton, FL, USA, 318.

71. Pal A, Paul A (2008) Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. Indian J Microbiol 48: 49-64.

72. Kim SY, Kim JH, Kim CJ, Oh DK (1996) Metal adsorption of the polysaccharide produced from *Methylobacterium organophilum*. Biotechnol Lett 18: 1161-1164.

73. Wang L, Yang J, Chen Z, Liu X, Ma F (2013) Biosorption of Pb(II) and Zn(II) by extracellular polymeric substance (EPS) of *Rhizobium Radiobacter*: Equilibrium, kinetics and reuse studies. Arch Environ Prot 39: 129-140.

74. Nocelli N, Bogino PC, Banchio E, Giordano W (2016) Roles of extracellular polysaccharides and biofilm formation in heavy metal resistance of Rhizobia. Materials 9: 418.

75. Mohite BV, Koli SH, Narkhede CP (2017) Prospective of Microbial Exopolysaccharide for Heavy Metal Exclusion. Appl Biochem Biotechnol 183: 582.

76. Alvarez VM, Jurelevicius D, Serrato RV, Bergter EB, Seldin L (2017) Chemical characterization and potential application of exopolysaccharides produced by *Ensifer adhaerens* JHT2 as a bioemulsifier of edible oils. Int J Biol Biomac 114.

77. Navon VS, Zosim Z, Gottlieb A, Legmann R, Carmeli S, et al. (1995) Alasan, a new bioemulsifier from *Acinetobacter radioresistens*. Appl Environ Microbiol 61: 3240-3244.

78. Sonawdekar S, Gupte A (2016) Production and characterization of exopolysaccharide produced by oil emulsifying bacteria. Int J Curr Microbiol App Sci. 5: 254-262.

79. Peele KA, Ravi TCV, Kodali VP (2016) Emulsifying activity of a biosurfactant produced by a marine bacterium. 3 Biotech 6: 177.

80. Calvo C, Martínez CF, Mota A, Béjar V, Quesada E (1998) Effect of cations, pH and sulphate content on the viscosity and emulsifying activity of the *Halomonas eurihalina* exopolysaccharide. J Ind Microbiol Biotechnol 20: 205-209.

81. Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev 61: 47-64.

82. Patel A, Prajapati JB (2013) Food and health applications of exopolysaccharides produced by lactic acid bacteria. Adv Dairy Res 1: 2.

83. Palaniraj A, Jayaraman V (2011) Production, recovery and applications of xanthan gum by Xanthomonas campestris. J Food Eng 106: 1-12.

84. Pathak M, Sarma HK, Bhattacharyya KG, Subudhi S, Bisht V, et al. (2017) Characterization of a novel polymeric bioflocculant produced from bacterial utilization of n- hexadecane and its application in removal of heavy metals. Front. Microbiol 7: 1-15.

85. Sparks TC, Dripps JE, Watson GB, Paroonagian D (2012) Resistance and cross-resistance to the spinosyns – A review and analysis. Pesticide Biochemistry and Physiology 102: 1-10.

86. Péchy-Tarr M, Bruck DJ, Maurhofer M, Fischer E, Vogne C, Henkels M D, et al. (2008) Molecular analysis of a novel gene cluster encoding an insect toxin in plant-associated strains of Pseudomonas fluorescens. Environ Microbiol 10: 2368-2386.

87. Chellat F, Tabrizian M, Dumitriu S, Chornet E, Magny P, et al. (2000) In vitro and in vivo biocompatibility of chitosan-xanthan polyionic complex. J Biome Mate Resea 51: 107.

88. Cho HM, Yoo B (2015) Rheological characteristics of cold thickened beverages

containing xanthan gum-based food thickeners used for dysphagia diets. J Acad Nutr Diet 115: 106-111.

89. Kang KS, Veeder GT, Mirrasoul PJ, Kaneko T, Cottrell IW (1982) Agar-like polysaccharide produced by a Pseudomonas species: Production and basic properties. Appli Environme Microbi 43: 1086-1091.

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

91. Barnhart D, Kaplan D, Winter W, Panilaitis B, Kiemle D (2012) Structural details of the polyelectrolytic exopolysaccharide (APE), the stabilizing component of the *Acinetobacter venetianus* RAG-1 emulsan complex. Carbohy Polym 88: 257-262.

92. Cruz M, Freitas F, Torres CAV, Reis MAM, Alves VD (2011) Influence of temperature on the rheological behavior of a new fucose-containing bacterial exopolysaccharide. Int J Biol Macromol 48: 695-699.

93. Ferreira ARV, Torres CAV, Freitas F, Reis MAM, Alves VD, et al. (2014) Biodegradable films produced from the bacterial polysaccharide FucoPol. Int J Biol Macromol 71: 111-116.

94. Freitas F, Alves VD, Gouveia AR, Pinheiro C, Torres CAV, et al. (2014) Controlled production of exopolysaccharides from *Enterobacter* A47 as a function of carbon source with demonstration of their film and emulsifying abilities. Appl Biochem Biotechnol 172: 641-657.

95. Wu S, Lu M, Chen J, Fang Y, Wu L, Xu Y, Wang S (2016) Production of pullulan from raw potato starch hydrolysates by a new strain of *Aureobasidium pullulans*. Int J Biol Macromol 82: 740-743.

96. Castellane TCL, Persona MR, Campanharo JC, Lemos EGM (2015) Production of exopolysaccharide from rhizobia with potential biotechnological and bioremediation applications. Int J Biol Macromol 74: 515-522.