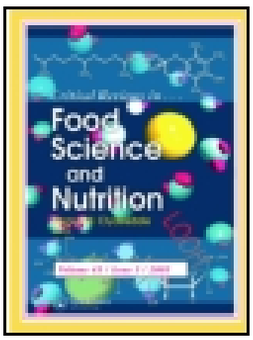

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REVIEW

Current status of biotechnological production and applications of microbial exopolysaccharides

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ABSTRACT

Microbial exopolysaccharides (EPS) are an abundant and important group of compounds that can be secreted by bacteria, fungi and algae. The biotechnological production of these substances represents a faster alternative when compared to chemical and plant-derived production with the possibility of using industrial wastes as substrates, a feasible strategy after a comprehensive study of factors that may affect the synthesis by the chosen microorganism and desirable final product. Another possible difficulty could be the extraction and purification methods, a crucial part of the production of microbial polysaccharides, since different methods should be adopted. In this sense, this review aims to present the biotechnological production of microbial exopolysaccharides, exploring the production steps, optimization processes and current applications of these relevant bioproducts.

KEYWORDS

EPS; EPS of industrial interest; EPS optimization; EPS purification; engineered exopolysaccharide

Introduction

Polysaccharides are macromolecules belonging to the carbohydrate group, constituting the most important and abundant group of compounds, being synthetized by bacteria, fungi and algae (Barbosa et al. 2004; de Souza and Garcia-Cruz 2004).

Microbial polysaccharides are classified depending on their position inside the microbial cell: (i) polysaccharides constituting the cell wall (structural polysaccharides), such as techoid acids, lipopolysaccharides (LPS) and peptidoglycans; (ii) polysaccharides which provide energy and act as carbon source for the cell, called cytosolic polysaccharides or intracellular polysaccharides (IPS); and (iii) exopolysaccharides (EPS), polysaccharides that are secreted in the form of biofilm or capsules to the extracellular medium (Bergmaier 2002; Boels et al. 2001; Kumar, Mody, and Jha 2007; Lahaye 2006; Ruas-Madiedo and de los Reyes-Gavilán 2005).

Polysaccharides obtained through biotechnological processes represent an available market, especially EPS, which can be produced by various classes of microorganisms, included bacteria, molds and yeasts, due to its water solubility with constant physical and chemical properties (Aranda-Selverio et al. 2010; Banik, Kanari, and Upadhyay 2000; de Souza and Garcia-Cruz 2004; Sutherland 2001). The microbial polysaccharides' market is expanding since the use of renewable sources is possible and for presenting various desirable characteristics, being use as polymers substitute for improvement of rheological properties of several products in food and pharmaceutical industry, and for its antitumor and immunostimulatory properties (Bergmaier 2002; Nwodo, Green, and Okoh 2012; Rühmann, Schmid, and Sieber 2015a).

One of the main exopolysaccharides produced nowadays is xanthan gum by the bacteria *Xanthomonas campestris*, with a production of 50,000 tons per year with worldwide market evaluated between U$600 and U$800 million per year (Subhash, Jadhav, and Jana 2015). Xanthan gum accounts for 6% of the polysaccharides market, mainly used by food industries in sauces, frozen food, juices, and desserts. Another relevant EPSs are gellan, dextran and pullulan gums, which are used specially to improve rheological properties of a range of food and pharmaceutical products (Bueno and Garcia-Cruz 2006; Freitas, Alves, and Reis 2011; Welman and Maddox 2003).

EPS are divided into two groups: homopolysaccharides (e.g. dextran) and heteropolysaccharides (e.g. xanthan and gellan). Homopolysaccharides are composed by only one type of monosaccharide while heteropolysaccharides are constituted of several monosaccharides, in which synthesis takes place inside the cell generating complex structures. The majority of bacterial EPS can be classified as heteropolysaccharides (Aranda-Selverio et al. 2010; Bergmaier 2002; Czaczyk and Myszka 2007; De Baets et al. 2002; Lahaye 2006).

The EPS performs important functions for the microbial cell, such as protection against biotic and abiotic stress (De Baets et al. 2002; Limoli et al. 2015; Nwodo, Green, and Okoh 2012). Besides, its production ensures greater resistance, affects the way microorganism interacts with the environment, and allows its use as a substrate for microorganism growth (Czaczyk and Myszka 2007; Ozturk, Aslim, and Suludere 2010).

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The wide range of EPS applications, which is based on their physical and chemical properties, demonstrates the potential of these substances for industries and the importance of further studies to better understand their production. In this sense, this review aims to present the biotechnological production of microbial EPS, in a general way, exploring all the production steps, optimization processes and current applications of these relevant bioproducts, as can be seen in Fig. 1.

Biotechnological production of exopolysaccharides

The biotechnological production of EPS offers advantages over chemical and plant-derived production such as energy efficient (when produced by algae), quick production in a matter of days independently of location and season, and the chance to use industrial and agricultural wastes as substrate. However, some limitations as cost of production may hinder the process due to the requirements needed (González López et al. 2009; Rutering et al. 2016; Thompson and He 2006).

Recent researches focus on nontoxic EPS and "greener" production, where one-step in-cell functionalization and polymerization are desirable at a optimize level, avoiding the harvesting of oceans, when it comes to alginate, and the use of catalysts (Anderson, Ahsanul Islam, and Prather 2018). In this way, some aminopolysaccharides, e.g. chitin and chitosan, have their microbial production studied in order to avoid its obtainment through acid hydrolysis from shellfish, and Hyaluronic acid to avoid its obtainment thorough animal tissues purification (Anderson, Ahsanul Islam, and Prather 2018). Newly discovered nontoxic EPS, such as Microbactan, has bioabsorption potential when it comes to cadmium, providing an alternative for the remediation of heavy metals especially at industrial waters (Camacho-chab et al. 2018). Other examples are: the EPS produced by *Rhodotorula mucilaginosa* UANL-001L co-cultured with *E. coli*, responsible for inhibit bacterial colonization with multiples uses at medical and industrial areas (Vazquez-rodriguez et al. 2018) and the EPS from *Lactobacillus plantarum* BR2, with high antioxidant activity, antidiabetic and cholesterol lowering potential possessing great potential for functional foods (Sasikumar et al. 2017).

Currently, many EPS are produced through biotechnology. Each EPS has its own biosynthetic pathway and, consequently, factors that may influence these pathways. Normally, the biosynthesis and accumulation of EPS occurs after the growth phase of the microorganism, and their synthesis can be divided into 4 different steps: (i) assimilation of the carbon source, (ii) synthesis of the oligosaccharide repeating units or direct synthesis by successive or progressive activity of glycosyltransferases, (iii) assembly of the polysaccharide from the repeating units, and (iv) EPS' exportation to the extracellular medium (Becker 2015).

The production of EPS by bacteria can also be described by 4 general mechanisms: (i) the so called Wzx/Wzy-dependent pathway where individual repeating units are assembled by several glycosyltransferases, giving rise to heteropolymers; (ii) the ATP-binding cassette (ABC) transporter-dependent pathway, producing capsular polysaccharides; (iii) the synthase-dependent pathway, in which homopolymers are produced; and (iv) the extracellular synthesis by the use of a single sucrase protein (Schmid, Sieber, and Rehm 2015).

During EPS biosynthesis, enzymes are responsible for almost all steps. Located at different parts of the microbial cell, they can be divided into 4 groups: (i) intracellular enzymes involved in other metabolic processes, such as hexokinase; (ii) enzymes that are believed to be intracellular, which are the source of monosaccharides residues; (iii) enzymes located at the cell periplasmic membrane, such as glycosyltransferases; and (iv) enzymes situated outside the cell membrane responsible for the polymerization of macromolecules (Kumar, Mody, and Jha 2007).

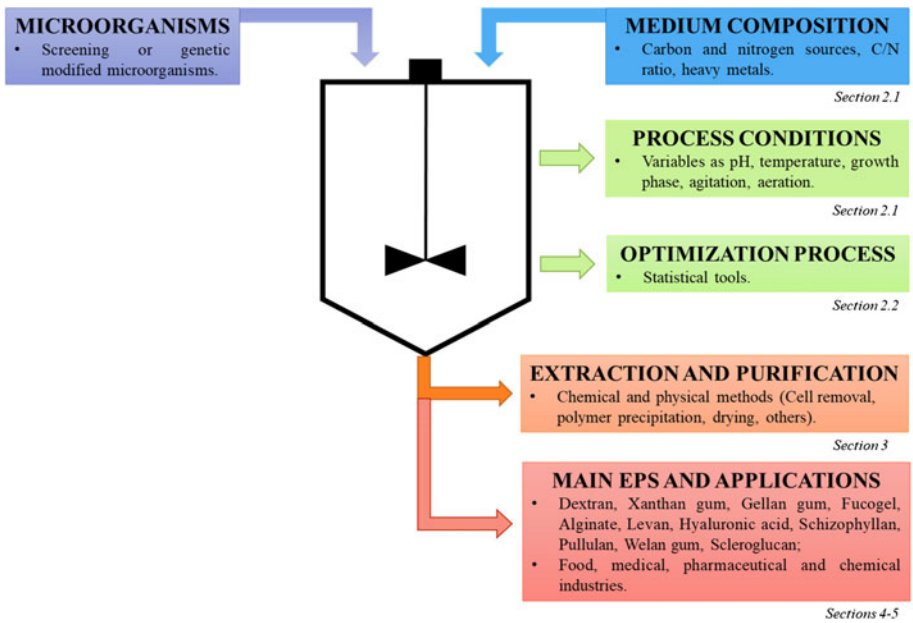

Figure 1. General scheme of the biotechnological route to obtain the main microbial EPS.

Thus, as EPS biosynthesis is dependable of several biological variables, improvements in their production are still necessary and desirable for industries and further applications. For the achievement of higher values of EPS production, an increase in the rate of synthesis, enzyme management, and genetic modifications are needed.

Process conditions and medium composition influence on EPS production

The amount of EPS produced is directly linked to the process conditions and medium composition (Kumar, Mody, and Jha 2007). Availability of carbon and nitrogen sources, temperature, pH, growth phase, agitation, and aeration plays major influence on EPS production by microorganism, especially bacteria (Barbosa et al. 2004; Czaczyk and Myszka 2007). How these variables influence the EPS production is related to the microorganism used.

The most influent conditions for EPS production is availability of carbon (e.g. glucose, sucrose) and nitrogen (e.g. ammonium sulfate, peptone, sodium nitrate, yeast extract). These conditions have contradictory effects, since greater the amount of carbon, better yields are achieved, and the opposite is observed with nitrogen, since large amounts of carbon can extend the growth phase of the microorganism and large amounts of nitrogen can increase the production of degrading enzymes (Barbosa et al. 2004; Czaczyk and Myszka 2007; Kumar, Mody, and Jha 2007). The relation between the carbon and nitrogen sources is called C/N ratio and it can be used as a production parameter. Is observed that greater the C/N ratio, maximal production of EPS can be achieved, with 10:1 considered the most favorable ratio (de Souza and Sutherland 1994).

The EPS production is also dependent on growth phase and microorganism. Many reports have described major production rates associated to the stationary phase of the microorganism, normally occurring 24 h after the incubation initial (Petry et al. 2000; Raguenes et al. 1996; Skaliy and Eagon 1972). However, it's also dependent on the microorganism used, with different phases reported for different microorganisms, such as stationary phase for *Rhodopseudomonas acidophila*, late log phase for *Bacillus megaterium*, and late log phase to initial stationary phase for *Saccharophagus degradans* and *Vibrio harveyi strain VB23* (Chug et al. 2016).

For incubation temperature, those below to optimal for biocatalyst growth can provide higher EPS production. This is supported since the enzymes presented at EPS synthesis, have different optimum temperatures from the microorganism (Kojic et al. 1992; Kumar, Mody, and Jha 2007).

A constant pH is desirable for maximum production of EPS, with some studies suggesting that the pathway for EPS production may be directly dependent on pH values (Gorret et al. 2001; Kumar, Mody, and Jha 2007). The enzyme glycosyl-hydrolase is capable of degrading EPS after long fermentations, which reduce the molar mass. In this way, the optimum pH would be capable to balance the effects of production and degradation (Laws, Gu, and Marshall 2001).

According to some authors, high aeration and agitation can give better results to EPS production since almost all EPS's producers are aerobic (Lee et al. 2001; Yang and Liau 1998). This impact may be explained since molecular oxygen is used as a primary source of energy metabolism, which lead to an increase in EPS production. Since the EPS production in submerged medium can lead to higher viscosity, irregular distribution of oxygen is observed, affecting negatively the EPS synthesis and quality. These problems can be avoid with agitation rates depending on the microorganism used (Kumar, Mody, and Jha 2007).

Using *Lactobacillus delbrueckii* subsp. *bulgaricus*, Petry et al. (2000) defined the factors that affect its EPS production using a chemically defined medium. Four main factors were observed: oxygen, pH, temperature, and medium constituents. The controlled pH at 6.0 was the most influential factor, leading to an increase in EPS production. However, changes at oxygen, temperature and medium constituents did not affect the EPS composition (Petry et al. 2000).

Rutering and coworkers (2016) described the EPS production by *Paenibacillus* sp. The influence of carbon and nitrogen sources on EPS composition and biosynthesis was investigated using different carbon (sucrose, glucose and glycerol) and nitrogen (peptone and $NaNO_3$) sources with concentrations of 30 g/L and 0.05 mol/L, respectively, incubated at 30 °C, 150 rpm, for 48 h with initial pH of 7.0. The authors observed that different carbon and nitrogen sources induced different biosynthesis pathways of EPS for *Paenibacillus*, obtaining a levan-type polymer and a heteropolysaccharide when using sucrose and peptone, and levan when using sucrose and $NaNO_3$ (Rutering et al. 2016).

A culture of the algae *Cylindrotheca closterium* was tested at temperatures of 4 °C, 10 °C, 15 °C, 25 °C and 35 °C, chosen based on normal environmental temperature of the microorganism for EPS production. The highest results, 9 μg/mL and 11 μg/mL, were observed during the stationary phase (after 13 days of process) at 15° and 25 °C, respectively, with a higher influence of temperature occurring during the growth phase (Wolfstein and Stal 2002).

The EPS production by the fungal strain *Ganoderma lucidum* was evaluated under different initial pH, varying from 3.5 to 7.0. The increase in pH value led to an increase in biomass production. However, the pH reduction, with value close to 3.5, resulted in higher EPS production, indicating a prolonged EPS production phase at lower pH (Fang and Zhong 2002). A similar result was observed for *Lactobacillus casei* CRL 87, where the maximum cell viability occurred at pH 6.0, but the maximum EPS yield was close to pH 4.0 (Mozzi et al. 1996).

Wisbeck, Furlan, and Ninow (2009) studied the effect of initial pH (ranging from 4.0 to 6.0) and concentration of glucose (from 20 to 40 g/L) for the production of EPS by the fungal *Pleurotus ostreatus* DSM 1833. Maximum productivity suffered significant influence of the interaction between glucose and pH, especially when fixing pH value at 6.0 and changing glucose concentration from 20 g/L to 40 g/L, achieving maximum productivity, with values four times higher (Wisbeck, Furlan, and Ninow 2009).

The effect of the C/N ratio was studied for the EPS production by a bacterial strain under semi anaerobic and anaerobic conditions. Three different C/N ratios were used: 13.6 g carbon/g nitrogen, 6.8 g carbon/g nitrogen and 3.4 g carbon/g nitrogen. For all the experiments, the production of cellulose, the main EPS produced, was dependent on the C/N ratio, except the ones with strictly anaerobic conditions. The best result was achieved with a C/N ratio of 13.6 g carbon/g nitrogen, reaching 23.3 g/g carbon, confirming that higher C/N ratio benefits EPS production (Miqueleto et al. 2010).

Some studies also show the influence of heavy metals in the EPS production and its capacity of further metal removal. Mota and coworkers (2015) analyzed the effects of different concentrations of heavy metals (lithium, copper, lead and cadmium) on *Cyanothece* sp. CCY 0110 growth and EPS production. Different heavy metals influence the cells at different manners triggering different responses as tune down the metabolic rate for detoxification mechanisms with remarkable recovery; nevertheless, EPS production continued to follow the growth pattern (Mota et al. 2015).

Using chromium, cadmium and a metal mixed (chromium + cadmium), it was investigated the removal ability and EPS production by *Synechocystis* sp. BASO671. The removal ability was direct linked to the exposure metal and EPS production increased when metal concentration was changed from 15 ppm to 35 ppm, altering EPS' monomers composition (Ozturk et al. 2014).

Thus, is possible to observe the importance of process conditions and medium composition on EPS production, leading to higher yields and even tailor-made polymers. A viable approach to study all of these variables is the use of optimization tools in order to obtain process increments.

Optimization of process conditions and medium composition

The biotechnological production of EPS is a promising alternative for the production of new and different biopolymers. Optimization procedures are indispensable for the achievement of maximum production, aiming to obtain the better medium, parameters and conditions of the process, especially when it comes to fermentations (Sirajunnisa et al. 2016). Thus, the optimization of process and medium conditions represents an alternative for faster production with higher yields. Furthermore, it may have the ability of improve EPS mass and consequently change its rheological properties (Chug et al. 2016).

Some of the statistical tools used for this purpose are One Factor at a Time, Response Surface Methodology (RSM), Orthogonal Matrix, Full Factorial design, Central Composite Design (CCD), among others.

As the microbial production of EPS is a unique process dependable of each microorganism, the most influential parameters may vary among different species.

Growth media macronutrients, e.g. carbon and nitrogen sources, exert influence on the EPS production with wide ranges of concentrations and types. *Bacillus polymyxa* reaches 54 g/L of EPS when sucrose and potassium nitrate were used (Lee et al. 1997). Meanwhile, *Armillaria mellea* has best production results when high concentration of glucose (40 g/L) and yeast extract (3 g/L) are presented at the growth media (Lung and Huang 2010); *Klebsiella* sp. H-207 when sucrose (31.93 g/L) and KNO_3 (2.17 g/L) (Qiang et al. 2013), *L. delbrueckii* subsp. *bulgaricus* when bacto-casitone (30 g/L) (Kimmel, Roberts, and Ziegler 1998)

The use of industrial residues as substrates for the process optimization is an interest, natural and low cost alternative for the development of cost-effective process, reducing the cost of the fermentation medium (Habibi and Khosravi-Darani 2017). The EPS production by *Bacillus subtilis* increased when sucrose was substituted by cane molasses and rice bran at the same concentration (20 g/L), achieving 4.86 g/L of EPS (Razack, Velayutham, and Thangavelu 2013).

The optimization of micronutrients of growth media also presents great influence at EPS production, as proven for *Paenibacillus polymyxa* SQR-21, reaching maximum yields when using iron and calcium (Raza et al. 2011); *Klebsiella* sp. H-207, when using phosphorus (Qiang et al. 2013); *Chlamydomonas reinhardtii* strain RAC when a medium consisting of 74 mg/L of $CaCl_2$, 422 mg/L of $NaNO_3$, 10 mg/L of K_2HPO_4 and 200 mg/L of $MgSO_4$ is used (Bafana 2013); *Rhizobium* sp. when using calcium carbonate (Duta, de Franca, and de Almeida Lopes 2006); and *Paenibacillus polymyxa* EJS-3 using calcium (Liu et al. 2010).

Other strategies for the enhancement of EPS production is the use of additives, such as Tween 80, and different fermentation strategies, such as fed-batch fermentation, as demonstrated for *Bionectria ochroleuca* M21 (Li, Guo, and Zhu 2016) and *Tremella mesenterica* NRRL Y-6158 (De Baets et al. 2002), respectively.

Table 1 presents in a comprehensive way the main examples of microorganisms, process and culture media optimized, and yields of EPS obtained.

Engineered exopolysaccharide production

The use of genetic engineering for the biotechnological production of microbial polysaccharides is a promising alternative and an ongoing process focused on improve the commercial scale production and the possibilities of applications in several fields. The most viable genetic tools to achieve these goals are (i) the transfer of genetic determinants of microbial polysaccharides to more efficient hosts, enhancing the production; or (ii) the change of the repeat unit of the polysaccharide, offering tailor-made EPS (Freitas, Alves, and Reis 2014; Van Kranenburg et al. 1999).

Attractive options such as increase the number of glycosyltransferases, the enzyme responsible for the synthesis of the repeating unit presented at the polysaccharide; introducing new or exchanging the existing enzymes in the EPS microorganism-producer; and the use of different hosts, can lead to a reduction at production costs, increasing production yields, and allow the use of cheaper substrates or other cost-effective fermentation conditions (Van Kranenburg et al. 1999).

Table 1. Microorganisms, optimum conditions for EPS production and yields obtained.

Microorganism	Optimization methodology	Optimum conditions for EPS production	Yield	Reference
Bacteria				
Bacillus polymyxa	One factor at a time	Sucrose and potassium nitrate as carbon and nitrogen sources, respectively. EPS production growth associated and the best pH was 7–8	54 g/L	(Lee et al. 1997)
Paenibacillus polymyxa SQR-21	One factor at a time and Fractional Factorial design	Iron (242 μM), calcium (441 μM) and galactose (48.5 g/L)	3.44 g/L	(Raza et al. 2011)
Paenibacillus polymyxa EJS-3	Fractional Factorial design	188.2 g/L of sucrose, 25.8 g/L of yeast extract, 5 g/L of K_2HPO_4 and 0.34 g/L of $CaCl_2$	35.26 g/L	(Liu et al. 2010)
Lactobacillus delbrueckii subsp. *bulgaricus*	RSM	38 °C, pH of 5.0, and 30 g/L of bacto-casitone	2.25 g/L	(Kimmel, Roberts, and Ziegler 1998)
Lactobacillus reuteri	One factor at a time	Softer dough, with pH 4.7, sucrose up to 350 mM and rye bran as substrate	–	(Kaditzky and Vogel 2008)
Bacillus subtilis	One factor at a time	Sucrose (20 g/L), yeast extract (5 g/L), NaCl (7 g/L), $CaCl_2$ (0.5 g/L), L-asparagine (0.05 g/L), and ascorbic acid (0.05 g/L)	Sucrose: 2.98 g/L. Cane molasses: 4.86 g/L, Rice bran : 2.14 g/L	(Razack, Velayutham, and Thangavelu2013)
Bacillus subtilis	–	Nutrient broth medium with pH of 7.0, 96 h and 37 °C	35.33 mg of dry mass	(Chug et al. 2016)
Klebsiella sp.	One factor at a time	31.93 g/L of sucrose, 2.17 g/L of KNO_3 and 5.47 g/L of K_2HPO_4 and 28.9 °C	15.05 g/L	(Qiang et al. 2013)
Azotobacter beijreinckii	One factor a time	Nutrient broth medium with pH of 7.0, 24 h and 30 °C	21.33 mg of dry mass	(Chug et al. 2016)
Fungi				
Tremella mesenterica NRRL Y-6158	One factor at a time	25 °C, aeration of 1.6 L/min, agitation at 150 rpm and without controlled pH.	Yield 2.2 times higher	(De Baets et al. 2002)
Rhodotorula bacarum	One factor at a time	8% glucose, 2% soybean cake, pH 7.0, 28 °C and 180 rpm	5,9% (w/v)	(Chi and Zhao 2003)
Collybia maculate	Orthogonal Matrix	30 g/L of glucose, 20 g/L of Martone A-1, 1 g/L of K_2HPO_4, and 1 g/L of $CaCl_2$	2.4 g/L	(Lim et al. 2004)
Agrocybe cylindracea	Orthogonal Matrix	Maltose 60g/L, Martone A-1 6 g/L, $MgSO_4{\cdot}7H_2O$ 0.9g/L, and $CaCl_2$ 1.1g/L	3.0 g/L	(Kim et al. 2005)
Coriolus (Trametes) versicolor	Full Factorial design	Incubation for 9 days at 28 °C at 180 rpm and media with 15 g/L of glucose and pH of 5.5	0.64 g/L	(Tavares et al. 2005)
Rhizobium sp.	RSM	Temperature and pH constant (30 °C and 7.0) with 1.1 g/L of calcium carbonate, aeration of 1.3 vvm and 800 rpm	0.35 g/g	(Duta, de Franca, and de Almeida Lopes 2006)
Fomes fomentarius	Orthogonal Matrix	50 g/L glucose, 15 g/L silkworm chrysalis, 3 g/L yeast extract, 0.5 g/L $CaCl_2$, and 0.8 g/L $MgSO_4$	3.64 g/L	(Chen et al. 2008)
Morchella esculenta SO-02	Orthogonal Matrix	Bran 200 g/L, glucose 30 g/L, yeast extract 1 g/L, and the optimum parameters for cultivation were: temperature 25 °C, cultivation time 4 days, volume of medium 100 ml, rotary speed 200 rpm and initial pH 6.5	2.9 ± 0.2 g/L	(Meng, Liu, et al. 2010)
Armillaria mellea	One factor at a time	Glucose 40 g/L, yeast extract 3 g/L, KH_2PO_4 4 g/L and $MgSO_4$ 2 g/L at an optimal temperature of 22 °C and pH 4.0	0.27 g/L	(Lung and Huang 2010)
Aureobasidium pullulans	Plackett Burman design	Sweet potato 10%, yeast extract 0.75%, pH of 5.5, and 100 h	9.3 g/L	(Padmanaban et al. 2015)
Cordyceps militaris	Plackett Burman design	48.67 g/L glucose, 12.56 g/L peptone, 1 g/L KH_2PO_4, 10 g/L yeast extract, and 0.5 g/L $MgSO_4{\cdot}7H_2O$	1.96 g/L	(Cui and Jia 2010)
Bionectria ochroleuca M21	CCD	4 days of fermentation in a 5 liter bioreactor, using 55.7 g/L of glucose, 6.04 g/L of yeast extract, 0.25 g/L of $MgSO_4$ and 0,1% (v/v) of Tween80	2.65 g/L	(Li, Guo, and Zhu 2016)
Algae				
Chlamydomonas reinhardtii	Plackett Burman and RSM	74 mg/L of $CaCl_2$, 422 mg/L of $NaNO_3$, 10 mg/L of K_2HPO_4 and 200 mg/L of $MgSO_4$ with a pH 7.0	0.63 g/L	(Bafana 2013)

Stingele and coworkers (1999) employed *Streptococcus thermophilus* Sfi6, a specie able to produce an EPS composed of glucose, galactose and *N*-acetylgalactosamine, isolated and transferred its gene cluster to *Lactococcus lactis* MG1363, a non-producer EPS host. The authors were able to produce a new EPS with high molecular weight and different structure, indicating that glycosyltransferases can have multiple specificities for the donor and acceptor, and the polymerase is able to polymerize the repeating unit in different ways (Stingele et al. 1999).

A homologous overexpression of the gene *gal*U, which code for enzymes in the central metabolism, was made by *Streptococcus thermophilus* LY03 leading to an increase in EPS yield when used in combination with *pgmA*, showing that EPS production can be improved by altering the level of certain enzymes (Levander, Svensson, and Rådström 2002).

In another studied, EPS production was increased by elevating the expression of the gene cluster pNZ4000 in *Lactococcus lactis* NIZO B40, a possible strain for polysaccharide production. This finding suggests that the EPS production is limited by the activity level of expression of the pNZ4000 gene cluster rather than by the level of EPS precursors (Boels et al. 2003).

The use of genetic and metabolic engineering for production of EPS is still little used due to the lack of genetic tools (Shin et al. 2016). The development and obtainment of engineered tailored polymers can lead to higher performance products, opening their potential application in several industrial segments (Schmid, Sieber, and Rehm 2015).

Extraction and purification methods

Extraction methods of EPS can be divided into chemical and physical methods. Chemical methods include the use of alkaline, ethylenediamine tetraacetic acid (EDTA) and cation exchange resin, which can contaminate the extracted products (Comte, Guibaud, and Baudu 2006). Meanwhile, physical methods include centrifugation, ultrasonication, and heating, which normally present lower efficiencies when compared to chemical methods (Liang et al. 2010; Liu and Fang 2002).

A large number of methods for the extraction of microbial EPS has been developed and optimized in the last years. Freitas, Alves, and Reis (2011) described three fundamental steps, which include chemical and physical methods, for EPS extraction from culture broth: cell removal, polymer precipitation, and drying. The cell removal is normally obtained using centrifugation, with speed and time depending on the polysaccharide, while the precipitation is done using a precipitating agent which is a water miscible solvent (e.g. methanol, ethanol, isopropanol, or acetone). When the polysaccharide is thermally stable, heat can be applied for the destruction of enzymes that can degrade the EPS (Freitas, Alves, and Reis 2011; Singha 2012).

When a high level of purity is desirable, the polysaccharide can be submitted to one or more additional methods, such as re-precipitation, deproteinization by physical or chemical methods, and membrane processes combined (Roca et al. 2015). In this way, the best extraction method must be a procedure that causes minimum cell lysis, does not disrupt the exopolysaccharide (Gehr and Henry 1983), and avoids a large amount of DNA and protein released with the EPS (Liu and Fang 2002). Zhang and coworkers (2014) tested the selectivity and influence of process parameters to release polysaccharides and proteins from yeast cells using ultrasound. It was observed that selected-release is possible by only manipulating the sonication temperature, in which high temperatures (65–85 °C) favors the release of polysaccharides. In general, elevated temperatures can lead to denaturation and coagulation of proteins (Zhang et al. 2014).

Some studies have compared the different chemical and physical extraction methods. One of the first studies was made using aerobic, acidogenic, and methanogenic sludges and different conditions of extraction: only centrifugation, extraction with different chemicals (EDTA 2%; at 4 °C for 3 h; cation exchange resin Dowex 50 × 8, Fluka, USA; at 4 °C for 1 h; formaldehyde at 4 °C for 1 h, formaldehyde plus NaOH 1 N; at 4 °C for 3 h; and formaldehyde plus ultrasonication 60 W for 2.5 min) followed by centrifugation. In these sludges, formaldehyde and NaOH were the most effective for the extraction, although they have guaranteed the extraction of a limited portion of the EPS (Liu and Fang 2002).

Liang et al. (2010) compared four extraction methods divided in 3 chemical (EDTA, NaOH, cationic exchange resin) and one physical (ultrasound) also on sludges. EDTA and ultrasound were more effective for EPS extraction since higher activity of glucose-6-phosphate dehydrogenase was obtain from cell lysis. In this study, the presence of DNA and glucose-6-phosphate dehydrogenase was used to determine the degree of rupture of the cells (Liang et al. 2010).

Another work compared six different methods, including ultrasonication, heating, formaldehyde + NaOH, H_2SO_4, glutaraldehyde, and EDTA, for the extraction of EPS from *B. megaterium* TF10, isolated from a soil sample with high EPS-producing capacity. The methods were compared based on EPS yields and compositions, cell lysis, flocculation activities, and spectrum characteristics of extracted EPS, which led to the follow results: heating, formaldehyde + NaOH and H_2SO_4 led to a high EPS yield compared to ultrasonication or EDTA, while the ultrasonication and H_2SO_4 caused much more cell lysis than the formaldehyde + NaOH. They also concluded that the extraction method could affect the structure and composition of the EPS (Sun et al. 2012).

Meng and coworkers (2010) optimized the extraction of EPS from *Morchella esculenta* SO-01 and analyzed by Plackett–Burman (PB) experimental factors that affected the extraction (concentration temperature, varying from 70 to 90 °C; precipitation time, from 12 to 24 h; and pH, from 7.0 to 9.0), and to optimize the extraction conditions using RSM. The best extraction conditions found were at 84.07 °C, precipitation with 3 volumes of cold (4 °C) ethanol for 22.19 h and pH 8.44, yielding 5.45 g/L of EPS (Meng, Zhou, et al. 2010).

The extraction procedure of EPS is not simple and cannot guarantee the extraction of all the microbial polysaccharide

produced. Thus, the optimization of a method is required for each case, taking into account the characteristics of the EPS and final aim of the extraction (qualitatively or quantitatively) (Donot et al. 2012).

Additionally, a purification step is needed after the extraction, since residues of DNA, proteins, and even chemicals may be present. The polymer can be subjected to re-precipitation from an aqueous solution (<1.0 g/L); chemical deproteinization (e.g. salting out or protein precipitation with trichloroacetic acid, or enzymatic methods (e.g. proteases)); and membrane processes (e.g. ultrafiltration and diafiltration) (Ayala-Hernández et al. 2008; Bahl et al. 2010; Freitas, Alves, and Reis 2011; Kumar, Mody, and Jha 2007; Wang et al. 2007).

Kanmani and coworkers (2011) described the use of gel filtration for EPS purification produced by *Streptococcus phocae* through a phenyl Sepharose column with the sample eluted by phosphate buffer with 2 mL/min of flow rate (Kanmani et al. 2011). Meanwhile, Kumar et al. (2004) showed the EPS purification produced by *Bacillus* sp. I-450 by treating with cetylpyridinium chloride (CPC), which precipitate the anionic cell wall of the polymer (Kumar et al. 2004).

For the suitable choice of EPS purification method, is necessary to analyze their impact on the polymer properties, product recovery, and purity since some methods can decrease the product recovery or have negative impact on its properties (Freitas, Alves, and Reis 2011). It is noteworthy that the development of processes which integrated production and recovery with higher EPS yield and quality is a suitable choice for reduction of costs (Antunes et al. 2017).

Applications of microbial exopolysaccharides

Microbial EPS applications have a very broad range from the food to medical industry, due to the vast variability in EPS molecular structure, resulting in a wide range of properties and potential applications (Freitas, Alves, and Reis 2014; Singha 2012).

One of the oldest applications of microbial polysaccharides, especially EPS, is the change or improvement of rheological properties within the food industry (Nwodo, Green, and Okoh 2012) by chemical or enzymatic means (Singha 2012). The EPS can be used as a viscosity agent, stabilizers, emulsifiers, gelling agents, or water-binding agents (Donot et al. 2012), mainly used in dairy industries (Duboc and Mollet 2001; Madhuri and Prabhakar 2014). The main example is gellan gum, considered the most used and known EPS in the food industry. This gum has been able to provide adequate controlled flavor release in a wide range of pH, while also improving texture and physical stability of food products (Banik, Kanari, and Upadhyay 2000).

An important role at medical industry is also observed. Microbial EPS are becoming promising material for drug release systems, due to the ability of retain a large amount of water and still remaining insoluble, and drug-targeting carriers, based on the particular binding and penetrating features to cellular receptors (Moscovici 2015).

Another highly relevant property of microbial EPS is the ability to create films, also called polysaccharide-based membranes. Within this classification, some of the most used EPS are pullulan, gellan gum, levan, curdlan, hyaluronan, and bacterial alginates (Freitas, Alves, and Reis 2014).The main uses of this type of microbial EPS include:

i. Medical and pharmaceutical biomaterials for tissue regeneration, drug delivery agents, adhesives, surgical sealants, and coating of medical devices, specially pullulan (Cheng, Demirci, and Catchmark 2011; Costa et al. 2013; Mishra, Vuppu, and Rath 2011);
ii. Food applications, as edible and/or biodegradable membranes. Polysaccharides such as gellan, pullalan, xanthan, curdlan, and GalactoPol have been already applied as films with effective gas barrier properties (Alves et al. 2011; Flores et al. 2010; Shih, Daigle, and Champagne 2011; Trovatti, Fernandes, Rubatat, Freire, et al. 2012; Trovatti, Fernandes, Rubatat, Perez, et al. 2012; Yang, Paulson, and Nickerson 2010; Yang and Paulson 2000); and
iii. Solvent dehydration and wastewater treatment, due to the hydrophilic character and charge, with a high adsorption capacity for dyes, heavy metal ions and aromatic compounds (Freitas, Alves, and Reis 2014).

EPS obtained from fungal strains (e.g. lentinan, schizophyllan and glucan) are most known for their medical properties, being often described as 'Biological Response Modifiers'. These compounds are able to trigger a nonspecific reaction against tumor cells, viral and bacterial infections, and inflammation (Giavasis 2014). Limin and coworkers (2016) proved the antioxidant activity of EPS from *Fomitopsis pinicola* with strong scavenging abilities of DPPH, hydroxyl radical and protective effects on yeast cells by UV and H_2O_2 induced oxidative damage (Limin et al. 2016). The EPS from *Cordyceps gracilis* also had their antioxidant activity studied, proving a significant DPPH and ABTS radical scavenging activities, iron chelating activity and reducing power (Sharma, Nandini, and Atri 2015). Also, the EPS of *Ganoderma neojaponicum* showed potential to be used as immunomodulating agents stimulating the immune system to fight infectious diseases (Ubaidillah, Abdullah, and Sabaratnam 2015).

EPS obtained from lactic acid bacteria are widely used in the food industry to improve the rheological properties of several fermented dairy products in terms of stability, mouthfeel, firmness and texture. Some studies have also shown prebiotic, anti-gastric, antiulcer and cholesterol lowering effects (Jaiswal et al. 2014; Patel and Prajapat 2013).

Main exopolysaccharides of industrial interest

As shown, EPS are molecules of great interest, both at laboratory and industrial scale. Despite laboratory research, some polymers are already recognized by the industry and widely applied, as can be seen below in Fig. 2.

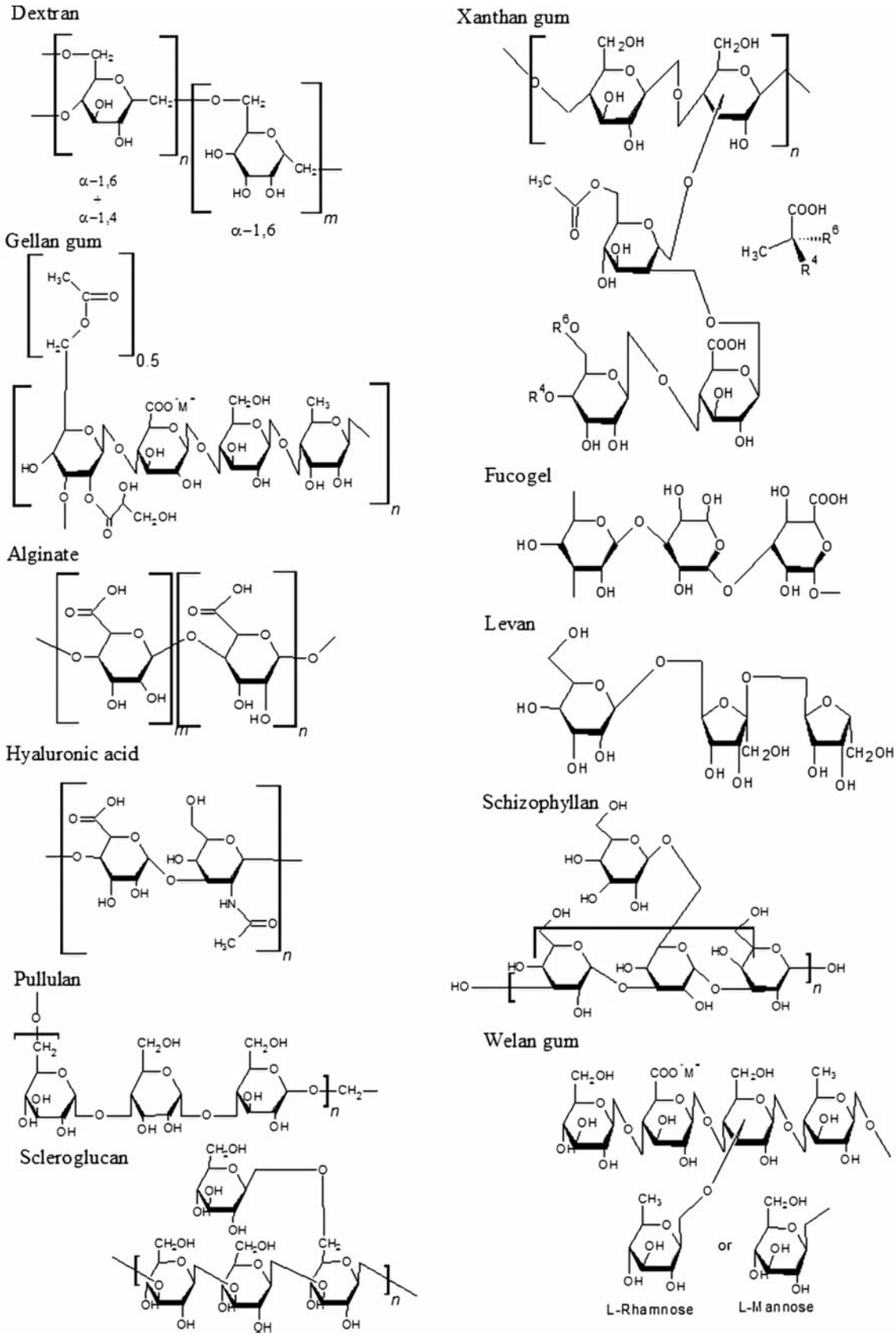

Figure 2. Main exopolysaccharides of industrial interest.

Dextran

Dextran is a homopolysaccharide of glucose with α-(1-6)-linkages and molecular weight varying from 10 to 2000 kDa. This compound can be synthesized by *Leuconostoc*, *Streptococcus, Weissella* and *Lactobacillus* species, outside the microbial cell from sucrose by dextransucrase enzymes, with

optimum conditions of temperature between 25 °C and 30 °C, and pH of 6–6.9. The commercial dextran is produced by *L. mesenteroides* and *L. dextranicum* (Ahmed et al. 2012; Andhare et al. 2014; Vu et al. 2009).

The dextransucrase uses two pathways for the production of dextran: (i) hydrolyzing the sucrose and binding the glycosyl moiety; (ii) building up the dextran by an insertion mechanism (Moosavi-Nasab, Alahdad, and Nazemi 2009). The dextran size and structure are synthesized based on the dextransucrase produced by the microbial strain, which justifies the differences in size and molecular weight of the various dextran (Purama et al. 2009).

Dextran, considered the most important polysaccharide obtained by fermentation, first reported in 1874, starting the study and development of microbial exopolysaccharides in industrial scale (Barbosa et al. 2004).

Dextran presents many industrial uses due to its good stability and nonionic character, being used, mostly, as an indirect food additive, as a stabilizer and viscosity agent. Additionally, this polysaccharide can be also applied in the medical and pharmaceutical industries, being used as a plasm expander and blood flow adjuvant (Biliaderis and Izydorczyk 2007). Derivatives of dextran such as Sephadex®, a crossed linked dextran gel launched by Pharmacia in 1959, are used in gel filtration for separation and purification of products such as proteins (Moosavi-Nasab, Alahdad, and Nazemi 2009; Sutherland 1998). The potential of the dextran produced by *Lactobacillus sakei* MN1 as an antiviral agent and as a prebiotic in aquaculture was recently reported (Nácher-Vázquez et al. 2015; Nácher-Vázquez et al. 2017).

Low-cost substrates for the production of dextran are reported, including molasses and cheese whey. The influence of such substrates, in the isolated form or in mixtures, was studied. The highest yield of dextran was obtained when a mixture of molasses and 10% cheese whey was used, and the lowest using a 2% mixture. No dextran was produced at only whey medium (Moosavi-nasab, Layegh et al. 2010).

Nowadays, companies that produce and commercialize dextran are: Pharmacosmos, Sigma-Aldrich, Pharmachem Corporation and Amersham Biosciences (de Oliveira 2013). Among those, Pharmacosmos is the number one company for the production and distribution of dextran for pharmaceutical uses, an expert at fractionation techniques, possessing a great number of different dextran in their catalog (Pharmacosmos 2017).

Xanthan gum

The xanthan gum is a heteropolysaccharide produced by the plant pathogenic, gram-negative bacteria *Xanthomonas* sp., mainly by *Xanthomonas campestris, Xanthomonas pelargonii, Xanthomonas phaseoli* and *Xanthomonas malvacearum*. This polymer is composed of repeating units of D-glucose, D-mannose, D-glucoronic acid, acetal linked pyruvic acid, and *D*-acetyl groups with high molecular weight, varying from 2×10^6 and 20×10^6 Da (Habibi and Khosravi-Darani 2017; Patricia Ruas-Madiedo, Hugenholtz, and Zoon 2002; Schmid, Sieber, and Rehm 2015).

Discovered in 1950, its commercialization started in 1960 by Kelco, being the first natural polymer produced in industrial scale. The consumption of this polysaccharide is estimated at US$23 million per year, with an annual worldwide growth of 6–7%. The xanthan gum is one of the most expensive microbial polysaccharides due to the use of only glucose and sucrose (expensive substrates costing US$4,000–5,000 per ton) as carbon sources, and the cost of the downstream process (approximately 50% of the final cost), since a high purity level is required when is used in the food industry (Li, Guo, and Zhu 2016; Subhash, Jadhav, and Jana 2015).

The production of xanthan gum is a non-continuous process operating at pH 7.0, 28 °C and high agitation rates (400–800 rpm) (Freitas, Alves, and Reis 2011; Habibi and Khosravi-Darani 2017; Subhash, Jadhav, and Jana 2015). When a high average molecular mass is desirable, the fermentation must occur at temperatures below 25 °C, in which the use of surfactants, such as Tween-40 and Tween-80, can improve the production (Habibi and Khosravi-Darani 2017; Kumar, Mody, and Jha 2007). Sucrose and glucose are the main carbon sources used at the industrial production, being enhanced by the carbon concentration but negatively affected by nitrogen concentration enhancement. Nitrogen is only needed for the growth of the bacteria, and it is not necessary for the production of xanthan gum, which occurs during the stationary phase. In this way, a two-step fermentation is proposed, firstly conducted with a low C/N ratio (for the bacteria growth), followed by a high C/N ratio (for the xanthan gum production) (Bueno and Garcia-Cruz 2006; Habibi and Khosravi-Darani 2017).

The downstream process of xanthan gum production consists of precipitation and recovery using acetone or alcohols. Heat treatment is necessary due to the pathological nature of the bacteria (Kumar, Mody, and Jha 2007; Rühmann, Schmid, and Sieber 2015b).

The main properties of xanthan gum are the high viscosity (even at low concentrations), water solubility, good stability at ample pH range (1–12), and resistance to degradation at high temperatures (Habibi and Khosravi-Darani 2017; Patel and Prajapat 2013). Due to these particular characteristics, this gum can be widely used as a stabilizing, thickening and suspending agent (Velu, Velayutham, and Manickkam 2016) at food, pharmaceutical and cosmetic industries (Biliaderis and Izydorczyk 2007). It can also be used for enhanced oil recovery (Gao 2016; Jang et al. 2015), as fat replacer (Rather et al. 2015), mucoadhesive polymer (Bhatia, Ahuja, and Mehta 2015), treating osteoarthritis (Han et al. 2017), soil strengthening (Chang et al. 2015), and controlled drug release carrier (Benny, Gunasekar, and Ponnusami 2014; Moscovici 2015). Its use with other polymers, e.g. starch, chitosan and zein protein, is reported for the development of biodegradable films with potential use in the food and pharmaceutical industries (Freitas, Alves, and Reis 2014; Lima et al. 2017).

The biotechnological production of xanthan gum can still be extensively explored and developed from the use of new producer strains, with high efficiency, and the use of cheap substrates. Some of the alternative substrates reported

include the use of kitchen waste (Li, Guo, and Zhu 2016), milk whey (Nitschke, Rodrigues, and Schinatto 2001), cheese whey (Niknezhad et al. 2015), sugar cane broth (Faria et al. 2011), glycerol (Wang et al. 2017), green coconut shells (Nery, Cruz, and Druzian 2013), rice straw (Jazini, Fereydouni, and Karimi 2017), and sugar cane molasses (Zakeri, Pazouki, and Vossougi 2015), for the low cost production of xanthan gum.

In the recent years, the biggest companies which commercialized xanthan gum are Merck and Pfizer (USA), Rhone Poulenc, Mero Rousselot-Santia and Sanofi – EIF (France), Saidy Chemica (China), and Jungbunzlauer (Austria) (Habibi and Khosravi-Darani 2017).

Gellan gum

The gellan gum was discovered in 1978 and had its first use in food products ten years later, in Japan. Only in 1990, the FDA approved its use in the USA and Europe as a stabilizer and thickener, being patented and first commercialized by Kelco. This gum is an anionic linear heteropolysaccharide with repeating units of α-rhamnose, two residues of *β-D*-glucose and *β-D*-glucuronate possessing high viscosity and thermal stability (Banik, Kanari, and Upadhyay 2000; Gidley and Grant Reid 2006; Moscovici 2015).

Gellan belongs to the sphingan family (Schmid, Sieber, and Rehm 2015), and it is mainly produced by aerobic submerged fermentation by *Sphingmonas paucimobilis*, formerly known as *Pseudomonas elodea*, a gram-negative bacteria, in batch culture (Gidley and Grant Reid 2006).

The fermentation process is conducted at 30 °C with a pH range of 6.0–7.0, during 30–60 h with agitation of 250 rpm, and precipitation made using alcohol, in which the extraction conditions can be varied to obtain a range of gellan gums with different degrees of esterification. Gellan gum production is direct related to microbial growth, in which factors that affect negatively the microbial growth can reduce its production. It is known that the use of yeast extract as nitrogen source and sucrose as carbon source gives the maximum production of gellan gum (Prajapati, Jani, and Khanda 2013; Zhang et al. 2015).

Other properties and uses for gellan gum include film formation with mechanical and water vapor barrier properties (Yang, Paulson, and Nickerson 2010; Yang and Paulson 2000), e.g. application in paper cups for hot drinks (Zhang et al. 2017) and the combined use with purple sweet potato for pH monitoring (Wei et al. 2017); vehicle for ophthalmic drugs and drug release (D'Arrigo et al. 2014; Mahdi, Conway, and Smith 2014; Osmałek, Froelich, and Tasarek 2014; Prezotti, Ferreira Cury, and Evangelista 2014); gelling agent in dental and personal care (Banik, Kanari, and Upadhyay 2000); wound healing and tissue engineering (Becker 2015; Bonifacio et al. 2017; Douglas et al. 2016; Hadjizadeh and Doillon 2010); inhibition of acid corrosion of iron cast (Rajeswari et al. 2013); and the use of gellan hydrogel for paper cleaning (Mazzuca et al. 2014).

The gellan gum is one of the most used exopolysaccharides due to its ability to form a transparent gel in the presence of multivalent cations, with good thermal and acid stability, being also thermo-reversible. Some commercial gellan products include Gelrite® and Kelcogel® being a potential replacement for agar and gelatin (Banik, Kanari, and Upadhyay 2000; Madhuri and Prabhakar 2014; Prajapati, Jani, and Khanda 2013).

Alginates

The alginates represent a family of polysaccharides composed of mannuric acid and guluronic acid first extracted from brown algae, patented at Oxford in 1881. These are linear polysaccharides with units of *β*-D-mannuronic acid and α-L-guluronic acid linked by 1-4 bonds (Ertesvåg and Valla 1998; Gacesa 1988). Normally, they are obtained from marine brown algae but can also be produced by soil bacteria such as *Azotobacter* and *Pseudomonas*, being quite abundant in nature (Gidley and Grant Reid 2006; Goh, Sia Heng, and Chan 2012).

The production of alginates is estimated in 30,000 tons per year (Gidley and Grant Reid 2006), with most commercial available alginates from brown algae, due to the economic unfeasibility of those produced by bacteria for commercial applications, being still confined to small scale studies (Goh, Sia Heng, and Chan 2012) using bacteria such as *Pseudomonas aeruginosa* (Schürks et al. 2002; Stapper et al. 2004), *Pseudomonas putida* (Chang et al. 2007) and *Pseudomonas syringae* (Laue et al. 2006).

The alginates have an important role at the food, pharmaceutical, textile, and paper industries, used as stabilizers and thickeners for foods and for color pigments, adhesive agents and fillers in the paper industry, and encapsulation material in the pharmaceutical industry (Gidley and Grant Reid 2006; Goh, Sia Heng, and Chan 2012). The biomedical applications study their use for therapeutical cell entrapment and immunologic properties (Draget and Taylor 2011). All these uses are based on the physiochemical properties of the alginates, such as high viscosity, sol-gel transformation, thermostability and potential for controlled drug release (Goh, Sia Heng, and Chan 2012).

Fucose containing EPS

Fucose is a rare sugar, not commonly found in nature, presenting interesting physical and bioactive properties, being already used in the pharmaceutical, cosmetics and food industry. The potential applications of these polysaccharides include its use in anticarcinogenic and anti-inflammatory drugs, justifying the interest in EPS containing fucose, which includes Fucogel, Clavan and FucoPol (Roca et al. 2015).

Fucogel is a linear anionic polymer commercialized by Solabia (France) and mainly used in the formulation of skin care cosmetics due to its moisturizing properties and action as an anti-aging agent. This polymer can be produced by *Klebsiella pneumoniae* with repeating units of galacturonic acid, L-fucose, and D-galactose (C. Robert, Robert, and Robert 2003; Robert et al. 2004; Roca et al. 2015).

Clavan, produced by *Clavibacter* strains, especially *C. michiganensis*, is composed of units of L-fucose, D-glucose, and D-galactose, being able to form high viscosity solutions (Vanhooren and Vandamme 2000).

FucoPol is synthesized by *Enterobacter* A47 presenting high molecular weight, composed mainly by fucose (32–36 mol%), galactose (25–26 mol%), glucose (28–37 mol%) and glucuronic acid (9–10 mol%) (Torres et al. 2015). The film-form ability of FucoPol makes possible its use as an inner layer at multilayer packaging material, featuring ductile mechanical properties, hydrophilic character and good barrier properties to gases when low water content is used (Ferreira et al. 2014). These properties were enhanced using chitosan, which provides the use for a broader ranging of food (Ferreira et al. 2016). Other uses of FucoPol are based on its adhesive properties (Araújo et al. 2016); emulsion stabilizing and flocculating capacities (Roca et al. 2015). Recently, the production of FucoPol using tomato-paste by-product was presented, showing the possibility of using low-cost substrates to produce this high-value product (Antunes et al. 2017).

Hyaluronic acid or hyaluronan

The hyaluronic acid (HA), also called hyaluronan, is a linear heteropolysaccharide, extremely hydrophilic with units of β-D-glucuronic acid and β-D-N-acetyl-glucosamine residues linked via β-(1-4) and β-(1-3)-glycosidic bonds produced by *Streptococcus* strains, belonging to group C. The main properties of these polysaccharides are due to their ability of high water retention, biocompatibility, and viscous behavior, being used mostly in the medical and cosmetics industries (Ruffing and Chen 2006; Schmid, Sieber, and Rehm 2015; Sutherland 1998).

The HA was discovered in 1934 and first obtained from animal tissues. Nowadays, the production is strictly from recombinant bacteria (Moscovici 2015). The use of HA at high-priced segments, with a world market of US$500 million and a selling price of US$100,000.00 per kg, motivates the use of metabolic engineering approaches, which leads to the enhanced HA synthesis (Saranraj and Naidu 2013; Schmid, Sieber, and Rehm 2015).

For most of the biomedical uses, HA is processed in the form of hydrogels being increasingly versatile, as they can be used as cell-based and regenerative therapies (Highley, Prestwich, and Burdick 2016); tissue-engineering cartilage constructor (Collins and Birkinshaw 2013; Levett et al. 2014); and drug delivery (Dosio et al. 2016; Mero and Campisi 2014). Other uses for HA are wound healing; osteoarthritis treatment (intra-articular injection) (Ahrq 2015; Fakhari and Berkland 2013); and eye surgery (commercial names: Healon® from Abbott, and Hylan GF-20® from Genzyme) (Liang, Jiang, and Noble 2016; Moscovici 2015).

Levan

Levan is a high molecular mass homopolysaccharide synthesized from sucrose by levansucrose outside the cell (similar mechanism to dextran), formed by groups of β-(2-6)-D-fructans with some β-(2-1)-branches. This compound is produced mainly by bacteria such as *Bacillus, Zymomonas, Halomonas, Pseudomonas, Rahnella, Aerobacter, Erwinia, Streptococcus, Microbacterium*, but also by some yeasts and fungal strains (Andhare et al. 2014; Biliaderis and Izydorczyk 2007; Kumar, Mody, and Jha 2007; Moscovici 2015; Poli et al. 2009; Sutherland 2001). Is noteworthy that levan produced by different organisms have different molecular weight and degree of branching which impacts on their possible applications (Abdel-Fattah et al. 2012).

Some studies reported the use of levansucrose by different microorganisms for the efficient biosynthesis of levan, e.g. *Brenneria goodwinii* (Liu et al. 2017), *Pseudomonas syringae* (Bondarenko et al. 2016), *Bacillus subtilis* (Esawy et al. 2013). The enzyme can be used in crude, recombinant, purified or immobilized forms and achieved satisfactory yields which showed potential for industrial use (Esawy et al. 2013; Liu et al. 2017). The use of immobilized cells can also offer advantages for industrial production, facilitating the product isolation and biocatalyst reuse (Shih, Chen, and Wu 2010).

Some of the main properties of levan include neutral charge, low viscosity, high water solubility but non-swelling, biological activity: anti-tumor, antioxidant and anti-inflammatory and cholesterol lowering effect (Abdel-Fattah et al. 2012; Moosavi-Nasab, Layegh et al. 2010), adhesive strength, film-forming capacity, and it is also used as thickener and binder in pet feed (Andhare et al. 2014; Freitas, Alves, and Reis 2014; Karunaratne 2012). In the food industry, levan may have potential application as prebiotic, since upon acid hydrolysis is converted into small fructo-oligosaccharides (FOS) (Srikanth et al. 2015). Levan from *Lactobacillus sanfranciscensis* had also demonstrated its influence on bread texture and volume (Kaditzky and Vogel 2008).

Low-cost substrates are being tested for the production of levan. One example includes the use of molasses and sugar cane sirup by the bacteria *Zymomonas mobilis*. The product obtained was 2.53 g/L and 15.46 g/L with molasses and sugar cane sirup, respectively, and although these values are still lower than the production using commercial sucrose (21.69 g/L), there is a great potential in the use of alternative substrates considering the cost and sustainability aspects (de Oliveira et al. 2007). Date sirup was also tested with *Microbacterium laevaniformans* obtaining a yield of 10.48 g/L (Moosavi-Nasab, Layegh et al. 2010).

Although levan is a remarkable polysaccharide for the industry, the scale up process presents technical issues due to the difficulty to handle large amounts of alcohol (used for precipitation of the polymer), unavailability of ultrafiltration equipment for multi-ton operations, impracticality of dialysis and the necessity of enzyme inhibition at the end of the fermentation (Oner, Hernández, and Combie 2016).

Nowadays, commercialization of levan is made by several companies with Rahn® selling the most finished products (Proteolea® and Slimexir®) containing this polysaccharide. Natural Polymers Inc., Bainbridge, GA, USA using *Bacillus subtilis*, Real Biotech Co. (Chungnam, Korea), using *Z.*

mobilis and Advance Co. (Tokyo, Japan), using *Streptococcus salivarius*, are the main companies that produce and commercialize levan (Oner, Hernández, and Combie 2016).

Pullulan

The pullulan is a linear homopolysaccharide consisting of maltotriose units connected by α-(1-4) glycoside bonds whereas consecutive maltotrioses are connected by α-(1-6) glycoside bonds. This polysaccharide is produced industrially by the yeast-like fungi *Aureobasidium pullulans* during late exponential and early stationary phase with average molecular weight of 362–480 kDa (Mishra, Vuppu, and Rath 2011; Singh, Saini, and Kennedy 2008; Welman and Maddox 2003). The application of pullulan as a food ingredient has been approved since 2002 in the USA and Europe (Moscovici 2015), mainly as a dietary fiber or a prebiotic, due to the partial degradation by human amylases (Giavasis 2014).

Pullulan has been widely used in the food, medical and pharmaceutical industries due to properties such as water solubility, capacity of mimic petrochemical-derived polymers, pH stability at a broad range, low viscosity, excellent adhesive properties, good oxygen barrier, and film-forming properties (Freitas, Alves, and Reis 2014; Padmanaban et al. 2015; Prajapati, Jani, and Khanda 2013; Ravella et al. 2010; Singh, Saini, and Kennedy 2008). Some of the uses consist of partial replacement of starch, edible films and packaging material in the food industry (Andhare et al. 2014; Farris et al. 2014; Shih, Daigle, and Champagne 2011; Xiao et al. 2017); for granulation and coating of tablets (Plantcaps-Capsugel, Inc.) (Jahanshahi-Anbuhi et al. 2014), drug-carrier for cancer treatment (Balasso et al. 2017; Huang et al. 2017), oral (Ravi et al. 2014) and transmucosal (Dionísio et al. 2013) drug delivery and wound care products such as Listerine® in the pharmaceutical industry (Ram et al. 2017); and engineering tissue (Moscovici 2015; Sarup et al. 2016), and RNA protection (Hsieh et al. 2017) in the biomedical area.

The production of pullulan is performed by an aerobic fermentation, conducted in batch or fed-batch with high aeration rates, optimum pH of 4.5 and temperature ranging from 24 to 30 °C, during 100 h. It is possible to use several different carbon sources, e.g. sucrose, glucose, fructose, maltose, starch, or malto-oligosaccharides, with the best nitrogen sources being ammonium and complexes nitrogen sources (Singh, Saini, and Kennedy 2008). During the production of pullulan, the total consumption of the nitrogen source can be observed (Kumar, Mody, and Jha 2007), with an increase of pullulan production under nitrogen-limitation conditions (Wang et al. 2015). The positive influence of Tween 80, a surfactant, during the production of pullulan was reported, favoring pullulan release and uptake of nutrients (Sheng et al. 2016).

One of the main obstacles found in pullulan fermentation by *A. pullulans* is melanin pigmentation that turns the broth to dark green or black color (Chi and Zhao 2003; Singh, Saini, and Kennedy 2008). This color requires the use of special solvents for the polysaccharide precipitation, being preferred solvents with relatively low hydrophilicity, like propyl alcohol, isopropyl alcohol, tetrahydrofuran, dioxane, with further purification made with ultrafiltration or ion exchanges resins (Singh, Saini, and Kennedy 2008). The production of pullulan by non-pigment strains of *Rhodotorula bacarum* had been reported (Chi and Zhao 2003), preventing this major obstacle. Another study reported the use of light-emitted diode to produce low melanin pullulan from sugarcane bagasse by a wild strain of *A. pullulans*, obtaining satisfactory results with higher yield (15.77 g/L) and low melanin content (4.46 UA_{540nm}/g) when compared to the untreated strain (11.75 g/L and 45.70 $UA5_{40nm}$/g) (Terán et al. 2017).

The pullulan was one of the most expensive polysaccharides, with its market value being three times higher than dextran and xanthan (Singh, Saini, and Kennedy 2008). Optimization of process conditions and medium composition, the use of genetic tools and low-cost substrates, such as sweet potato (Padmanaban et al. 2015), coconut and palm kernel (Sugumaran et al. 2013), potato starch (An et al. 2017; Wu et al. 2016), cassava bagasse (Sugumaran, Jothi, and Ponnusami 2014), and corn steep liquor (Mehta, Prasad, and Choudhury 2014), could contribute to a more competitive overall process cost (Yoon et al. 2012).

Schizophyllan (SPG) or sizofiran

Schizophyllan is a fungal beta-glucan polysaccharide composed of β-(1-3)-D-glucopyranose backbone which is branched with a single β-(1-6)-D-glucopyranose residue at every third glucose unit, produced by the edible mushroom *Schizophyllum commune* with triple helical conformation and molecular weight varying from 100,000 to 200,000 Da (Giavasis 2014; Moscovici 2015).

This glucan is a water soluble and nonionic polysaccharide produced by submerged fermentation with pH of 4.8 in a glucose rich medium, precipitated after 4–8 days by adding water-miscible organic solvents (e.g. methanol) (Zhang et al. 2013). Some low-cost substrates have been used for the production of schizophyllan, such as date sirup (Jamshidian et al. 2016) and corn fiber with satisfactory results suggesting the potential industrial use (Leathers et al. 2016).

Most of the applications of schizophyllan are based on its immunostimulatory activity probably due to its triple helical conformation, being a high-value polysaccharide, used for the treatment of various types of cancer, such as breast, gastric, lung, cervical and colorectal (Kumar, Auroshree, and Mishra 2016; Smirnou et al. 2017; Zhong et al. 2015; Zhou et al. 2015). It also contributes to the prevention of metastasis and reduces the side effects of chemotherapy (Giavasis 2014). The schizophyllan is also used in skin care products as an anti-aging and healing agent, having a suggested potential to enhance oil recovery (Gao 2016).

Scleroglucan

The scleroglucan is a fungal beta-glucan with the similar structure (β-D-glucan (1→3 and branch at 1→6)) as

schizophyllan, produced mainly by *Sclerotium glucanicum* with the triple helical conformation and, consequently, antitumor activity (Andhare et al. 2014; Barbosa et al. 2004).

The growth of *Sclerotium glucanicum* occurs as pallets surrounded by a layer of scleroglucan, which reduces the polysaccharide production due to the reduction in the mass transfer rate to/from cells (Kumar, Mody, and Jha 2007). The production of oxalic acid, an undesirable by-product of the process, can be observed below 28 °C. Optimum conditions for scleroglucan production by *Sclerotium glucanicum* are described as 20–37 °C, pH of 4.0–5.5, with vigorous agitation and high supply of oxygen (Castillo, Valdez, and Fariña 2015; Survase and Saudagar 2007; Wang and McNeil 1995). During the production of this compound, the total consumption of nitrogen from the medium can be noted (Kumar, Mody, and Jha 2007). When it concerns the downstream process, the method chosen can affect stability and flow behavior of the polysaccharide, originating different scleroglucan variants (Viñarta, Delgado, et al. 2013). The polymer can be precipitated by ethanol or isopropanol, with high levels of purity obtained when isopropanol is used (Viñarta, Yossen, et al. 2013).

Different substrates with economic and ecological benefits are being tested for the production of scleroglucan, such as coconut water, sugar cane molasses, sugar cane juice (Survase, Saudagar, and Singhal 2007), and condensed corn solubles, a by-product of corn-based ethanol production (Fosmer, Gibbons, and Heisel 2010).

The production of scleroglucan by other *Sclerotium* strains is also reported, with *Sclerotium rolfsii* being the most studied. The use of different strains makes the process more attractive industrially providing an option for the production of scleroglucan with similar properties of the one produced by *Sclerotium glucanicum*, once different *Sclerotium* strains can produce polysaccharides with different molecular weight, number and length of side chains, degree of polymerization and rheological characteristics (Fariña et al. 2001; Survase, Saudagar, and Singhal 2006; Viñarta et al. 2006; Viñarta et al. 2007).

Scleroglucan has a potential industrial interest due to its high molecular weight, water solubility, high viscosity, stability in the presence of salts, high temperature and extreme pH, biocompatibility, and nonionic character (Viñarta et al. 2006; Viñarta et al. 2007). Due to these features, this compound is reported for potential oil recovery, with antimicrobial and immunostimulatory activity (Barbosa et al. 2004; Wang and McNeil 1995), syneresis prevent agent (Viñarta et al. 2006), drug delivery vehicle (Coviello et al. 2005; Coviello et al. 2007), suspension stabilizer and emulsifier (Viñarta et al. 2007), film forming ability (Franois et al. 2011), and compatibility and synergism with industrial thickeners (Viñarta, Yossen, et al. 2013).

This polysaccharide was first introduced to the market under the name Polytran® and commercialized by CECA S.E. (France) with the name Biopolymer CS®. After, Satia, a division of Mero-Rousselot (France), sold scleroglucan with the trade name Acti-gum CS6®. Sanofi Bio-Industries (Carentan, France), obtained the rights from Satia and CECA, and became the biggest producer and distributor of scleroglucan, being sold to Degussa Food Ingredients (Germany) in 1995. In 2006, Cargil (Germany) acquired Degussa, selling the polysaccharide as Actigum™ CS (Survase and Saudagar 2007).

Welan gum

Welan gum, another polysaccharide from the sphingans class, is an anionic, non-gelling heteropolysaccharide produced by *Sphingomonas* sp. and a mutant strain of *Alcaligenes*, with the follow structure [→3)-β-Glcp-(1→4)-β-D-GlcpA-(1→4)-β-D-Glcp-(1→4)-α-L-Rha-(1→], where Glcp is glucose, GlcpA is glucuronic acid, Rha is rhamnose, with singular side chains containing either L-rhammose or L-mannose substituted on C3 of every 1,4-linked glucose repeating units (Biliaderis and Izydorczyk 2007; Freitas, Alves, and Reis 2011; Kaur et al. 2014).

The welan gum can be produced by submerged fermentation with optimum temperature ranging between 28 °C and 35 °C, pH of 6.5–7.5, and high agitation rates (800 rpm), since its synthesis is limited by dissolved oxygen levels (Kaur et al. 2014; Li et al. 2012; Liu et al. 2017). In addition, the use of surfactants, such as Tween-40, showed positive potential in the production of this compound (Li et al. 2012).

Welan gum is easily precipitated with alcohols and acetone (Rühmann, Schmid, and Sieber 2015b) and its purified form can be obtained by 3 different ways: (i) dissolved in NaCl, precipitated with isopropanol and vacuum dried; (ii) use of dialysis; and (iii) dissolved in aqueous solution of NaOH, neutralized with HCl followed by centrifugation and filtration (Kaur et al. 2014).

The uses of welan gum are based on its strong temperature (20–120 °C) and pH (2–12) tolerance (Liu et al. 2017), being used as thermostable thickener for oilfield application (Xu et al. 2014); suspending, stabilizing, emulsifying and thickening agent in the food industry; coating materials; concrete additive (Lachemi et al. 2004); mud thickener (Gao 2015a), and enhance oil recovery (Gao 2015b). This polysaccharide presents higher viscoelasticity than xanthan gum even with a lower molecular weight, resisting a higher shear, temperature, and salinity (Xu et al. 2013).

This gum is one of the most valuable polysaccharides due to its use as an enhancing oil recovery, and at high-value products. It is commercialized by CP Kelco, but still remains as one of the most expensive bio-gums due to its inefficient conversion from carbon sources and low yields (Kaur et al. 2014). To reduce fermentation costs and for a better commercial exploitation, efficient and cost-effective substrates are being studied, such as cane molasses (Ai et al. 2015).

Conclusion

Microbial EPS are renewable, biodegradable and biocompatible substances with a wide range of applications, such as improve or change of rheological properties of various products. In this sense, these compounds can be used in competitive markets within new industrial applications

in a diversity of sectors, such as food, medical and pharmaceutical.

However, despite their potential, there are still some difficulties in their production and purification processes that may hinder their scale of production and, consequently, the extension of commercial applications, due to the high cost and lower yields obtained. In this way, several researches aim to circumvent some of these problems for the development of a viable process. Some of these activities include the isolation of new strains and the use of low-cost substrates. Additionally, many studies are taking place to optimize the production of EPS and to better understand how process conditions may affect their biosynthesis, followed by the best methods for extraction and purification.

Thus, considering their properties and broad fields of application, the production of microbial EPS aiming to ally the scale of production, specific properties, purity and quality is necessary to meet the industrial demands.

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Ethical statement

Authors declare that there is no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

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