Microbial Production of Extracellular Polysaccharides from Biomass Sources

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Abstract

The interest in bio-based polymers, especially extracellular polysaccharides (EPSs), has increased considerably in recent years due to their useful physicochemical and rheological properties and diverse functionality. Microbial polysaccharides have many commercial applications in different industrial sectors like chemical, food, petroleum, health, and bionanotechnology. Although microbial EPS production processes are regarded as environmentally friendly and in full compliance with the biorefinery concept, EPSs constitute only a minor fraction of the current polymer market due to their cost-intensive production and recovery. For that reason, much effort has been spent to the development of cost-effective production processes by using cheaper fermentation substrates

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K.G. Ramawat, J.-M. Mérillon (eds.), *Polysaccharides*,
DOI 10.1007/978-3-319-16298-0_51

such as low-cost biomass resources. These resources are generally either in liquid form like syrups, molasses, juices, cheese whey, and olive mill wastewater or solid-like lignocellulosic biomass and pomaces. In this chapter, after a brief description of microbial polysaccharides, submerged and solid-state fermentation processes utilizing cheap biomass resources are discussed with a special focus on the microbial production of EPSs with high market value.

Keywords
EPS • Microbial exopolysaccharides • Polysaccharides • Biomass resources • Fermentation

1 Introduction

Since the beginning of the twentieth century, bio-based technologies such as the production of biomolecules like enzymes, antibiotics, metabolites, and polymers have developed to a great extent. Currently, microorganisms are used for commercial production of several products such as pesticides, fertilizers, and feed additives in the agrochemical sector, biopharmaceuticals and therapeutics in the healthcare sector, and biopolymers and biofuels in the energy and environment sectors (Toksoy Oner 2013). Globally, the market for bio-based products has been increasing significantly such that from 2005 to 2010, it increased from 77 to 92 billion € and is expected to increase up to 228 and 515 billion € in 2015 and 2020, respectively (without biofuels and pharmaceuticals) (Fava et al. 2013).

Substrates used by microorganisms for the production of bio-based products are an important cost consideration. Hence, using cheaper substrates such as wastes or by-products of food processing or agro-industry instead of synthetic media leads to a significant decrease in the production costs. In the EU, the annual amount of waste or by-products of agro-industrial, organic household, yard/forestry, and food processing reaches up to 1,000, 200, 550, and 250 million tons, respectively. In 2008, the recovery rate of waste (excluding energy recovery) was 46 % (Fava et al. 2013). Hence the use of wastes or by-products as substrate for microbial production of biotechnologically important molecules like exopolysaccharides (EPSs) has a remarkable potential for not only the recovery of waste but also for reducing the production cost (Kaur et al. 2014).

EPSs are high-molecular-weight polymers that are composed of sugar residues and are secreted by microorganisms to the surrounding environment. Many microorganisms including many species of gram-positive and gram-negative bacteria, archaea, fungi and some algae are known to produce EPSs. These natural, nontoxic, and biodegradable polymers not only protect microorganisms against environmental extremes such as Antarctic ecosystems, saline lakes, geothermal springs, or deep-sea hydrothermal vents but also play important roles in various biological mechanisms such as immune response, adhesion, infection, and signal transduction (Jones et al. 2014; Poli et al. 2011; Kumar et al. 2007; Sutherland 1998) as well as in

biofilm formation, biofouling, and quorum sensing (Garg et al. 2014; Fazli et al. 2014). Some EPSs produced by microorganisms such as cellulose are also produced by higher-order plants. But, due to their slow production rate (3–6 months) as well as their dependence on seasonal conditions and high agricultural land, controlled microbial fermentations are preferred over plants for sustainable and economical production of EPSs at industrial scale (Toksoy Oner 2013).

Due to their useful physicochemical and rheological properties and diverse functionality, the EPSs have been recognized as new biomaterials and been found to have a wide range of applications. They can be used as thickeners, bioadhesives, stabilizers, probiotics, gelling agents, emulsifiers, biosorbents, and bioflocculants not only in many industrial sectors like textiles, detergents, adhesives, microbial enhanced oil recovery (MEOR), wastewater treatment, dredging, brewing, downstream processing, cosmetology, pharmacology, and food additives but also in health and bionanotechnology sectors (Kreyenschulte et al. 2014; Toksoy Oner 2013; Donot et al. 2012; Freitas et al. 2011). However, despite these diverse industrial applications, EPSs still represent only a small fraction of the current polymer market, mostly due to the processes associated with their costly production and recovery (Kreyenschulte et al. 2014). Hence, significant effort has been devoted to the development of efficient downstream processing and cost-effective EPS production processes such as investigating the potential use of biomass resources as cheaper fermentation substrates.

In this chapter, after a brief description of microbial polysaccharides, submerged and solid-state fermentation processes utilizing cheap biomass resources are discussed with a special focus on the microbial production of EPSs with high market value.

2 Microbial Extracellular Polysaccharides

Polysaccharides are high-molecular-weight carbohydrate polymers of sugar residues that are linked by glycosidic bonds. While some polysaccharides like starch or glycogen serve for energy storage, others like chitin or cellulose function as structural support. In microorganisms, they are present either at the outer membrane as lipopolysaccharides (LPS) that mainly determine the immunogenic properties or secreted as capsular polysaccharides (CPSs) forming a discrete surface layer (capsule) associated with the cell surface or excreted as extracellular polysaccharides (EPSs) that are only loosely connected with the cell surface (Cuthbertson et al. 2009). While CPSs are responsible for pathogenicity like resistance to specific and nonspecific host immunity and adherence (Taylor and Roberts 2005), EPSs are related with adhesion, cell-to-cell interactions, biofilm formation (Mann and Wozniak 2012), and cell protection against environmental extremes (Kumar et al. 2007).

These microbial polysaccharides exhibit considerable diversity in their composition and structure. Based on their monomeric composition, they are generally classified as homopolysaccharides and heteropolysaccharides (Sutherland 1982).

Whereas homopolysaccharides consist of one type of monosaccharide connecting each other with either linear chains (pullulan, levan, bacterial cellulose, or curdlan) or ramified chains (dextran), heteropolysaccharides consist of two or more types of monosaccharides being usually present as multiple copies of oligosaccharides, containing three to eight residues (xanthan or gellan) (Purama et al. 2009; Sutherland 2007). Among the EPS producer microorganisms, bacteria are dominant for industrial or technical production. Some bacteria species such as *Xanthomonas*, *Leuconostoc*, *Sphingomonas*, and *Alcaligenes* which produce xanthan, dextran, gellan, and curdlan are the best known and most industrially used (Toksoy Oner 2013).

Dextran, curdlan, and cellulose are neutral bacterial glucans that are homopolysaccharides of glucose monomers. Dextran is produced by *Leuconostoc mesenteroides* cultures (Nasab et al. 2010a) together with levan (Siddiqui et al. 2014). Commercial applications for dextran are generally in the pharmaceutical industry, but new applications are being considered in the food and textile industries (Nasab et al. 2010a; Sarwat et al. 2008). Curdlan produced by the alkaline-tolerant mesophilic bacteria *Alcaligenes faecalis* (Matsushita 1990) can form aqueous suspensions which can form high-set gels upon heating. Curdlan can be used as a gelling agent in food and pharmaceutical industries. Curdlan is also produced by *Cellulomonas flavigena* as an extracellular storage polymer (Kenyon and Buller 2002). On the other hand, bacterial cellulose is produced by many species of bacteria, such as those in the genera *Gluconacetobacter*, *Agrobacterium*, *Aerobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*, *Salmonella*, *Enterobacter*, *and Escherichia* and several species of cyanobacteria. Contrary to plant cellulose, bacterial cellulose has many desirable properties such as high purity (free of lignin and hemicelluloses), high crystallinity, high degree of polymerization, a nanostructured work, high wet tensile strength, high water holding capacity, and good biocompatibility (Hungund et al. 2013).

Pullulan from *Aureobasidium pullulans* (Özcan et al. 2014; Singh et al. 2008) and scleroglucan produced by *Sclerotium glucanicum* (Survase et al. 2007) are fungal glucans that are the most known uncharged homopolysaccharides having industrial production for a long time. Whereas the major market for pullulan is still in food industry, there are several reports for its potential applications in pharmaceutical, biomedical, and environmental remediation areas (Özcan et al. 2014). Due to its exceptionally high stability, the first application of scleroglucan was in the oil recovery; however, other applications in the cosmetic, pharmaceutical, and agriculture sectors have also been reported (Schmid et al. 2011; Survase et al. 2007).

Besides these glucan-type EPSs, levan is a linear, fructan-type homopolymer of fructose residues. It is a water-soluble, strongly adhesive, and film-forming EPS, which can be used not only in the food industry as an emulsifying, thickening, and encapsulating agent but also in medicine such as an immunomodulator and a blood plasma substitute (Kang et al. 2009; Silbir et al. 2014). Xanthan, which is after dextran, the second EPS to be approved by the FDA as a safe food ingredient, is an industrially important EPS produced by *Xanthomonas campesteris* through aerobic fermentation (Gunasekar et al. 2014; Palaniraj and Jayaraman 2011). It is a branched, anionic heteropolysaccharide composed of glucose, mannose, and

glucuronic acid monomers. Due to its exceptional rheological properties, xanthan has a considerable market. Another anionic EPS is the gellan gum that is a linear polymer of glucose, rhamnose, and glucuronic acid. Gellan produced by *Sphingomonas paucimobilis* (formerly *Pseudomonas elodea*) is gaining increasing attention due to its novel property of forming thermo-reversible gels and showing good stability over a wide pH range (3.5–8.0). Due to the diversity of its structure and properties, gellan gum has a great commercial potential for food, pharmaceuticals, and predominantly environmental bioremediation (Bajaj et al. 2007; Jin et al. 2003). Hyaluronan, composed of glucuronic acid and acetylglucosamine residues, is widely used in regenerative medicine and cosmetic applications due to its water binding and retention capacity and immune compatibility characteristics (Sutherland 2007). Another heteropolysaccharide is alginate that is an anionic polymer of glucuronic acid and mannuronic acid residues. Alginate produced by species of *Pseudomonas* and *Azotobacter* is widely used as a thickening, stabilizing, and gelifying agent in food, textile, paper, and pharmaceutical industries (Hay et al. 2014, 2009; Gaona et al. 2004).

3 Microbial EPS Production

Microbial EPS production is usually not confined to just one type of EPS as product but rather a mixture of different polymers, each being synthesized by a certain gene cluster. For example, *Pseudomonas aeruginosa* has the genetic capacity to produce three different EPSs, namely, Pel, Psl, and alginate, which are synthesized via different mechanisms (Hay et al. 2014; Franklin et al. 2011). Similarly, *L. mesenteroides* cultures can produce both dextran and levan (Siddiqui et al. 2014). Generally, the availability of the precursors encoded by the related genes has a high impact on the yield and structure of the EPS excreted by the cell (Sutherland 2007). The synthesis of homopolysaccharides is carried out in the extracellular environment through the action of specifically secreted glycoside hydrolase (sucrase) enzymes that act on sucrose and catalyze the transglycosylation reactions forming the polymer chain (Rehm 2009; van Hijum et al. 2006). On the other hand, biosynthetic pathways of heteropolysaccharides are more complex and involve five distinct steps, namely, the uptake of sugar subunits and their activation with a high-energy bond through their conversion into sugar nucleotides, assembly of the repeating monosaccharide unit on an isoprenoid lipid carrier by sequential transfer of monosaccharides from sugar nucleotides by glycosyltransferases, addition of any acyl groups, polymerization of the repeating unit, and secretion of the polysaccharide from the cell membrane into the extracellular environment (Sutherland 2007).

Recent studies on microbial EPS production use systems-based approaches to elucidate the associated biosynthesis mechanisms; to modify physicochemical and/or rheological properties of the biopolymer by changing its composition, length, or degree of branching; and also to improve the microbial productivity via strain improvement strategies. For such an approach, whole genome sequencing (WGS) projects employing next-generation sequencing (NGS) technologies play a

central role by enabling high-throughput genomic data at very high speed with a relatively low cost. Genome data was used in comparative genomic studies of EPS biosynthesis by *Bifidobacterium* (Hidalgo-Cantabrana et al. 2013), *Crocosphaera watsonii* (Bench et al. 2013), and *Salipiger mucosus* DSM 16094^T (Riedel et al. 2014) as well as in functional genomic studies for the xanthan production by *Xanthomonas campestris* (Vorhölter et al. 2008), EPS biosynthesis by *P. aeruginosa* PA01 (Franklin et al. 2011; Hay et al. 2010; Rehm 2005), alginate biosynthesis by *Azotobacter vinelandii* (Kumar et al. 2007), and levan production by the halophilic strain *Halomonas smyrnensis* AAD6^T (Ates et al. 2011, 2013).

4 Microbial Production Processes

Microbial EPS production is significantly affected by fermentation conditions such as temperature, pH, oxygen concentration, bioreactor configuration, and culture medium. Furthermore, the chemical structure of EPS such as molecular weight, monomer composition, and physicochemical and rheological properties of the final product could also change with the type and age of strain (Özcan et al. 2014). Generally, typical microbial EPS fermentations start with the growth phase followed by the production phase (Toksoy Oner 2013). During the cultivations, severe changes in rheological properties of the microbial culture such as highly viscous and non-Newtonian broth may result in serious problems of mixing, heat transfer, oxygen supply, and also instabilities in the quality of the end product (Seviour et al. 2011). Such challenges are encountered in the microbial production of pullulan (Cheng et al. 2011) and xanthan (Palaniraj and Jayaraman 2011) but not in the production of low-viscosity polymers such as levan (Küçükaşık et al. 2011) or in high-temperature processes where thermophiles are utilized as microbial producers (Nicolaus et al. 2010).

Conventional modes of operations for the fermentation processes are batch, fed batch, and continuous. In these operations, different fermenter configurations can be used such as submerged fermentation (SmF) bioreactors including pneumatically or mechanically agitated types as well as solid-state fermentation (SSF) bioreactors. The mode of operation and the fermenter design depend on the microbial system used, and since the production is highly subject to change with the physiological and biochemical requirements of the microbial strain, each process requires a specific design by avoiding generalities (Nicolaus et al. 2010). Hence, to realize the industrial production of EPSs with the desired specifications and standardization, the parameters affecting the process should be well defined via effective optimization methods such as response surface methodology (Özcan et al. 2014).

5 Biomass Resources

Almost 30 % of the cost for a microbial fermentation can be represented by fermentation medium. Using complex media is not attractive due to its high content of expensive nutrients such as yeast extract, peptone, and salts. In order to reach

high production titers at reasonable costs, the fermentation medium should be carefully designed to make the end product compatible with its synthetic petrochemical counterpart. Until the 1990s, studies were concentrated on the recovery and chemical characterization of pure EPSs, and consequently, to avoid impurities, fermentations were preferentially performed under defined culture conditions. However, recently, studies are mainly concerned about the economical aspects of microbial production, and hence a growing number of studies focus on maximizing the cost-effectiveness of the process by replacing synthetic or complex media with appropriate biomass resources as cheaper alternatives (Kaur et al. 2014; Toksoy Oner 2013).

Biomass resources are generally of two physical states, namely, liquid resources like syrups, molasses, juices, cheese whey, and olive mill wastewater and solid resources like lignocellulosic biomass and pomaces. Whereas EPS production from liquid biomass resources is realized through submerged fermentation processes, solid-state fermentation is preferred for the latter state. Furthermore some physical and chemical pretreatment methods can also be applied to biomass resources for removal of heavy metals, colored substances, and other impurities that would interfere with the fermentation or subsequent downstream processing. In Table 1, various biomass resources have been listed for some microbial EPS producers together with the EPS yields obtained after a certain fermentation period.

6 Submerged Fermentation Processes

Submerged fermentation (SmF) processes define fermentations carried out in liquid media. The scale of the process may vary from small scale like shake flasks up to large scale like fermenters. Fermenters designed for SmF can be classified according to their agitation so that they are either pneumatically agitated such as bubble column and airlift bioreactors or mechanically agitated such as stirred tank reactors (Doran 1995). Syrups, molasses, cheese whey, and olive mill wastewater are common biomass resources for microbial production of EPS via SmF.

6.1 Syrups and Molasses

Syrups and molasses have been used as substrates for fermentative production of commercial polysaccharides such as xanthan (Kalogiannis et al. 2003), pullulan (Israilides et al. 1998; Roukas 1998; Lazaridou et al. 2002; Göksungur et al. 2004), dextran (Vedyashkina et al. 2005), levan (Küçükaşık et al. 2011; Han and Watson 1992; Oliveira et al. 2007), scleroglucan (Survase et al. 2007), and gellan (Banik et al. 2007) due to their many advantages like high sucrose and other nutrient contents, low cost, and easy availability. The chemical composition of syrups and molasses includes high ion concentrations such as K^+, Na^+, Fe^{2+}, and Zn^{2+} which could be additional stress factors that trigger the formation of the EPS (Abdel-Aziz et al. 2012a). Not only crude form of syrups and molasses but also pretreated form

Table 1 Biomass resources and applied fermentation types for some microbial EPSs

EPS	Microorganism	Biomass resource	Fermentation type	Yield (time)	Reference
Curdlan	*Agrobacterium* sp. ATCC 31749	Condensed corn solubles	SmF	7.72 gL^{-1} (120 h)	West and Nemmers 2008
Dextran	*L. mesenteroides* NRRL B512	Carob extract	SmF	8.56 gL^{-1} (12 h)	Santos et al. 2005
Dextran	*L. mesenteroides* NRRL B512	Carob extract and cheese whey	SmF	7.23 gL^{-1} (12 h)	Santos et al. 2005
Dextran	*L. mesenteroides* V-2317D	Sugar beet molasses	SmF	50 gL^{-1} (9 days)	Vedyashkina et al. 2005
Dextran	*L. Mesenteroides* NRRL B512	Combination of molasses and cheese whey	SmF	9.51 gL^{-1} (48 h)	Nasab et al. 2010a
Gellan	*S. paucimobilis* ATCC-31461	Sugar beet molasses	SmF	13.81 gL^{-1} (48 h)	Banik et al. 2007
Gellan	*S. paucimobilis* NK2000	Soybean pomace	SmF	7.33 gL^{-1} (3 days)	Jin et al. 2003
Levan	*Halomonas* sp. AAD6	Starch molasses	SmF	12.4 gL^{-1} (210 h)	Küçükaşık et al. 2011
Levan	*Paenibacillus polymyxa* NRRL B-18475	Sugar beet molasses	SmF	38.0 gL^{-1} (5 days)	Han and Watson 1992
Levan	*P. polymyxa* NRRL B-18475	Sugarcane syrup	SmF	19.6 gL^{-1} (5 days)	Han and Watson 1992
Levan	*Zymomonas mobilis* ATCC 31821	Sugarcane molasses	SmF	2.53 gL^{-1} (24 h)	Oliveira et al. 2007
Levan	*Z. mobilis* ATCC 31821	Sugarcane syrup	SmF	15.5 gL^{-1} (24 h)	Oliveira et al. 2007
Pullulan	*Aureobasidium* sp. NRRLY	Condensed corn solubles	SmF	4.5 gL^{-1} (9 days)	Leathers and Gupta 1994
Pullulan	*A. pullulans* SU-M18	Carob extracts	SmF	6.5 gL^{-1} (3 days)	Roukas and Biliaderis 1995
Pullulan	*A. pullulans*	Olive mill wastewater	SmF	8 gL^{-1}	Cormenzana et al. 1995
Pullulan	*A. pullulans* NRRLY-6220	Olive mill wastewater	SmF	10.7 gL^{-1} (7 days)	Israilides et al. 1998

Pullulan	*A. pullulans* NRRLY-6220	Grape pomace	SmF	22.3 gL^{-1} (7 days)	Israilides et al. 1998
Pullulan	*A. pullulans* NRRLY-6220	Sugar beet molasses	SmF	6.0 gL^{-1} (7 days)	Israilides et al. 1998
Pullulan	*A. pullulans*	Sugar beet molasses	SmF	32.0 gL^{-1}	Roukas 1998
Pullulan	*A. pullulans* P 56	Sugar beet molasses	SmF	24 gL^{-1} (144 h)	Lazaridou et al. 2002
Pullulan	*A. pullulans* P 56	Sugar beet molasses	SmF	35 gL^{-1} (96 h)	Göksungur et al. 2004
Pullulan	*A. pullulans* RBF 4A3	Corn steep liquor	SmF	77.92 gL^{-1} (96 h)	Sharma et al. 2013
Pullulan	*A. pullulans* RBF 4A3	De-oiled jatropha seed cake	SmF	83.98 gL^{-1} (120 h)	Choudhury et al. 2012
Scleroglucan	*Sclerotium rolfsii* MTCC 2156	Sugarcane juice	SmF	23.87 gL^{-1} (72 h)	Survase et al. 2007
Scleroglucan	*S. rolfsii* MTCC 2156	Sugarcane molasses	SmF	19.21 gL^{-1} (72 h)	Survase et al. 2007
Scleroglucan	*S. rolfsii* MTCC 2156	Coconut water	SmF	12.58 gL^{-1} (72 h)	Survase et al. 2007
Scleroglucan	*S. rolfsii* MT-6	Waste loquat kernel	SmF	12.08 gL^{-1} (72 h)	Taşkın et al. 2010
Scleroglucan	*S. glucanicum* NRRL 3006	Condensed corn solubles	SmF	14.8 gL^{-1} (144 h)	Fosmer and Gibbons 2011
Xanthan	*Xanthomonas campestris*	Carob extracts	SmF	0.126 gL^{-1} h^{-1}	Roseiro et al. 1992
Xanthan	*X. campestris* PD 656	Apple pomace	SSF	52.1 gL^{-1} (6 days)	Stredansky and Conti 1999
Xanthan	*X. campestris*	Grape pomace	SSF	10 gL^{-1} (6 days)	Stredansky and Conti 1999
Xanthan	*X. campestris* PD 656	Tangerine peels	SSF	32.9 gL^{-1} (6 days)	Stredansky and Conti 1999
Xanthan	*X. campestris* NRRL-B-1459	Sugar beet pulp	SmF	1.19 gL^{-1} (4 days)	Yoo and Harcum 1999

(*continued*)

Table 1 (continued)

EPS	Microorganism	Biomass resource	Fermentation type	Yield (time)	Reference
Xanthan	*X. campestris* NRRL-B-1459	Olive mill wastewater	SmF	4 gL^{-1} (5 days)	Lopez et al. 2001a
Xanthan	*X. campestris* T646	Olive mill wastewater	SmF	7.7 gL^{-1} (5 days)	Lopez et al. 2001b
Xanthan	*X. campestris* EBK-4	Ram horn hydrolysate	SmF	25.6 gL^{-1} (48 h)	Kurbanoglu and Kurbanoglu 2007
Xanthan	*X. campestris* 1182	Cheese whey	SmF	26.35 gL^{-1} (72 h)	Silva et al. 2009
Xanthan	*X. campestris* PTCC 1473	Date syrup	SmF	8.9 gL^{-1} (96 h)	Nasab et al. 2009
Xanthan	*X. campestris* NRRL-B-1459	Sugar beet molasses	SmF	9.02 gL^{-1} (120 h)	Nasab et al. 2010b
Xanthan	*Xanthomonas* sp.	Potato peel	SSF	2.9 g/50 g peel (6 days)	Vidhyalakshmi et al. 2012
Xanthan	*X. campestris* NCIM 2954	Tapioca pulp	SmF	7.1 gL^{-1} (72 h)	Gunasekar et al. 2014
Bacterial cellulose	*Gluconacetobacter hansenii* CGMCC 3917	Waste beer yeast	SmF	7.02 gL^{-1} (10 days)	Lin et al. 2014
Bacterial cellulose	*Gluconacetobacter xylinus* PTCC 1734	Date syrup	SmF	43.5 gL^{-1} (336 h)	Nasab and Yousefi 2011
Bacterial cellulose	*Gluconacetobacter persimmonis*	Watermelon	SmF	5.98 gL^{-1} (14 days)	Hungund et al. 2013
Bacterial cellulose	*G. persimmonis*	Orange juice	SmF	6.18 gL^{-1} (14 days)	Hungund et al. 2013
Bacterial cellulose	*G. Persimmonis*	Muskmelon	SmF	8.08 gL^{-1} (14 days)	Hungund et al. 2013
β-Glucan	*Botryosphaeria rhodina*	Olive mill wastewater	SmF	17.2 gL^{-1} (120 h)	Crognale et al. 2003
EPS	*Paenibacillus jamilae* CECT 5266	Olive mill wastewater	SmF	2.5 gL^{-1} (100 h)	Morillo et al. 2009

EPS	*P. jamilae* CP-38	Olive mill wastewater	SmF	5 gL^{-1} (72 h)	Aguilera et al. 2008
EPS	*Halomonas* sp. AAD6	Sugar beet pulp	SSF	2.22 gL^{-1} (3 days)	Söğütçü et al. 2011
EPS	*Bacillus subtilis*	Sugarcane molasses	SmF	4.86 gL^{-1} (48 h)	Razack et al. 2013
EPS	*P. fluorescens*	Sugarcane molasses	SmF	2.9 gL^{-1} (48 h)	Sirajunnisa et al. 2012
EPS	*Streptococcus Thermophilus* BN1	Skimmed milk	SmF	548 mgL^{-1} (17 h)	Rabha et al. 2012
EPS	*S. Thermophilus* BN1	Whole milk	SmF	325 mgL^{-1} (17 h)	Rabha et al. 2012
EPS	*S. Thermophilus* BN1	Cheese whey	SmF	375 mgL^{-1} (17 h)	Rabha et al. 2012
EPS	*Paenibacillus jamilae* CECT 5266	Two-phase olive mill waste	SmF	2 gL^{-1} (5 days)	Morillo et al. 2006
EPS	*Grifola frondosa* MBFBL 662 and MBFBL 21	Oak sawdust	SSF	3.5 gL^{-1} (45 days)	Mikiashvili et al. 2011
EPS	*Bacillus licheniformis* UD061	Squid processing by-product and maize cob	SSF	14.68 mg/gds	Fang et al. 2013
EPS	*Pleurotus eryngii*	Mushroom hydrolysate powder	SmF and SSF	312 mgL^{-1} (8 days)	Chen et al. 2013
EPS	*Morchella esculenta*	Detoxified loquat kernel extract	SSF	5.2 gL^{-1} (3 days)	Taşkın et al. 2011
EPS	*M. esculenta*	Loquat kernel extract	SSF	4.1 gL^{-1} (3 days)	Taşkın et al. 2011
EPS	*M. esculenta*	Chicken feather hydrolysate	SmF	4.8 gL^{-1}	Taşkın et al. 2012

of syrups and molasses can be used as substrate. Some pretreatment methods for syrups and molasses are acid treatment, pH adjustment (Roukas 1998; Küçükaşık et al. 2011), activated carbon treatment (Lazaridou et al. 2002; Göksungur et al. 2004), ion exchange chromatography (Kalogiannis et al. 2003), and centrifugation followed by filtration (Oliveira et al. 2007).

X. campestris was used for producing xanthan gum using date syrup, prepared from low-quality dates, as a substrate. Fermentation was carried out with date syrup and sucrose syrup. The results showed that EPS concentration increased with an increase in fermentation time with a maximum yield of 8.9 gL^{-1} after 96 h which was much higher than that of the sucrose-containing medium (0.18 g/100 mL) (Nasab et al. 2009). The same group used date syrup, for the production of bacterial cellulose using *Gluconacetobacter xylinus*. Static batch fermentation for the purpose of cellulose production by *G. Xylinus* PTCC 1734 was studied using date syrup and sucrose solution as fermentation media. Results showed that maximum yields of bacterial cellulose after 336 h fermentation were 4.35 and 1.69 g/100 ml of date syrup and sucrose media, respectively (Nasab and Yousefi 2011). Fruit juices are also a good alternative as biomass resource like syrups. For instance, bacterial cellulose was produced by *Gluconacetobacter persimmonis* using some fruit juices as cheaper carbon sources (Hungund et al. 2013). Hungund et al. used various fruit juices including pineapple, pomegranate, muskmelon, water melon, tomato, and orange and also molasses, sugarcane juice, coconut water, and coconut milk as alternative carbon sources for bacterial cellulose production. Out of which, muskmelon juice gave the highest cellulose yield of 8.08 gL^{-1}. Survase et al. (2007) used various dilutions of coconut water, sugarcane molasses, and sugarcane juice which were not subjected to any pretreatment methods before their use for the scleroglucan production by filamentous fungi *S. rolfsii* MTCC2156, and the highest yields (23.87 gL^{-1} in 72 h) were observed using sugarcane juice. Coconut water and sugarcane juice were also used for EPS production by *Lactobacillus confusus* cultures (Seesuriyachan et al. 2011).

Muhammadi (2014) used different carbon sources such as glucose, fructose, sucrose, sugar beet, and sugarcane molasses to produce EPS by *Bacillus* strain CMG1403. The study showed that under optimum culture conditions, sugar beet and sugarcane molasses could be superior and efficient alternatives to synthetic carbon sources providing way for an economical production of EPS. Razack et al. (2013) replaced sucrose with sugarcane molasses in the optimized medium, for an enhanced production of EPS from a soil isolate, *Bacillus subtilis*. Sugarcane molasses at a concentration of 2 % gave higher EPS yield (4.86 gL^{-1}) than that obtained in medium with sucrose (2.98 g EPS/L). Sugarcane molasses was also used to produce EPS from *P. fluorescens*. Sucrose and sugarcane molasses as the carbon substrates at different concentrations (1–7 %) and different incubation times were investigated in this study. Maximum production was obtained in the medium containing 5 % sugarcane molasses and was found to be 2,843 mgL^{-1} at 48 h after which the production decreased. The EPS production using sugarcane molasses gave comparatively a higher yield than sucrose, which could be commercialized for

a cost-effective production of these viscous to plastic polymers (Sirajunnisa et al. 2012). Banik et al. (2007) used response surface methodology to optimize the production of gellan gum by *S. paucimobilis* ATCC-31461 using crude sugarcane molasses and reported a maximum yield of 13.81 gL^{-1} gellan. Abdel-Aziz et al. (2012b) stated that EPS, synthesized by the fungus *Mucor rouxii*, was found to play an important role for the protection of cells against abiotic stress such as extreme pH values or elevated temperature. An acidic pH-shock was found to be the strongest stressor for synthesizing the EPS exploiting beet molasses as an inexpensive carbon source. Beet molasses was also used as a carbon source in other studies to produce xanthan by *X. campesteris* NRRL-B-1459 (Nasab et al. 2010b) and to produce dextran by *L. mesenteroides* (Vedyashkina et al. 2005).

6.2 Cheese Whey

Whey is the major by-product of the dairy products, especially cheese, industry. The nutrient composition of whey is based on the nutrient composition of milk from which it is derived, and it is affected by many factors including how the milk was processed. The major component of whey is lactose which is about 70 % of the total solids of whey. Whey has a rich pool of nutrients and growth factors that have the potential to stimulate the growth of microorganisms. On the other hand, the suitability of whey for EPS production highly depends on the ability of the microorganism to utilize lactose (Toksoy Oner 2013).

Fialho et al. (1999) used the media containing lactose, glucose, and sweet cheese whey as substrates for the production of gellan by *S. paucimobilis* ATCC 31461. The strain was known to grow on lactose and to produce highly viscous gellan directly from lactose (Pollock 1993). Silva et al. (2009) produced xanthan by two strains of *X. campesteris* using cheese whey as carbon source. Although both strains reached comparable yields, the polymers were found to differ in their chemical characteristics such as chemical composition and ionic strength. Nasab et al. (2010a) used a combination of molasses and cheese whey for the production of dextran by *L. mesenteroides*. Results showed that maximum dextran yield was achieved in combination of molasses-whey 10 % (M-W 10 %) and no dextran was produced using only whey. The report showed that the combination of nutrients and minerals in molasses and cheese increased the EPS yield.

Not only crude whey but also partially hydrolyzed whey by protease/peptidase complex can be used for the production of EPS. Fermentation of the hydrolyzed whey using *Lactobacillus delbrueckii* ssp. *bulgaricus* RR (RR, an EPS-producing bacterium) resulted higher EPS yield than fermentation of the unhydrolyzed whey (Briczinski and Roberts 2002). Another pretreatment method for cheese whey is precipitation of protein. Rabha et al. (2012) partially precipitated the residues of milk proteins present in the whey samples in their study which is related to EPS production by *Streptococcus thermophilus* BN1 using skimmed milk, whole milk, and cheese whey as cheap culture media.

6.3 Olive Mill Wastewater

Olive mill wastewater (OMW) is the by-product of the olive oil industry. OMW is a dark-colored juice that consists of a mixture of water from the olive, machinery cooling waters, fruit washings, and remainder of the fruit (Toksoy Oner 2013). About 15 % of OMW is organic material that is composed of carbohydrates, proteins, and lipids as well as a number of other organic compounds including monoaromatic and polyaromatic molecules (Aguilera et al. 2008). OMW also comprises toxic ingredients being mainly derived from its extremely high organic load and the presence of recalcitrant organic compounds such as polyphenols with strong antimicrobial properties. Valorization of OMW produced by the olive oil industry has long been an environmental concern in the Mediterranean countries (Morillo et al. 2009). Among several conventional technological treatment methods applied, biovalorization of OMW to value-added chemicals is considered as the most cost-effective and environmentally compatible alternative (Mantzavinos and Kalogerakis 2005). OMW has been used as a suitable substrate for the production of EPS such as pullulan (Ramos-Cormenzana et al. 1995) and xanthan (Lopez and Ramos-Cormenzana 1996) due to its composition with high carbon-to-nitrogen ratio and other valuable nutrients.

Some pretreatment methods which can be applied before microbial fermentation to reduce the inhibitory effect of phenols in OMW, are filtration, clarification by centrifugation, dilution with water, and saline neutralization (Lopez et al. 2001a; Crognale et al. 2003). Lopez et al. (2001b) used OMW as a substrate to the production of xanthan using four *X. campesteris* strains. Differences among strains were found in the range of tolerance to OMW concentration and the xanthan amount obtained. *X. campestris* NRRL-B-1459 S4LII was chosen by its capability for xanthan production from 50 % to 60 % OMW as the sole nutrient source.

Morillo et al. (2006) investigated the use of a two-phase olive mill waste (TPOMW) which is a thick sludge that contains water and pieces of pit and pulp of the olive fruit as substrate for the production of EPS by *Paenibacillus jamilae* which is able to grow and produce EPS in aqueous extracts of TPOMW as a unique source of carbon. Maximal polymer yield in 100-mL batch-culture experiments (2 gL^{-1}) was obtained in cultures prepared with an aqueous extract of 20 % TPOMW (w/v). The effects of the addition of inorganic nutrients (nitrate, phosphate, and other inorganic nutrients) were also investigated, but the nutrient supplementation did not increase yield.

In addition to EPS production, some microorganisms can be used for bioremediation of OMW. The *Paenibacillus* genus having the high phenol biodegradation ability was not only proposed for the production of a metal-binding EPS that could be used as a biofilter but also for the bioremediation of OMW (Aguilera et al. 2008).

The high amount of phenols which negatively affects the microbial fermentation is the main constraint associated with the use of OMW, and dilution of OMW can be required. On the other hand, dilution of OMW limits the concentration of the used waste as culture medium (Aguilera et al. 2008). For instance, undiluted OMW was found to be a poor substrate for pullulan production by *A. pullulans* (Israilides et al. 1998).

6.4 Others

Carob which is grown on carob tree (*Ceratonia siliqua* L.) in the Mediterranean region has recently found its place in the food industry as a biomass substrate due to its very high sugar content (Turhan et al. 2010). Pretreated carob extracts with 25 gL^{-1} initial sugar content were used for pullulan production by *A. pullulans* SU-M18, and a pullulan productivity of 2.16 gL^{-1}/day was reached (Roukas and Biliaderis 1995). Carob extract can also be used for dextran production. Santos et al. used carob pod residues pretreated by milling and extracting by use of an acetate buffer to get its sugar content. Then carob extracts were used for the production of dextran by *L. mesenteroides* NRRL B512. 8.56 gL^{-1} dextran was produced within 12 h of the fermentation period in this study (Santos et al. 2005).

Waste beer yeast (WBY) being the second major by-product from the brewing industry can be used for bacterial cellulose production by *Gluconacetobacter hansenii* CGMCC 3917. Lin et al. (2014) used pretreated WBY as the only nutrient source for bacterial cellulose production. WBY hydrolysates treated by ultrasonication gave the highest bacterial cellulose yield (7.02 gL^{-1}), almost six times as that from untreated WBY (1.21 gL^{-1}). Tapioca pulp was used as a carbon source for xanthan production by *X. campestris* NCIM 2954. Maximum 7.1 gL^{-1} xanthan was produced using sulfuric acid-pretreated tapioca pulp (Gunasekar et al. 2014). Condensed corn solubles (CCS) containing changing levels of carbohydrates, proteins, vitamins, and nutrients are a by-product of the bioethanol industry (Smith et al. 2008). The use of diluted CCS was reported for the production of scleroglucan by *S. glucanicum* (Fosmer and Gibbons 2011; Fosmer et al. 2010), the poly-(3-hydroxybutyrate-*co*-3-hydroxyvalerate) by *Rhodospirillum rubrum* (Smith et al. 2008), pullulan by *Aureobasidium* sp. strain NRRL Y-12974 (Leathers and Gupta 1994), and curdlan by *Agrobacterium* sp. ATCC 31749 (West and Nemmers 2008). Mushroom hydrolysate powder (MHP) was used as a nitrogen source for mycelial biomass and EPS productions by *Pleurotus eryngii*. MHP gave higher mycelial biomass growth rate and EPS yield than those of the yeast extract (Chen et al. 2013). Waste loquat kernel is another potential biomass resource for EPS production due to its high protein and carbohydrate content Taşkın et al. 2010).

Pullulan is one of the most producing EPSs using various ranges of biomass resources. Abdel Hafez et al. (2007) produced pullulan by *A. pullulans* ATCC 42023 using molasses, cellulosic wastes, potato starchy waste, glucose syrup, sweet whey, and corn steep liquor. Maximum pullulan concentration (65.3 gL^{-1}) was obtained after 5 days of fermentation at 28 °C using 7 % corn steep liquor as the sole nitrogen source in a medium containing 20 % sucrose. Sharma et al. (2013) also used rice bran oil cake, soya bean oil cake, cotton seed oil cake, and mustard seed oil cake corn steep liquor (CSL) as agro-industrial nitrogen source for the production of pullulan by *A. pullulans* RBF 4A3. CSL was found to be the best production of 77.92 gL^{-1} pullulan under un-optimized conditions. Choudhury et al. (2012) produced pullulan using another nitrogen source, namely, de-oiled jatropha seed cake (DOJSC). Under optimized condition, 8 % DOJSC with 15 % dextrose gave 83.98 gL^{-1} of pullulan which is comparatively a higher yield than current reports.

Some animal wastes such as ram horn hydrolysates and chicken feather have been also reported to be a substrate for EPS production. Taşkın et al. (2012) investigated the usability of chicken feather hydrolysate (chicken feather peptone (CFP)) as substrate for mycelial biomass and EPS production from edible mushroom *Morchella esculenta*. Maximum 15.9 gL^{-1} biomass and 4.8 gL^{-1} EPS were obtained using CFP in this study. Kurbanoglu et al. (Kurbanoglu and Kurbanoglu 2007) used ram horn hydrolysates for xanthan production by *X. campestris* EBK-4 due to their high amino acid and mineral content.

7 Solid-State Fermentation Processes

Solid-state fermentation (SSF) defines bioprocess technologies including the growth of microorganisms on moist solid particles (Aydınoğlu and Sargın 2013). Over the last two decades, SSF has gained significant attention for the development of industrial bioprocesses, particularly due to lower energy requirement associated with higher product yields and cheap and eco-friendly process conditions (Thomas et al. 2013). The substrates used in SSF processes are often the product, by-product, or waste of agro-industrial, forestry, or food processing (Mitchell and Krieger 2006). SSF has been recently explored for the production of biopolymers such as EPS of which yields are comparable to those obtained from conventional submerged cultivation (Thomas et al. 2013). Agro-industrial wastes like lignocellulosic biomass and pomaces are common biomass resource used in SSF for production of EPS.

7.1 Lignocellulosic Biomass

Lignocellulosic biomass is a cheap and abundant substrate for EPS production in SSF, especially for microbial systems with hydrolytic capability via endoglucanases or cellobiose. Otherwise, it is utilized to a limited extend during the fermentation and hence requires pretreatments beforehand (Toksoy Oner 2013). Mikiashvili et al. (2011) investigated 14 strains of *Grifola frondosa* for lignin degradation, ligninolytic enzyme activities, protein accumulation, and EPS production. Experiments were carried out in SSF using oak sawdust as substrate. Among 14 strains, the strains MBFBL 21, MBFBL 662, and MBFBL 638 appeared to be good producers of EPS (3.5, 3.5, and 3.2 gL^{-1}, respectively). *Lentinus squarrosulus* Mont., a high-temperature-tolerant white rot fungus, is attracting attention due to its rapid mycelia growth and lignocellulolytic enzyme activity. Isikhuemhen et al. used cornstalks as carbon source to evaluate lignocellulolytic enzyme activity and EPS production of *L. squarrosulus* MBFBL 201 in SSF. The results showed that *L. squarrosulus* was able to degrade cornstalks significantly and maximum 5.13 gL^{-1} EPS could be recovered from the fermentation media (Isikhuemhen et al. 2012). Chowdhury et al. (2012) used lignocellulosic fibers of jute with 58–63 % cellulose content as a carbon source for EPS production by *Bacillus*

megaterium RB-05 cells with known cellulase activity in SSF. Considerable cellulase activity and maximum polymer yield of 0.297 g g^{-1} substrate were found after 72 h fermentation in this study.

7.2 Pomace

Pomace is also a common substrate for microorganism fermentation in SSF due to its content of pectin, crude fiber, and minerals such as K, Mg, Fe, and Mn (Shalini and Gupta 2010). Globally, about 10 million tons of grape pomace (seeds, skin, and stem) and 1 million tons of apple pomace are produced each year (Toksoy Oner 2013; Shalini and Gupta 2010). Stredansky and Conti (1999) produced xanthan by *X. campesteris* strains in SSF using agro-industry wastes or by-products, including spent malt grains, apple pomace, grape pomace, and citrus peels as solid substrate. Yields of the xanthan ranging from 32.9 to 57.1 gL^{-1} revealed a composition consistent with those of commercial xanthan analyzed by NMR spectroscopy. Xanthan was also produced by *X. campesteris* strains in SSF using potato peel as substrate in another study (Vidhyalakshmi et al. 2012). The ammonium nitrate is a nitrogen source for the production of gellan gum by *S. paucimobilis* NK2000. The production of gellan gum by *S. paucimobilis* NK2000 significantly increased using soybean pomace as a nitrogen source instead of ammonium nitrate (Jin et al. 2003). Using soybean pomace as nitrogen source also increased pullulan production by *A. pullulans* HP-2001 compared to using yeast extract as a nitrogen source (Seo et al. 2004).

7.3 Others

Moussa and Khalil (2012) used SSF for the production of dextran by *Saccharomyces cerevisiae* using date seeds. Different concentrations of date seeds were investigated, and the highest dextran production was achieved at 6 g/flask. The purified dextran was comparable to commercial ones. Seesuriyachan et al. (2010) compared the production of EPS by a lactic acid bacteria, *Lactobacillus confuses*, in SSF and SmF using coconut water and sugarcane juice as renewable wastes with agar medium. High concentrations of EPS (62 and 18 gL^{-1} of sugarcane juice and coconut water with agar, respectively) were obtained in SSF. Fang et al. (2013) used response surface methodology to optimize physical and nutritional variables for the production of antioxidant EPSs by *Bacillus licheniformis* UD061 in SSF using squid processing by-products and maize cobs as a carbon and nitrogen source. Succinoglycan is also produced using SSF. Various solid substrates such as spent malt grains, ivory nut shaving, and grated carrots were used for the production of succinoglycan by *Agrobacterium tumefaciens* in SSF. The highest succinoglycan yield in SSF was 30 g EPS/kg solid in this study (Stredansky and Conti 1999). Taşkın et al. (2010) used detoxified loquat kernel extract (DLKE) and neutralized loquat kernel extract (LKE) prepared from waste loquat kernels as main carbon

sources in the submerged and solid cultures of *M. esculenta* for the production of EPS having various biologic and pharmacologic activities, including antitumor, immunostimulating, and hypoglycemic activities. Maximum EPS concentrations using DLKE and KLE were 5.2 and 4.1 gL^{-1}, respectively, in this study.

Whereas liquid sugar beet molasses is commonly used in SmF, solid sugar beet pulp (SBP) which is another by-product of the sugar beet industry can be used in SSF as biomass resource for EPS production. SBP is the fibrous material left over after the sugar is extracted from sugar beets and is mainly composed of cellulose, hemicellulose, and pectin (Toksoy Oner 2013). Yoo and Harcum investigated the feasibility of using pretreated SBP as a supplemental substrate for xanthan gum production from *X. campestris*, and they reported a production yield of 0.89 g xanthan per gram of SBP in 4 days of fermentation time (Yoo and Harcum 1999). Söğütçü et al. (2011) used SBP to produce EPS by halophilic *Halomonas* sp. AAD6 cultures. Some pretreatment methods such as milling, dialyzing, and autoclaving were reported to increase the EPS yields in this study.

8 Conclusion/Prospects

The constant increase in population and industrialization has created enormous quantities of industrial waste biomass and associated environmental and health hazards. Hence biomass management via biorefinery approach has become an important issue for sustainable development and economic competitiveness. The utilization of biomass requires intensive research activities for the development of feasible pretreatment, fermentation, and downstream processing techniques. The most challenging part in the whole process is to combine the production stream with the suitable waste stream.

Microbial EPS production is strictly dependent on the nutritional and environmental requirements of the microbial culture. In this respect, omics technologies and systems biology tools provide ample knowledge on the genetic capabilities of the microbial producer cultures; hence, by functional and comparative genomics, the most appropriate biomass resource leading to high polymer titers can be identified. Such systems-based approaches are expected to have an ever-increasing importance in microbial EPS production and in general industrial biotechnology.

Acknowledgments The financial support provided by TUBITAK through project 111M232 is gratefully acknowledged.

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