Production of microbial polysaccharides for use in food

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Abstract: Microbial polysaccharides comprise a large number of versatile biopolymers produced by several bacteria, yeast and fungi. Microbial fermentation has enabled the use of these ingredients in modern food and delivered polysaccharides with controlled and modifiable properties, which can be utilized as thickeners/viscosifiers, gelling agents, encapsulation and film-making agents or stabilizers. Recently, some of these biopolymers have gained special interest owing to their immunostimulating/therapeutic properties and may lead to the formation of novel functional foods and nutraceuticals. This chapter describes the origin and chemical identity, the biosynthesis and production process, and the properties and applications of the most important microbial polysaccharides.

Key words: biosynthesis, food biopolymers, functional foods and nutraceuticals, microbial polysaccharides, structure–function relationships.

16.1 Introduction

Microbial polysaccharides form a large group of biopolymers synthesized by many microorganisms, as they serve different purposes including cell defence, attachment to surfaces and other cells, virulence expression, energy reserves, or they are simply part of a complex cell wall (mainly in fungi). Many of them have been used for many years in the food industry and in human diet, either as an ingredient naturally present in food (e.g. in edible mushrooms or brewer's/baker's yeast) or mainly as a purified food additive recovered from microbial fermentation processes, as well as in pharmaceuticals (as bioactive compounds, or media for encapsulation and controlled drug release), cosmetics and other industrial applications, such as oil drilling and recovery, film formation, biodegradable plastic, tissue culture substrate, and other applications which go beyond the scope of this chapter (Sutherland, 1998). Their broad spectrum of applications is due to their diverse and modifiable properties as viscosifiers thickeners, gelling and film-forming

agents, stabilizers, texturizers and emulsifiers. In addition, research in recent years has revealed that some microbial polysaccharides posses significant immunomodulating properties (anti-tumour, anti-inflammatory, antimicrobial), or hypocholesterolaemic and hypoglycaemic properties, thus making them perfect candidates for use in 'functional foods' or 'nutraceuticals' (Giavasis and Biliaderis, 2006). The world market for this type of foods is currently expanding and scientific interest in this field is growing, as consumers realize the importance of food to the quality of life (Hardy, 2000).

In comparison with polysaccharides isolated from plant sources (carrageenan, guar gum, modified starch, cereal glucans, etc), which are also used for similar purposes, microbial polysaccharides have the advantages of well-controlled production processes in a large scale within a comparatively limited space and production time, stable chemical characteristics and unhindered availability in the market, as opposed to plant derivatives whose availability, yearly production and chemical characteristics often vary (Reshetnikov *et al.*, 2001). However, in some cases, high production costs, low polysaccharide yields, and tedious downstream processing needed for isolation and purification are still a matter of concern for microbial processes, and appropriate strategies for bioprocess optimization have to be adopted (Kumar *et al.*, 2007).

Apart from well-established microbial polysaccharides, such as xanthan, gellan, curdlan, pullulan or scleroglucan, many new polysaccharides from fungi, yeasts or bacteria emerge, as research on polysaccharide-producing strains continues and the properties and functionality of these biopolymers become better elucidated. The present chapter discusses the types and sources, the physicochemical and biological properties, and the applications of a number of well-established, commercial microbial polysaccharides, such as xanthan, gellan, alginate, curdlan, pullulan, scleroglucan and some less industrialized or less studied biopolymers such as elsinan, levan, alternan, microbial dextrans, lactic acid bacteria (LAB) polysaccharides and last but not least, mushrooms polysaccharides, such as lentinan, ganoderan, grifolan, zymosan, and soon.

16.2 Types, sources and applications of microbial polysaccharides

Microbial polysaccharides are found in many microorganisms, being part of the cell wall (such as fungal β-glucans), or serving as an energy reserve for the cell (such as polyhydroxybutyrate), or as a protective capsule or a slime-facilitating attachment to other surfaces (such as xanthan and gellan), the latter being characteristic of pathogens, especially plant pathogens (Giavasis *et al.*, 2000). Cell wall polysaccharides are generally difficult to isolate and purify, as cell lysis and fractionation are needed to remove other cell impurities prior to alcohol precipitation, while extracellular polysaccharides

(EPS), which are excreted out of the cell, can generally be separated by filtration or centrifugation which removes cells, followed by precipitation. The main producers of microbial polysaccharides are fungi of the Basidiomycetes family, and several Gram negative (*Xanthomonas, Pseudomonas, Alcaligenes*, etc) and Gram positive (LAB) bacteria. Some yeasts may also synthesize polysaccharides in significant quantities, mostly belonging to the *Saccharomyces* genus (Giavasis and Biliaderis, 2006).

16.2.1 Bacterial polysaccharides

Xanthan is probably the most common bacterial polysaccharide used as a food additive owing to its viscofying and stabilizing properties. It is produced by *Xanthmonas campestris*, a Gram negative plant pathogen which yields xanthan as a means of attachment to plant surfaces (Kennedy and Bradshaw, 1984). It was discovered in 1963 at Northern Regional Research Center of the United States Department of Agriculture (USDA) and commercial production for use in the food industry started soon after. Xanthan was approved by the United States Food and Drug Administration (FDA) for use in food additive without any quantity limitations, as it is non-toxic (Kennedy and Bradshaw, 1984). Xanthan comprises a linear (1,4) linked β-D-glucose backbone with a trisaccharide side chain on every other glucose at C-3, containing a glucuronic acid residue (1,4)-linked to a terminal mannose unit and (1,2)-linked to a second mannose of the backbone (Jansson *et al.*, 1975; Casas *et al.*, 2000). Its chemical structure is shown in Fig. 16.1. Its molecular weight ranges from 2000,000–20,000,000 Da (Daltons), depending on bioprocess conditions and the level of aggregation of individual chains (Casas *et al.*, 2000). Native xanthan is pyruvylated by 50% at the terminal mannose and acetylated at non-terminal mannose residues at C-6.

Xanthan has found multiple uses as a viscosifier and stabilizer in syrups, sauces, dressings, bakery products, soft cheese, restructured meat, and so on, where it is characterized by thermal stability even under acidic conditions, good freeze–thaw stability, and excellent suspending properties (Casas *et al.*, 2000; Sharma *et al.*, 2006; Palaniraj and Jayaraman, 2011). In bakery products xanthan gum is used to improve volume and texture (especially of gluten-free breads), water binding during baking and shelf life of baked foods, freeze–thaw stability of refrigerated doughs, to replace egg white in low calorie cakes and to increase flavour release and reduce syneresis in creams and fruit fillings (Sharma *et al.*, 2006). In dressings, sauces and syrups xanthan gum facilitates emulsion stability to acid and salt and a stable viscosity over a wide temperature range; it impart desirable body, texture and pourability and improved flavour release. In buttered syrups and chocolate toppings xanthan offers excellent consistency and viscosity and freeze–thaw stability (Sharma *et al.*, 2006; Rosalam and England, 2006). Xanthan is also an effective stabilizer and bodying agent in cream cheese where it improves

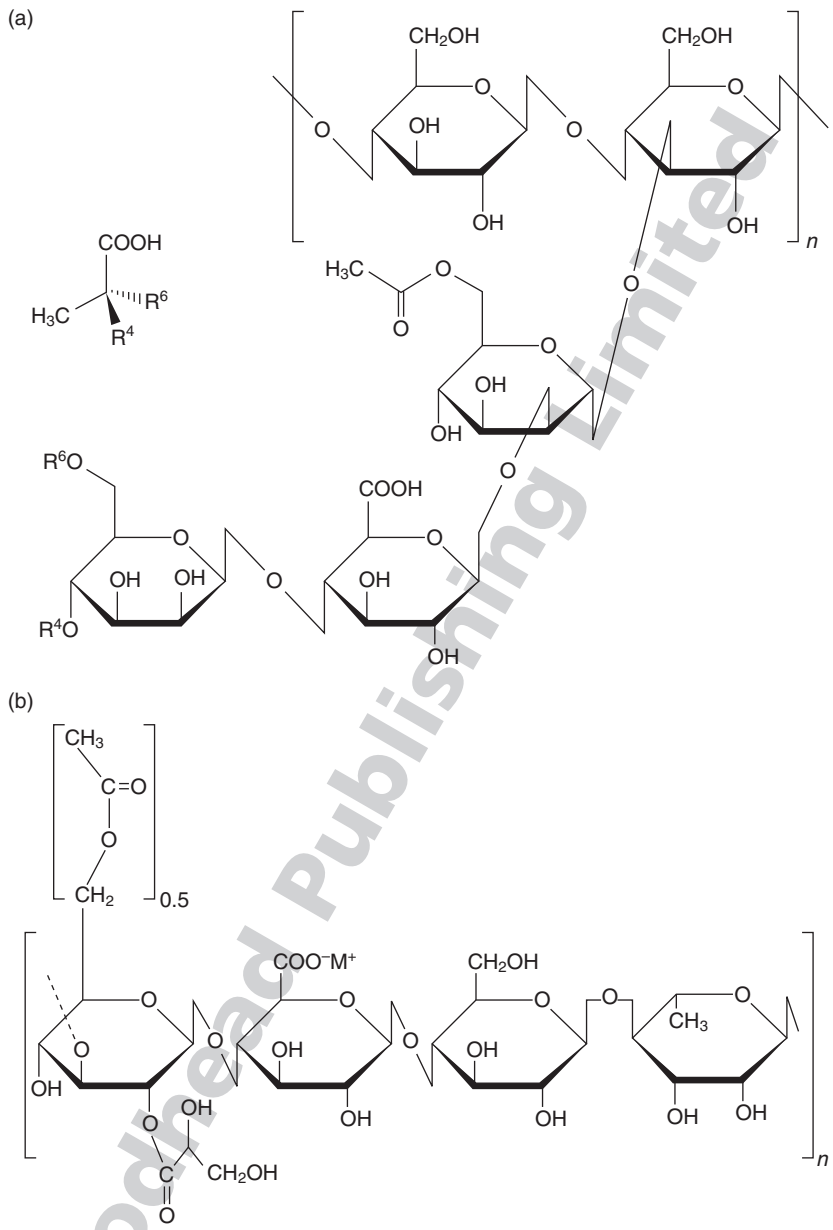

Fig. 16.1 Structures of some important bacterial polysaccharides. (a) xanthan repeating unit, (b) native gellan repeating unit (acetylated), (c) dextran repeating unit, (d) levan repeating unit.

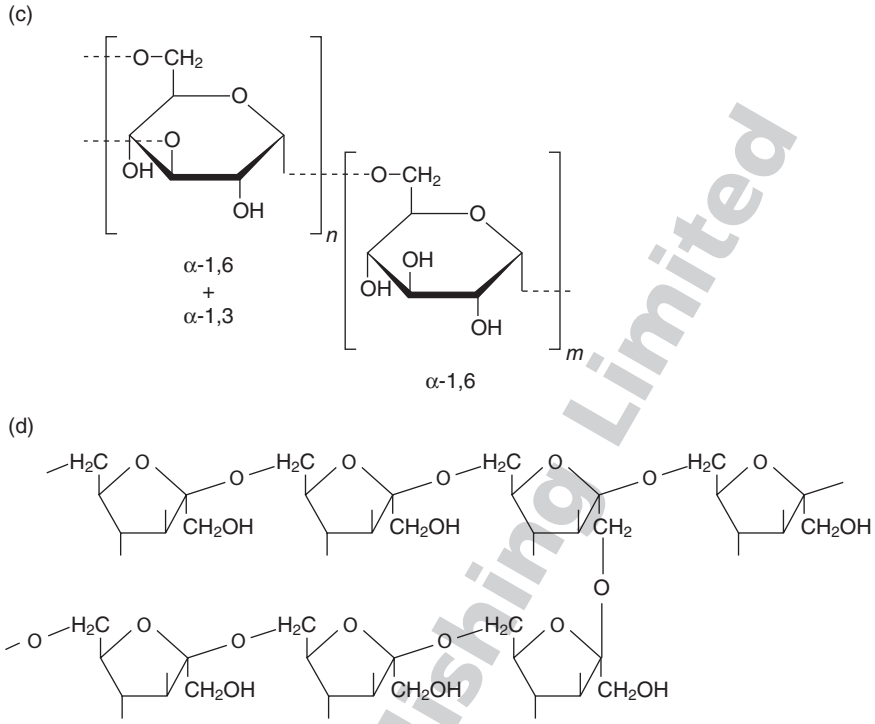

Fig. 16.1 *Continued*

flavour, self life, heat-shock protection and reduces syneresis, and is also suitable for beverages as it is soluble and stable at low pH and improves the suspension of insoluble partices (e.g. in fruit juices) and the body and mouthfeel of the products (Sharma *et al.*, 2006; Palaniraj and Jayaraman, 2011).

Acetan (also known as xylinan) is another EPS structurally related to xanthan and is produced by *Acetobacter xylinum*, a strain that is used in the food industry for the production of a sweet confectionery and vinegar (van Kranenburg *et al.*, 1999). It is an anionic heteropolysaccharide with a MW of approximately 1000,000 Da, consisting of a pentasacchride main chain where the (1, 2)-D-mannose residue of the main chain and the (1,3,4)-D glucose residue are *O*-acetylated (Ridout *et al.*, 1994, 1998; Ojinnaka *et al.*, 1996).

The same microorganism is the best industrial producer of microbial cellulose, a β-(1,4)-linked glucopyranose biopolymer with a low degree of branching or no branching at all, which lacks the hemicellulose, pectin and lignin moieties of plant-derived cellulose. It was granted a 'GRAS' (generally recognized as safe) status by FDA in 1992 for food applications (Khan

et al., 2007). *Acetobacter xylinum* cellulose also differs from plant-derived cellulose in that it has high purity and crystallinity, gel strength, moldability and increased water-holding capacity (Jonas and Farah, 1998; Iguchi *et al.*, 2000; Khan *et al.*, 2007). It is used mainly in Asian speciality food '*nata*', for instance '*Nata de Coco*', a jelly food with coconut water used in confectionery and desserts (Iguchi *et al.*, 2000; Khan *et al.*, 2007). Other potential food applications of microbial cellulose include dressings, sauces, icings, whipped toppings and aerated desserts, frozen dairy products where it functions as a low-calorie additive, thickener, stabilizer and texture modifier (Okiyama *et al.*, 1993; Khan *et al.*, 2007).

Another plant pathogen, *Sphingomonas paucimobilis* (formerly *Pseudomonas elodea*), produces gellan, an EPS of approximately 500,000 Da on average, which facilitates cell attachment to plant surfaces, such as water lilies, the plants from which it was first isolated (Kang *et al.*, 1982; Pollock, 1993; Giavasis *et al.*, 2006). Native gellan is composed of a linear anionic tetrasaccharide repeat unit containing two molecules of D-glucose, one of D-glucuronic acid and one of L-rhamnose, as well as glucose-bound acyl substituents (one L-glycerate and two O-acetate substituents per two repeat units on average) (Jay *et al.*, 1998). Its structure is depicted in Fig. 16.1. In its industrial form, gellan gum is usually deacylated after an alkaline thermal treatment, which transforms the soft elastic gels of native gellan to hard, brittle, thermoreversible, acid-tolerant, transparent gels, especially after addition of divalent cations (Jay *et al.* 1998; Giavasis *et al.*, 2000; Rinaudo and Milas, 2000; Rinaudo, 2004). Commercial gellan is available in three forms with distinct degree of acetylation: no, low and high acyl content corresponding to the brand names of Gelrite®, Kelcogel® F and Kelcogel® LT100 (Fialho *et al.*, 2008). Gellan has found several food applications as viscosifier, stabilizer, gelling agent in dessert gels, icings, sauces, puddings and restructured foods, as a bodying agent in beverages, or as an edible film and coating agent when blended with other gums (Giavasis *et al.*, 2000; Fialho *et al.*, 2008; Stalberg *et al.*, 2011). Other species of the genus *Sphingomonas* produce other biopolymers structurally related to gellan, such as wellan, rhamsan, diutan and gums S-88 and S-657 (all with different acylation patterns compared to gellan), which lack the strong gelling properties of deacylated gellan, but perform well as suspension agents with high resistance to shear stress and have found several applications in the food industry (Kang and Pettitt, 1993; Rinaudo, 2004; Fialho *et al.*, 2008).

Dextrans are some of the most common bacterial polysaccharides, and some of the first to be produced on industrial scale, with applications in foods, as well as pharmaceuticals, separation technology and so on (Glicksman, 1982; Alsop, 1983; Leathers, 2002a). Although many bacterial strains belongining to the genera *Leuconostoc, Lactobacillus, Streptococcus, Acetobacter,* and *Gluconobacter* are capable of synthesizing dextrans, dextran is industrially produced by *Leuconostoc mesenteroides* strains grown on a

sucrose medium via the action of dextran sucrase (a glucosyltransferase) which catalyses sucrose to form D-fructose and D-glucose and transfers the latter to an acceptor molecule where polymerization takes place. Purely enzymatic (bioconversion) processes, involving polymerization via dextransucrases, have also been developed (Jeanes *et al.*, 1954; Brown and McAvoy, 1990; Khalikova *et al.*, 2005; Khan *et al.*, 2007). Microbial dextran was initially identified and characterized after attempts to solve the problem of thickening or ropeyness that occurred in sugar juices and wines in the 1980s, but soon its water-binding properties led to its utilization in several applications as a food thickener and viscosifier (Glicksman, 1982; Vandamme and Soetaert, 1995).

Commercial dextran is produced by the lactic acid bacterium *L. mesenteroides* strain NRRL-B512 and consists of a α-(1,6)-D-glucan backbone (by 95% or less) and α-(1,3)- branches (by 5% or more) (Leathers, 2002a). Its chemical structure is shown in Fig. 16.1. Crude dextran has relatively high MW, around or above 1000,000 Da, although much higher MW values have also been reported, probably caused by the tendency of dextran molecules to aggregate (Khalikova *et al.*, 2005). In industrial processes, dextran is partly hydrolysed (by acid or enzymatic hydrolysis) and fractionated, yielding a wide range of dextrans with different MW values (Khalikova *et al.*, 2005). When used in food applications MW ranges from 15,000 to 90,000 Da (Glicksman, 1982; Kumar *et al.*, 2007). Food applications of dextrans include confectionery products where they act as stabilizers and bodying agents (e.g. in puddings), as crystallization inhibitors (e.g. in ice cream), or as moisture retention agents and viscosifiers in food pastes (Khan *et al.*, 2007). Dextrans from *L. mesenteroides* or other lactic bacteria (e.g. *Lactobacillus curvatus*) have also been used as texturizers in bread, especially gluten-free bread, where they enhance water-holding capacity, elasticity and specific volume of bread (Ruhmkorf *et al.*, 2012). The α-(1,6) linkages of the molecule are resistant to depolymerization, which results in the slow digestion of dextran in humans (Glicksman, 1982).

Alternan is another glucan similar to dextran, yet with unusual structure. It is synthesized mainly by *L. mesenteroides* strain NRRL B-1355, which is grown in a complex sucrose-based medium, in a process that resembles that of dextran production and is mediated through alternan sucrases (Cote and Robyt, 1982; Raemaekers and Vandamme, 1997). Although several *L. mesenteroides* strains that produce alternan also synthesize dextrans as undesirable contaminants, genetically engineered strains producing only alternan have been isolated (Kim and Robyt, 1994; Monsan *et al.*, 2001). The unique characteristic of alternan is the alternating structure of α-(1,6) and α-(1,3) linkages, with approximately 10% branching through 3,6-disubstituted D-glucosyl units (Seymour and Knapp 1980; Leathers *et al.*, 2003).

Several *Agrobacterium* and *Rhizobium* species, can each produce exopolysaccharides such as curdlan, a neutral 1,3-β-D-glucan with a low MW

(around 74,000 Da) (Sutherland, 1998). Curdlan, along with xanthan and gellan, has been approved for use in food by FDA and it is industrially produced from *Agrobacterium sp.* ATCC 31749, or *sp.* NTK-u, or *Agrobacterium radiobacter* (Jezequel 1998; Zhan *et al.*, 2012). Curdlan is a homopolysaccharide formed in the stationary phase following depletion of nitrogen and is insoluble in cold water but can be dissolved in hot water or in dimethylsulphoxide, forming stable gels. Many food applications of curdlan utilize its thermo-irreversible gel form, its stability during freeze–thawing cycles or during deep-fat frying, its lipid-mimicking properties and the fact that it provides a pleasant mouthfeel compared to other biopolymers (Lo *et al.*, 2003; McIntosh *et al.*, 2005). Curdlan has been used in various food products, mainly freezable and low-calorie foods, since it is not degraded in the gastrointestinal tract (McIntosh *et al.*, 2005). In Japan, curdlan is commonly used in food as a texturizer and water-holding agent in pasta, tofu, jellies, fish pastes, and reconstituted food and confectionery (Sutherland, 1998; Laroche and Michaud, 2007). In addition to the above properties and applications, the sulphated derivatives of curdlan have shown important immunostimulatory, antitumour and antiviral properties which have been reported extensively (Goodridge *et al.*, 2009; Zhan *et al.*, 2012) and might be exploited in the formulation of novel neutraceuticals.

Rhizobium and *Agrobacterium* species, as well as microorganisms such as *Alcaligenes faecalis* var. *myxogenes* and *Pseudomonas* sp. also produce succinoglycan, an acidic biopolymer which is commercialized and used mainly in oil recovery, but is also suitable for food applications for its thickening and stabilizing properties, even under extreme process conditions (Freitas *et al.*, 2011; Moosavi-Nasab *et al.*, 2012). It comprises large (octasaccharide) repeating units of D-glucose and D-galactose and carries *O*-acetyl groups, *O*-succinyl half-esters and pyruvate ketals as substituents, which form a molecule of relatively high MW (in the order of 10^6 Da) (Ridout *et al.*, 1997; Sutherland, 2001). Natural and chemically modified succinoglycans show high stability under high temperature and pressure, high/low pH and high shear stress (Moosavi-Nasab *et al.*, 2012).

Many other extracellular polysaccharides (EPS) have been isolated from a large number of LAB, namely *Lactobacillus, Streptococcus*, *Lactococcus, Pediococcus,* as well as *Bifidobacterium* sp. and *Weissella* strains found in fermented dairy products (De Vuyst and Degeest, 1999, Notararigo *et al.*, 2012). They excrete linear or branched biopolymers of galactopyranose, glucopyranose, fructopyranose, rhamnopyranose or other residues (e.g. *N*-acetylglucosamine, *N*-acetylgalactosamine, or glucuronic acid), characterized by a large range of MW values (10^4–10^6 kDa); for instance, homopolysaccharides (α-glucans or β-glucans) such as reuteran from *Lactocccbacillus reuteri,* mutan from *Streptococcus mutans,* polygalactan from *Lactococcus lactis* H414, and heteropolysaccharides such as kefiran from *Lactobacillus hilgardii,* and several other EPS from *Lactobacillus bulgaricus, Lactobacillus helveticus, Lactobacillus rhamnosus, Lactococcus lactis* NIZO-B39 or

NIZO-B891 and *Streptococcus thermophillus* (Ruas-Madiedo *et al.*, 2002; Tieking *et al.*, 2005; Patel *et al.*, 2010). Most LAB produce polysaccharides extracellularly from sucrose by glycansucrases or intracellularly by glycosyltransferases from sugar nucleotide precursors (Ruas-Madiedo *et al.*, 2002). These molecules and the producer strains have been thoroughly studied, since they can improve rheological and textural properties in dairy and other food products where LAB are already used (Laws *et al.*, 2001). Also, EPS from LAB such as kefiran have been used in the formulation of edible films with various plasticizers (Ghasemlou *et al.*, 2011). Moreover, some of these slimy homo/heteropolymers are associated with anticarcinogenic and immunomodulating properties, or reported to act as prebiotics promoting the growth of the producer strain or other LAB (Oda *et al.*, 1983; Adachi, 1992; Nakajima *et al.*, 1995; Sreekumar and Hosono, 1998; Ruas-Madiedo *et al.*, 2002), which could be great assets in formulating novel foods with bioactivity and health-promoting properties.

In spite of the fact that LAB and their products are considered GRAS and acceptance and incorporation of these polysaccharides in traditional and new functional food products should be easy, the substantially low production yields of these biopolymers, especially of the heteropolysaccharides (i.e. 50–1000 mg l^{-1} compared to 15–25 g l^{-1} of xanthan gum), remain a serious drawback for their broad commercial application in foods, which could be overcome with the aid of genetic engineering and better understanding of microbial physiology (Laws *et al.*, 2001). An exception to these low yields are two types of homobiopolymers, a glucan and a fructan synthesized by *Lb. reuteri* strain LB 121 which can reach a concentration of nearly 10 g l^{-1} during fermentation on a sucrose-based medium (van Geel-Schutten *et al.*, 1999). Most applications of LAB polysaccharides are related to fermented dairy food, beverages and sour doughs where the specific LAB are either part of the natural fermenting microflora or inoculated in purified form in order to contribute to the improvement in texture and viscosity, owing to the synthesis of the above biopolymers (Elizaquível *et al.*, 2011; Natararigo *et al.*, 2012). Also in another application, the *in situ* production of EPS from LAB cultures was useful in the production of low-fat Mozzarella cheese where they improved moisture retention (Bhaskaracharya and Shah, 2000).

Another class of bacterial polysaccharides is levans, extracellular homopolysaccharides of D-fructose (fructans). These biopolymers are characterized by β-(2,6)-fructofuranosidic bonds in their main chain and β-(2,1)-linked side chains (Huber *et al.*, 1994). A typical levan structure is illustrated in Fig. 16.1. They are produced by several bacteria, such as *Streptococcus salivarius* (a bacterium of the oral flora), *Lactobacillus sanfranciscensis*, *Bacillus subtilis* and *Bacillus polymyxa*, *Acetobacter xylinum, Gluconoacetobacter xylinus, Microbacterium levaniformans, Zymomonas mobilis* and a few more microorganisms which express the biosynthetic enzyme levan sucrase in sucrose-rich culture media (Newbrun and Baker, 1967; Han, 1990;

Keith *et al.*, 1991; Yoo *et al.* 2004; Notararigo *et al.*, 2012). Alternatively, they can be synthesized enzymatically by levan sucrases using sucrose as subtrate (Jang *et al.*, 2001; Castillo and Lopez-Munguia, 2004). Levans often reach a very high MW value (over 10^6–10^7 Da), while low MW levans can also be produced, depending on the microorganism used and the fermentation/biocatalysis conditions (Newbrun and Baker, 1967; Calazans *et al.*, 2000; Shih *et al.* 2005).

The properties and potential applications of levan in food resemble those of dextrans, but levans from *Aerobacter levanicum* and *Z. mobilis* (an industrial ethanol-producing strain) have also exhibited immunostimulating and anti-tumour properties (Calazans *et al.*, 2000; Bekers *et al.*, 2002; Yoo *et al.*, 2004), as well as hypolipidaemic and hypocholesterolaemic effects (Kang *et al.*, 2004). Most food applications of levans utilize their texturizing, and water and air retention properties in doughs and breads, as well as their ability to act as a stabilizer, thickener, osmolegulator, cryoprotector, sweetener and a carrier of flavours and fragnances (Han, 1990; Bekers *et al.*, 2005; Tieking *et al.*, 2005; Kang *et al.*, 2009). Levan from *Lactobacillus sanfranciscensis* was reported to affect dough rheology and texture positively (Waldherr and Vogel, 2009). Also, Huber *et al.* (1994) proposed the use of levan as an ingredient for forming edible films. These are too brittle when levan is the sole ingredient, but when blended with other polymers, such as glycerol, elastic and extrudable films can be formed (Barone and Medynets, 2007). Furthermore, levan has exhibited anti-obesity and hypolipidaemic effects as well as antitumour and anti-radiation protective properties (Han, 1990; Kang *et al.*, 2004; Yoo *et al.*, 2004; Bekers *et al.*, 2005; Combie, 2006) which could be exploited in novel nutraceuticals.

Bacterial alginate is another biopolymer with food applications. It is currently produced from the marine brown algae on the industrial scale thanks to the comparatively low cost of this process, but can also be produced by liquid cultures of bacteria such as *Azotobacter vinelandii*, *Azotobacter chroococcum* and *Pseudomonas aeruginosa,* with *Azotobacter* being preferable for microbial alginate production, owing to the potential pathogenicity of *P. aeruginosa.* (Sabra *et al.*, 2001; Remminghorst and Rehm, 2006). Alginate is an acidic copolymer of β-D-mannuronic acid (M) and α-L-guluronic acid (G), with varying content of G and M and chain length (although alginate from *P. aeruginosa* lacks the G blocks). Its molecular weight is in the order of 10^6 Da (Sabra *et al.*, 2001; Celik *et al.*, 2008; Freitas *et al.*, 2011). Homopolymeric M and G groups are normally interconnected with alternating residues of both acids (MG groups) in *Azotobacter* and brown algae. Microbial alginates are acetylated on some mannuronic acid residues, which is a main difference from alginate derived from algae (Sabra *et al.*, 2001).

Alginic acid and its sodium calcium and, potassium salts are safe for use in food (GRAS) as thickeners, stabilizers, or gelling agents. They are usually added to jams, confectionery (candies, ice cream, milk shakes), beverages,

soups and sauces, margarine, liquors, structured meat and fish, as well as dairy products (Sabra *et al.*, 2001; Giavasis and Biliaderis, 2006). Calcium alginate is also a common medium for cell and enzyme immobilization and microencapsulation of bioactive molecules and can be used as an edible film coating (Freitas *et al.*, 2011). Recently, several physiological effects of alginate have been disclosed, including dietary fibre effects, anti-inflammatory (anti-ulcer) and immunostimulating properties, as well detoxifying properties (Khotimchenko *et al.*, 2001) which may establish this biopolymer as a functional ingredient in the manufacture of functional foods or nutraceuticals. In fact, a bioactive food additive ('Detoxal') containing calcium alginate can reduce lipid peroxidation products and normalize the concentrations of lipids and glycogen in the liver, while it has also shown antitoxic effects, for example against tetrachlorometan-induced hepatitis in mice, or via adsorption and elimination of heavy metals in humans (Khotimchenko *et al.*, 2001).

16.2.2 Fungal polysaccharides

One of the most common and well-studied fungal polysaccharides is pullulan. It was back in 1958 when Bernier (1958) observed that *Pullularia* (now *Aureobasidium*) *pullulans*, a yeast-like fungus, can synthesize an extracellular polysaccharide, a neutral glucan which was called pullulan a year later (Bender *et al.*, 1959). It was first commercialized by Hayashibara Biochemical Laboratories (Japan) and protected by patents for several years (Sugimoto, 1978; Singh *et al.*, 2008). Pullulan is a white, tasteless, water-soluble homopolymer of glucose consisting of repeating units of maltotriose with a regular alternation of two α-(1,4) linkages, and one α-(1,6) linkage on the outer glucosyl unit (ratio 2 : 1) as periodate oxidation, permethylation and infra-red spectrum analysis suggest (Bender *et al.*, 1959; Catley, 1970; Taguchi *et al.*, 1973a; Sandford, 1982; Le Duy *et al.*, 1988, Leathers, 2002b), although other structures comprising α-maltotetraose units and (1,3)-linked residues have also been proposed (Ueda *et al.*, 1963; Taguchi *et al.*, 1973b). This variance is not surprising since several extracellular polysaccharides have been isolated from the same microorganism (Sandford, 1982).

Figure 16.2 depicts a typical pullulan structure. The MW of pullulan is generally in the range 10,000–1000,000 Da with a average MW of 360–480 KDa, depending on process conditions and the strain used (McNeil and Harvey, 1993; Cheng *et al.* 2011), but the two main industrial products from Hayashibara Company Ltd, a food grade pullulan (PF) and a deionized pullulan (PI), have a mean molecular weight of 100,000 Da (PF-10 and PI-10), or 200,000 Da (PF-20 and PI-20) (Singh *et al.*, 2008). Pullulan can also form oil-resistant, water-soluble, odourless, thin and transparent films with low oxygen permeability which can act as edible food coatings that improve self life (e.g. of fruits and nuts) (Leathers, 2003;

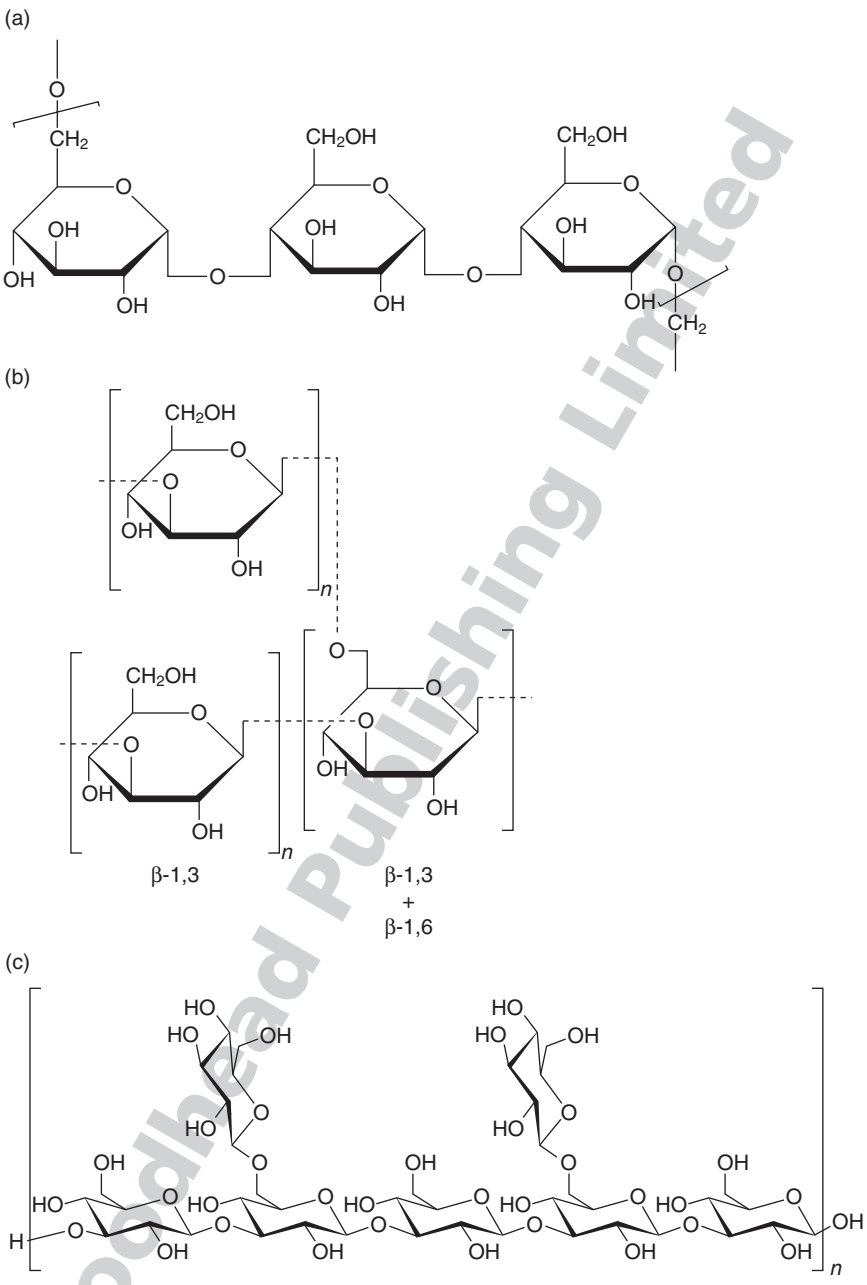

Fig. 16.2 Structures of some important fungal polysaccharides. (a) Pullulan repeating unit, (b) schizophyllan (sizofiran) repeating unit, (c) lentinan repeating unit.

Gounga *et al.*, 2008; Cheng *et al.*, 2011). These applications have been marketed in Japan, but are apparently limited elsewhere (Sutherland, 1998; Leathers, 2003).

Pullulan has been proposed as a replacement for starch in solid and liquid food, especially pastas and baked products, where it strengthens food consistency, moisture and gas retention and dispersibility. In addition, it can be used as a stabilizer/viscosifier in sauces and beverages, offering low but stable viscosity with temperature and pH changes, or as a binder in food pastes and confectionery products where its adhesive properties may be exploited (e.g. for adhesion of nuts on cookies). It has also been applied as a dietary fibre and as a prebiotic to promote growth of *Bifidobacterium* spp. owing to its partial degradation to indigestible short-chain oligomers by human salivary α-amylase (Okada *et al.*, 1990; Singh *et al.*, 2008; Cheng *et al.*, 2011). In food packaging, pullulan–polyethylene films could be used to offer high water and oxygen resistance, and better rigidity and strength comparable to expanded polystyrene films (Paul *et al.*, 1986).

The fungus *Elsinoe leucospila*, isolated from a white spot of tea leaves, produces elsinan, an extracellular, linear α-D-glucan composed of glucose units linked by approximately 70% (1,4)-linkages (maltotriose) and 30% (1,3)-linkages (maltotetraose) (Sandford, 1982; Misaki *et al.*, 1978, 1982). The proposed structure of elsinan, as determined by methylation and periodate oxidation studies, as well as partial acid hydrolysis, acetolysis and enzymic degradation by glucanases, is similar to that of pullulan, which has (1,6)-links instead of the (1,3)-links in elsinan (Misaki *et al.*, 1978, 1982; Misaki, 2004). Like pullulan, elsinan was manufactured by Hayashibara Biochemical Laboratories (Japan), but despite its viscosifying and film-forming properties it has found little application as a food additive so far (Misaki, 2004). However, there is a significant potential for food applications of elsinan owing to its dietary fibre properties (i.e. reduction of serum cholesterol in hypercholesterolemic rats) and its ability to form oxygen impermeable edible films and coatings, and viscous solutions which are stable over a wide range of pH (3–11), temperature (30–70°C) and salt concentrations (Misaki, 2004). It can also be used in food packaging as a biodegradable film (Yokobayashi and Sugimoto, 1979; Sandford, 1982). In experiments with oleic acid and fresh fish packed with elsinan films, no colorization caused by self-oxidation occurred over 3 and 4 months, respectively, while acidic conditions (pH 1 to 4) did not affect the stability of these films (Sandford, 1982). Moreover, its cholesterol-lowering and anti-tumour properties can be utilized in the formulation of novel functional foods (Shirasugi and Misaki, 1992; Misaki, 2004). Additionally, Shirasugi and Misaki (1992) have isolated a cell wall polysaccharide from *Elsinoe leucospila*, which exhibited antitumour activity. This polymer, obtained from cold alkali cell wall extract, was a β-D-glucan with a main chain of eight (1,3)-glucose residues and single β-D-glucosyl side groups at the O-6 position.

Scleroglucan is another extracellular glucose homopolysaccharide with a high MW (about or over 1000,000 Da) with a β-(1,3) linked backbone, where a single D-glucosyl side group is bound via a β-(1,6) linkage to every third or fourth unit of glucose in the main chain (Holzwarth, 1985; Giavasis *et al.*, 2002). The main producer microorganisms are the filamentous phytopathogenic fungi *Sclerotium glucanicum* and *Sclerotium rolfsii*. Scleroglucan was first brought into the market by Pillsbury Co (Minneapolis, USA), followed by CECA S.E. (France) and Satia S.A. (France), serving mainly as a viscosifier in chemically enhanced oil recovery, where it performs better than xanthan (Holzworth, 1985; McNeil and Harvey, 1993). In the food industry, scleroglucan would be ideal for the stabilization of dressings, sauces, ice creams and other desserts, as well as low calorie or thermally processed and acidic products (sterilization, salts and acids do not affect its stabilizing capacity), but its use in food is not yet approved in Europe and the USA (Survase, 2007; Schmid *et al.*, 2011). Nevertheless, there are several Japanese patents on the application of scleroglucan as a stabilizer and thickener in frozen or heat-treated food, such as steamed foods and bakery products (Schmid *et al.*, 2011), showing the interest that exists for such applications.

Vinarta *et al.* (2006) investigated the stabilizing properties of scleroglucan in cooked starch pastes and showed that scleroglucan offered high water retention and significantly reduced syneresis during refrigeration, and this effect was even more pronounced when scleroglucan was blended with corn starch before being added. Scleroglucan could also be utilized in the formation of edible films and tablets for neutraceuticals, owing to its chemical stability, biocompatibility and biodegradability (Grassi *et al.*, 1996; Coviello *et al.*, 1999). Although it does not act as a surfactant, it can stabilize oil-in-water emulsions, by preventing coalescence (Sandford, 1982). Additionally, this β-glucan has shown significant antitumour and antiviral activity (Jong and Donovick, 1989; Pretus *et al.*, 1991; Mastromarino *et al.*, 1997), which could be a great asset in designing functional foods.

Two similar polysaccharides (only of lower MW than scleroglucan), namely schizophyllan (also called sizofiran) and lentinan, are produced by the edible mushrooms *Schizophyllum commune* and *Lentinus edodes*, respectively (Giavasis *et al.*, 2002). They are two of the most well-studied immunostimulating microbial β-(1,3)-D-glucans, while *L. elodes*, is the most common edible mushroom in Japan (Maeda *et al.*, 1998). Their chemical structure is illustrated in Fig. 16.2. Both lentinan and schizophyllan are characterized by a main chain of β-(1,3)-D-glucose residues to which β-(1,6)-D-glucose side groups are attached (one branch to every third main chain unit), and an average molecular weight of about 500,000 Da (Misaki *et al.*, 1993). Their addition to food in purified form has not been commercialized yet, in contrast to several pharmaceutical applications where they are used (Giavasis and Biliaderis, 2006), but as they both come from edible

mushrooms, they have a great potential for use in novel foods and nutraceuticals.

(1,3)(1,6)-β-D glucans from *L. edodes* were used as a replacement for a portion of the wheat flour in baked foods such as cakes, in an attempt to produce a novel functional food with low calories and high fibre content (Kim *et al.*, 2011). In this application *L. edodes* glucans from mushroom powder which was incorporated in batter improved pasting parameters and increased batter viscosity and elasticity, without having any adverse effects on air holding capacity (volume index) or hardness compared to the control, when used at concentrations of 1 g pure glucan per 100 g of cake. Reduced volume and increased hardness were only observed when glucan concentration was 2% or more (Kim *et al.*, 2011). In similar studies, *L. edodes* glucan from unmarketable mushrooms was added to noodles as a partial wheat flour replacement and resulted in a fibre-rich functional food with antioxidant and hypocholesterolaemic effects and improved quality charasterictics (Kim *et al.*, 2008, 2009). In another study (Kim *et al.*, 2010) *L. edodes* mushroom powders (LMP) rich in β-glucans were utilized effectively as oil barriers and texture-enhancing ingredients in frying batters.

Several other mushrooms, many of which are part of the traditional diet in East Asian (especially Chinese and Japanese) or South American populations, contain a number of polysaccharides, mainly β-D-glucans, which have been associated with healthy diet have fortified the immune system of the consumers (Hobbs, 1995; Wasser, 2002; Giavasis and Biliaderis, 2006; He *et al.*, 2012) and could find novel applications as functional food ingredients. *Agaricus blazei*, for instance, is a well-known edible and medicinal mushroom originating from Brazil, containing several antitumour polysaccharides in its fruit body (Mizuno *et al.*, 1990). The water-soluble fraction of these polysaccharides includes a β-(1,6);β-(1,3) glucan an acidic β-(1,6);α-(1,3) glucan, and an acidic β-(1,6);α-(1,4) glucan. Unlike most known glucans, *A. blazei* glucans have a main chain of β-(1,6) glycopyranose, instead of the common β-(1,3) linked main chain (Mizuno *et al.*, 1990). The fruit body also contains a water-soluble proteoglucan with a α-(1,4) glucan backbone and β-(1,6) branches at a ratio of 4 : 1. It has a MW of 380,000 Da and it consists mainly of glucose (Fujimiya *et al.*, 1998). Moreover, the water-insoluble fraction of *A. blazei* fruit body, which has also shown immunostimulating activity, includes two heteroglucans consisting of glucose, galactose and mannose, one consisting of glucose and ribose, a xyloglucan and a proteoglucan (Cho *et al.*, 1999; Mizuno, 2002). Notably, submerged cultures of *A. blazei* synthesize somewhat different (medicinal) polysaccharides compared to those from the mushroom fruit body (Mizuno, 2002). Among these biopolymers, some of which are covered by patents (Hikichi *et al.*, 1999; Tsuchida *et al.*, 2001), an extracellular protein–polysaccharide polymer with significant antitumour properties and a high MW (1000,000–10,000,000 Da) has been isolated. The sugar components of this

biomolecule include mainly mannose, as well as glucose, galactose and ribose (Mizuno, 2002).

Ganoderma lucidum is another medicinal mushroom belonging to the *Basiomycetes* family, which has been used for many years in traditional East Asian medicine as a dry powder, or consumed as a hot water extract (in a type of bitter mushroom tea). The bioactive component of the fungi, termed 'ganoderan', is a typical β-(1,3) glucan branching at C-6 with β-(1,6) glucose untis and with a high (Bao *et al.*, 2002) or low (Misaki *et al.*, 1993) degree of branching, which can be isolated either from the water-soluble fraction of the fruit body (Misaki *et al.*, 1993; Bao *et al.*, 2002), or from the filtrates of liquid cultures of *G. lucidum* mycelia. The latter is a water-soluble β-D-glucan with a MW of 1.2–4.4 × 10^6 Da, degradable by pectinases and dextranases (Lee *et al.*, 1996; Xie *et al.*, 2012). Apart from the above glucans, a few more heteroglucans and proteoglucans are also present in fruit bodies of *G. lucidum* (Eo *et al.*, 2000). Kozarski *et al.* (2011, 2012) studied the antioxidant and immnomodulatory properties of glucans from *G. lucidum* and *Ganoderma applanatum* with respect to their potential application in food, and reported a significant free radical scavenging activity and protective action against lipid peroxidation, as well as significant enhancement of interferone synthesis in human blood cells.

Other antioxidant and immunostimulating basidiomycetal polysaccharides from edible mushrooms include krestin, a commercialized proteoglucan synthesized by the mushroom *Coriolous versicolor* (also called *Trametes versicolor*) which has a β-(1,3)-D-glucan moiety (Ooi and Liu, 2000) and grifolan, a gel-forming β-(1,3)-D glucan with β-(1,6) branches at every third glucopyranosyl residue, elaborated by the edible fungus *Grifola frondosa* (Adachi *et al.*, 1998; Laroche and Michaud, 2007), which could also be utilized as a food grade functional ingredient.

Kozarksi *et al.* (2012) also reported significant antioxidant properties of polysaccharides extracted from *T. versicolor* and *L. edodes* mushrooms, which exhibited chelating properties and inhibited lipid oxidation. The latter were correlated with the presence of an α-glucan and a phenolic (mainly tyrosine and ferrulic acid) moiety linked to the main β-glucan backbone by covalent bonds. He *et al.* (2012) studied the antioxidant properties of edible mushroom glucans, namely the water soluble β-glucans of *Agaricus bisporus* (one of the most popular edible mushrooms in Europe), *Auricularia auricula*, *Flammulina velutipes* and *L. edodes*. The glucans of the first three mushrooms were composed of D-mannose, D-galactose and D-glucose, while the glucan from *F. velutipes* contained L-arabinose, D-mannose, D-galactose and D-glucose. Based on their reducing capacity and their hydroxyl, superoxide ion and DPPH radical scavenging ability, the use of these biopolymers in food applications was suggested owing to their significant antioxidant properties (especially those of *A. bisporus* glucans which showed the highest antioxidant acitivity). Although commercial applications of the above glucans in the food industry are not available so far, there

are patents (especially in Japan) related to the use of Ganoderma, Agaricus and other mushroom glucans in edible film coatings and water-soluble capsules, for example inclusion of pickling liquids in soups and sauces (Laroche and Michaud, 2007) and a great potential exists for future food applications.

16.2.3 Yeast polysaccharides

Although most microbial polysaccharides derive from fungi and bacteria, *Saccharomyces cerevisiae*, probably the most common food grade yeast in fermented food and drinks, is known for the production of a food-related glycan which is extracted from yeast cells walls. Cell wall polysaccharides are usually insoluble in water, but their solubility and properties can readily be altered by chemical or enzymatic derivatization and facilitate their use in foods or pharmaceuticals. BYG is the general term for commercialized 'brewer's yeast glucan' (or more precisely glycan), which may also contain non-carbohydrate moieties, produced from *S. cerevisiae*. BYG is efficient in improving the physical properties of foods as a thickening and water-holding agent, or as a fat replacer giving a rich mouthfeel, and it also enhances gel strength in solutions, when used alone or in combination with other food grade polymers, such as carrageenan (Reed and Nagodawithana, 1991; Xu *et al.*, 2009). Firm gels of BYG can be formed after heating and subsequent cooling of solutions above 5–10% concentration. The glycan also has emulsifying properties and is reported to improve the organoleptic characteristics of the foods where it is added (Sandford, 1982). Thammakiti *et al.* (2004) studied the production of such a β-glucan with a β-(1,3)-glucose backbone chain and a minor branch (about 3%) of β-(1,6)-glucose with an additional 4.5–6.5% protein content from spent brewer's yeast after alkali extraction from homogenized cell walls, which had potential applications in food as an emulsion stabilizing agent, as it exhibited high viscosity and water holding and oil binding capacities.

Baker's yeast glycan is a similar product composed of D-glucose and D-mannose in 3 : 2 ratio and used mainly as stabilizer/emulsifier in dressings and desserts (Robbins and Seeley, 1977, 1978; Sandford, 1982). The same yeast has also been studied and utilized for the production of therapeutic glucans (Williams *et al.*, 1992). The wild type strain of *S. cerevisiae* excretes an extracellular β-(1,3)-D-glucan with a degree of branching (DB) of 0.2, and a genetically modified strain produces PGG (also known as Betafectin), a commercial bioactive (1,6)-β-D-glucopyranosyl-(1,3)-β-D-glucopyranose glucan with DB of 0.5 which has several pharmaceutical properties (Jamas *et al.*, 1991; Wakshull *et al.*, 1999; Kim *et al.*, 2006). In addition, *S. cerevisiae* is the industrial producer of zymosan, a complex immunoactive and anti-inflamatory glycan (proteoglucan) comprising a cell wall β-glucan with long (1,3)- and (1,6)-glucosyl groups, in conjunction with mannan, protein and nucleic acid (Ohno *et al.*, 2001; Goodridge *et al.*, 2009). These health-

promoting effects of glucans from edible yeast cell walls could find new applications in novel functional foods.

16.3 Production of microbial polysaccharides

A brief look at the literature on microbial polysaccharides shows that despite the numerous biopolymers that have been discovered and studied in the laboratory and the interesting properties and miscellaneous proposed applications, only a handful of these have made their way into industry and the market. The reasons for this vary, but it is principally the production process on a large scale and the problems related to it, which may make such an application economically unfeasible. High production costs, low polysaccharide yields, by-product formation and laborious downstream processing (separation and purification of the final product) are therefore issues that have to be resolved (Freitas *et al.*, 2011; Donot *et al.*, 2012). In this direction, the understanding of microbial physiology, polysaccharide biosynthesis and genetics, bioprocess (fermentation) conditions and separation/purification steps, are valuable tools. In addition, as can be deduced from the above description of microbial biopolymers, there is sometimes a diversity in structure and composition of polysachharides produced by the same microorganism, which can be a problem when commercializing these polymers. This is attributed partly to the cultivation/fermentation process conditions adopted, the composition of nutrients in the cultivation medium, and the fractionation and purification steps that are followed, which can altogether influence polysaccharide composition, branching and molecular weight. Besides this, fruit bodies of fungi generally contain more biopolymers than cultured mycelia (Wasser, 2002; Lee *et al.*, 2004; Giavasis and Biliaderis, 2006; Donot *et al.*, 2012). All these parameters have to be taken into account in the standardization of commercial products and will be briefly discussed below.

16.3.1 Biosynthesis

Microbial polysaccharides are either a part of the cell wall or excreted from the cell (extracellular polysaccharides) and are characterized as primary (e.g. several cell wall biopolymers) or secondary (e.g. several bacterial capsular biopolymers) metabolites. Their role in the cell can be to form an external slimy layer as a means of attachment to other cells and cell-to-cell interaction (a characteristic of many pathogenic speices) or a more rigid capsule or glycocalyx closely attached to the cell wall offering protection from unfavourable conditions (such as high acid or alkali concentrations, desiccation, oxygen stress, antibiotics, phagocytes, etc), the mechanical stability of the cell wall, the control of the diffusion of molecules into the cell and the export of other metabolites, or the formation of an energy reserve,

as some polysaccharide-producing microorganisms also possess degrading enzymes (polysaccharide lyases) in order to hydrolyse these biopolymers to sugar monomers (Sutherland, 1990; Herrera, 1991; Sharon and Lis, 1993; Whitfield and Valvano, 1993; McNeil, 1996; Sutherland, 1997, Kumar *et al.*, 2007).

The biosynthetic steps in polysaccharide production generally include the import and assimilation of sugar monomers inside the cell by passive or active transport, their conversion to activated sugar-phospho-nucleotides after intracellular phosporylation (e.g. uridine diphospate, UDP, and thimidine diphosphate, TDP) which act as sugar donors, the transfer of sugars to lipid carriers (located in the cytoplasmic membrane) by specific glycosyltranferases, and subsequent polymerization by polymerases (Whitfield and Valvano, 1993; Stephanopoulos *et al.*, 1998; Laws *et al.*, 2001; Sutherland, 2001; Freitas *et al.*, 2011). A key step in this process is the interconversion of glucose-6-phosphate (a glycolysis intermediate) into glucose-1-phosphate (which acts as sugar nucleotide precursor), which is catalysed by phosphoglucomutase (PGM), a key enzyme in polysaccharide biosynthesis (Patel *et al.*, 2010). From this point onwards, the biosynthesis of sugar nucleotides begins, which is the other crucial step in the assembly of the main repeat unit.

The biosynthetic route of sugar nucleotides involved in gellan formation is depicted in Fig. 16.3. Cell wall polysaccharides (e.g. mushroom polysaccharides) and many exopolysaccharides are synthesized totally intracellularly, but in the case of some exopolysaccharides, such as dextran, levan, alternan, mutan and reuteran, a simpler and partially extracellular process takes place, involving lipoprotein biosynthetic enzymes excreted at the cell surface (Vanhooren and Vandamme, 1998; Sutherland 2001; Patel

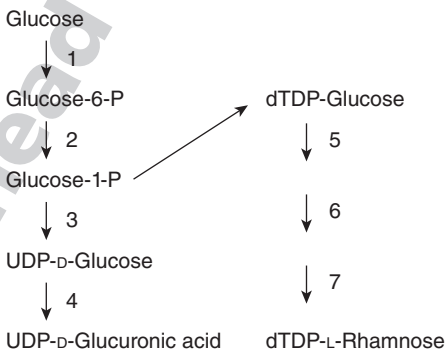

Fig. 16.3 Proposed pathway for biosynthesis of nucleotide precursors for gellan formation (adapted from Fialho *et al.*, 2008). (1) Phosphoglucomutase, (2) UDP-glucose pyrophosphorylase, (3) UDP-glucose dehydrogenase, (4) TDP-glucose pyrophosphorylase, (5) TDP-D-glucose-4,6-dehydratase, (6) TDP-6-deoxy-D-glucose-3,5-epimerase, (7) TDP-6-deoxy-L-mannose dehydrogenase.

et al., 2010). For instance, biosynthesis of levan is carried out via the dual action of levansucrases, which possess hydrolase activity to break down sucrose to fructose and glucose, and transferase activity, which is responsile for the transfer of the fructose moiety to a fructosyl-acceptor molecule (Han, 1990; Patel *et al.*, 2010). Similarly, in dextran synthesis by *Leuconostoc sp.* the major enzyme involved is a dextransucrase or D-glycosyl transferase which transfers glucose molecules to a monosaccharide or oligosaccharide acceptor, and polymerization takes place by the addition of D-glucose to the reducing end of the growing chain. Notably, these acceptors do not act as primers for dextran synthesis and their synthesis is competitive with dextran synthesis (Robyt *et al.*, 2008; Donot *et al.*, 2012). Dextran, as well as levan can also be synthesized by a purely enzymatic process, after isolation of the sucrases from cell cultures and mixing with sucrose. In the enzymatic process of dextran and levan synthesis it was observed that although biopolymer concentration increases at high enzyme concentration, the molecular weight of the polysaccharide is not proportional to sucrase concentration (Abdel-Fattah *et al.*, 2005; Robyt *et al.*, 2008).

In exopolysaccharide synthesis, apart from the biosynthetic enzymes, lipid transporters play a significant role in biosynthesis. They are long-chain phosphate esters and isoprenoide alcohols, similar to those involved in the biosynthesis of lipopolysaccharides, *O*-antigen and peptidoglycans (Sutherland, 1990). In EPS synthesis, lipid carriers are attached to the inner side of the cell membrane and are the anchor molecules on which the carbohydrate chain is orderly assembled. The chain is then transfered to the outer membrane where it is polymerized by a polymerase, although in some cases polymerization takes place on the inner side of the membrane and the whole chain is transferred out of the cell by exporter proteins linked to the lipid carrier (De Vuyst *et al.*, 2001; Donot *et al.*, 2012).

The biosynthetic route of heteropolysaccharides such as xanthan, gellan and LAB EPS are generally more complex than those of homopolysaccharides like fungal β-glucans. Xanthan is built up from cytoplasmic sugar nucleotides, acetyl-CoA and phosphoenolpyruvate with an inner-membrane polyisoprenol phosphate as an acceptor (Becker *et al.*, 1998). In xanthan synthesis, the repeating unit is formed by the sequential addition of glycosyl-1-phosphate from an UDP-glucose molecule to a polyisoprenol phosphate of a lipid carrier, followed by the transfer of D-mannose and D-glucuronic acid from GDP-mannose and UDP-glucuronic acid, while the acetyl groups attach to the internal mannose residue and pyruvate groups to the terminal mannose (Rosalam and England, 2006; Donot *et al.*, 2012).

The biosynthetic pathway of EPS production from LAB, although relatively complex, can be separated into four reaction sequences, one involved in sugar transport into the cytoplasm, one regulating the synthesis of sugar-1-phosphates, one responsible for activation of and coupling of sugars (formation of sugar nucleotides) and one regulating the export processes of the

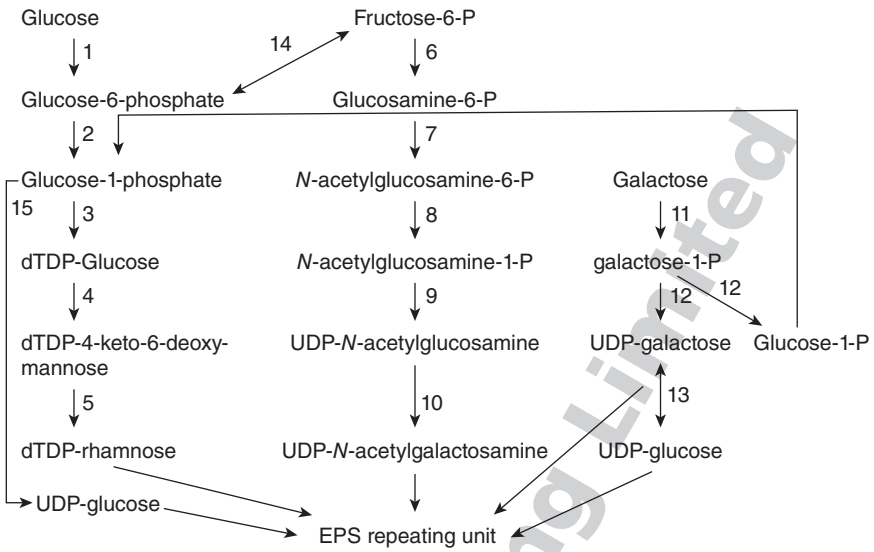

Fig. 16.4 Schematic representation of metabolic pathways for sugar nucleotide and heteropolysaccharide synthesis in LAB: (1) glucokinase, (2) phosphoglucomutase, (3) dTDP-glucose pyrophosphorylase, (4) dehydratase, (5) epimerase reductase, (6) glutamine-fructose-6-phosphate transaminase, (7) glucosamine-phosphate acetyltransferase, (8) acetylglucosamine-phosphate mutase, (9) UDP-glucosamine pyrophosphorylase, (10) UDP-*N*-acetylglucosamine-4-epimerase, (11) galactokinase, (12) galactose-1-phosphate uridylyl transferase, (13) UDP-galactose 4-epimerase, (14) phosphoglucose isomerase, (15) UDP-glucose pyrophosphorylase (adapted from DeVuyst *et al.*, 2001).

EPS (Laws *et al.*, 2001). The heteropolysaccharide biosynthetic route in LAB is described in Fig. 16.4.

Fungal glucans are in most cases not well studied at a biochemical and genetic level and identification of some enzymes involved in biosynthesis is still missing. However general postulated pathways have been described. Scleroglucan formation starts with the assimilation of glucose by glucose transporter(s) and its phosphorylation to glucose-6-phosphate via a hexokinase reaction. After isomerization to glucose-1-phosphate via the action of a phosphoglucomutase, UDP-glucose is formed by an UTPglucose-1-phosphate uridylyltransferase. A (1,3)-β-glucan synthase uses UDP-glucose for the synthesis of the main chain, while a (1 ~ 3);(1 ~ 6)-β-glucosyltransferase is postulated to mediate the addition of the (1,6)-β-linked glucosyl side chain into the (1,3)-β-glucan backbone (Schmid *et al.* 2011). Although the β-glucan synthase activity of *S. rolfsii* involved in the assembly of the (1,3)-β-glucan has been studied in membrane and protoplast fractions, the branching activity has not been assigned to a specific enzyme yet (Kottutz

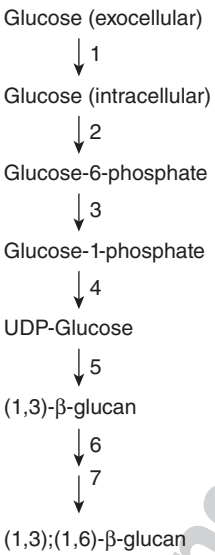

Fig. 16.5 Postulated pathway for biosynthesis of scleroglucan by *S. rolfsii*; (1) glucose transporter, (2) hexocinase, (3) phosphoglumutase, (4) UTP-glucose-1-phosphate-uridyltransferase, (5) (1,3)-β-glucansynthase, (6) glycosyltransferase, (7) glucosidase (adapted from Schmid *et al.*, 2010).

and Rapp, 1990; Schmid *et al.*, 2011). Fig. 16.5 summarizes a general proposed pathway for scleroglucan synthesis.

In pullulan biosynthesis, three key enzymes are nessesary for glucose to be converted into pullulan, namely α-phosphoglucomutase, uridine diphosphoglucose pyrophosphorylase (UDPG-pyrophosphorylase) and glucosyltransferase. Hexokinases and isomerases are needed for the convertion of sugars other than glucose to the key sugar nucleotide UDPG, which acts as the pullulan precursor by transferring a D-glucose residue to the lipid carriers (lipid hydroperoxides with a phosphoester bridge) to form a lipid-linked isomaltosyl and subsequently an isopanosyl residue. The latter is finally polymerized into the pullulan chain (Simon *et al.*, 1998; Cheng *et al.*, 2011). Notably, a somewhat distinct process has been proposed concerning the sugar utilization in pullulan biosynthesis, where it has been observed that *A. pullulans* cells are able to store sugars in the form of an intracellular storage polysaccharide (glycogen) which is broken down to monosaccharides from which pullulan is formed (Simon *et al.*, 1998; Cheng *et al.*, 2011).

The activity of these biosynthetic enzymes, the availability of lipid carrier or acceptor molecules (usually mono- or oligosaccharides) and the number of phosphorylated sugars and sugar nucleotides strongly influence the

degree of polymerization, molecular weight and total yield of polysaccharides. The (over)expression of these molecules and the regulation of the corresponding genes are the targets of metabolic and genetic engineering efforts for improved biopolymer processes (Stephanopoulos *et al.*, 1998; Van Kranenburg *et al.*, 1999; Ruffing and Chen, 2006).

For most exopolysaccharides synthesized intracellularly, a typical gene sequence of the order of 12–17 kb may be required for biosynthesis. One gene cluster usually regulates the synthesis of sugar nucleotides and acyl groups if required. A different gene cluster may control the assembly sugar precursors on lipid carriers, and a separate cluster seems to be responsible for polymerization and export (Kumar *et al.*, 2007). Gene size and complexity depend on the complexity of the polysaccharide structure and significant similarities in gene clusters have been observed among structurally similar polysaccharides (Sutherland *et al.*, 2001). Fig. 16.6 shows a proposed sequence of genes involved in the biosynthesis of LAB EPS, xanthan and gellan, which are some of the most well-studied biopolymers at a genetic level. In contrast, information on the gene cassette required for glucan synthesis in fungi is scarse.

During or after the biosynthetic process, polysaccharide lyases are activated in many microorganisms. The action of these enzymes is often triggered by glucose or carbon source depletion (i.e. in a prolonged fermentation process), or by the need to break down the extracullar slime or capsule in order to improve mass and oxygen transfer into the cell, which may be hindered otherwise. In addition, many of these degrading enzymes are necessary during the polymerization process, to control the size of the biopolymer and cleave parts of it, if necessary, and deletion of the genes encoding polysaccharide lyases may be detrimental to the synthesis of the biopolymer (Mattysse *et al.*, 1995). For instance, several hydrolases may appear upon a prolonged process of LAB EPS production (Degeest *et al.*, 2001), or during the stationary or death phase of *S. paucimobilis* in gellan production (for instance under high aeration rate conditions) (Giavasis *et al.*, 2006), or during alginate formation by *Azotobacter* or *Pseudomonas* sp. (Sutherland, 2001), while a β-1,3-endoglucanase and a β-glucosidase may hydrolyse scleroglucan to glucose molecules owing to carbon exhaustion (Rapp, 1989).

Interestingly, acetyl groups of gellan, alginate and acetan seem to have an inhibitory effect on the corresponding lyases, while xanthan lyases are unaffected by the presence of acyl groups (Sutherland, 1995). The susceptibility of several other glucans, such as lentinan, and *S. scerevisiae* and *Candida albicans* glucans has also been exhibited (Cutfield *et al.*, 1999; Minato *et al.*, 1999; Fernandez *et al.*, 2003). In pullulan production the decline in MW with processing time is attributed to the action of α-amylases (Manitchotpisit *et al.*, 2010). The reduction in MW and DB as a result of polysaccharases is usually undesirable since it may deteriorate the rheological properties of the biopolymers and thus process conditions have to aim

Fig. 16.6 (a) Gene clusters regulating EPS biosynthesis by *L. lactis* ssp. *cremoris* NIZO B40, localized in plasmid (adapted from Laws *et al.*, 2001). (b) Gene clusters regulating xanthan biosynthesis (adapted from Sutherland, 2001 and Becker *et al.*, 1998). (c) Gene clusters regulating gellan biosynthesis (adapted from Fialho *et al.*, 2008).

at minimal activity of these enzymes, especially after the biopolymer is formed at high concentrations. Having said this, the complete elimination of polysaccharide lyases in mutant strains may result in reduced biopolymer production as they may also be involved in biosynthesis (Sutherland, 2001; Giavasis *et al.*, 2006).

16.3.2 Industrial production

The industrial production of most microbial polysaccharides involves the batch or fed-batch cultivation (so-called 'fermentation') of the selected industrial strain in a bioreactor (or 'fermentor') under controlled conditions of agitation, aeration, pH, temperature, dissolved oxygen and process medium composition. The latter is usually a liquid synthetic medium with a standard composition based on a glucose or sucrose carbon source as the main ingredient, or a complex, carbohydrate-rich, non-synthetic medium derived from agricultural by-products (such as molasses, fruit pulp, potato pulp, corn syrup, deproteinized whey, etc). The exception to this 'fermentation' process is the biocatalytic synthesis of some polysaccharides using cell-free biosynthetic enzymes (Lopez-Romero and Ruiz-Herrera, 1977; Finkelman and Vardanis, 1987, Abdel-Fattah *et al.*, 2005), or the cultivation of mushrooms on solid media (soil, manure, sawdust, straw, etc), to produce fruit bodies from which the polysaccharides are extracted.

The control and modulation of bioprocess conditions normally has a significant effect on the quantity and the quality of the biopolymer and is a key parameter for process optimization, along with metabolic and genetic engineering, as well as downstream process efficiency. The latter is the process of isolation and purification of the end product in its final form via centrifugation or filtration, chromatographic separation, precipitation and drying, all of which play a significant role in the total cost and efficacy of the production process.

In terms of bioprocesss optimization, although plenty of data exist for polysaccharides such as xanthan, gellan, pullulan and scleroglucan, there is limited research on other microbial polysaccharide processes. However, some general rules apply to most processes. The way process parameters like nutrient composition affect polysaccharide production depends largely on whether the products are growth associated (primary metabolites) or non-growth associated (secondary metabolites). For example, a high carbon (e.g. glucose)/nitrogen (e.g. ammonium nitrate) ratio in the process medium, and subsequent nitrogen limitation, enhances scleroglucan (Farina *et al.*, 1998) and pullulan (Harvey, 1993) synthesis, by reducing the utilization of glucose for biomass (cell) synthesis. Taurhesia and McNeil (1994) reported that scleroglucan was produced at a higher concentration in a phosphate-limited medium (18.9 g l^{-1}) than in a nitrogen-limited medium (11.4 g l^{-1}). In xanthan and gellan synthesis, a high C/N ratio is required. Carbon sources are preferably used at 20–40 g l^{-1} and nitrogen sources, despite being

essential nutrients for growth, should be exhausted before polysaccharide formation arises (De Vuyst *et al.*, 1987; Garcia-Ochoa *et al.*, 2000; Giavasis *et al.*, 2006). Also, in the case of xanthan, a high concentration of nitrogen sources (low C/N ratio) has led to a low pyruvilation degree of the polymer (Casas *et al.*, 2000).

Culdlan synthesis is also boosted by nitrogen limitation and in this case it was observed that this was linked to enhanced levels of nucleotide precursors under these conditions (Kim *et al.*, 1999; McIntosh *et al.*, 2005). Conversely, cell wall polysaccharides from *S. cerevisiae* which depend on the rate of cell growth are accumulated at higher concentrations when a low carbon/nitrogen (C/N) ratio exists (Aguilar-Uscanga and François, 2003), probably due to the necessity of nitrogen for cell growth and proliferation. Carbon and nitrogen demands in exopolysaccharide-producing lactic acid bacteria differ between mesophilic species (*Lactococcus cremoris, Lactobacillus casei*) where EPS are not growth associated, and thermophilic species (*L. bulgaricus, L. helveticus*), where the synthesis of EPS is usually growth related (Cerning *et al.*, 1992; DeVuyst *et al.*, 2001).

The type of carbon and nitrogen source, also influences biopolymer production via fermentation. For instance levan from *B. subtilis* and *Z. mobilis* is elaborated at high amounts only in a sucrose medium, as opposed to glucose-based media or more complex carbon sources such as molasses and corn syrup (Senthilkumar and Gunasekaran, 2005; Shih *et al.*, 2005; De Oliveira *et al.*, 2007). Also, the composition of the carbon source(s) in the fermentation medium may influence the ratio of sugar monomers in heteropolysaccharides from LAB (Grobben *et al.*, 1996). In homopolymers from LAB, sucrose is an inducer of dextran synthesis by *L. mesenteroides*, while fructose represses dextran sucrase in the same microorganism and stimulates levan sucrase activity (Dols *et al.*, 1998; Leathers, 2002a). In gellan production, when lactose was used as the main carbon source instead of the commonly used glucose, acyl levels increased, having a negative effect on the rheological properties of lactose-derived gellan (Fialho *et al.*, 1999). Xanthan can be produced by various carbon sources and the best production yields in declining order were obtained by glucose, sucrose, maltose and soluble starch (Leela and Sharma, 2000).

The feeding strategy is another parameter influencing the efficacy of a biopolymer producing process. Although batch cultures are usually adopted on an industrial scale, in many cases a fed-batch process with a stepwise addition of the carbon source (and other nutrients) can improve the concentration of the final product, by eliminating potential substrate inhibition. For instance, optimization via a fed-batch approach has been reported for curdlan (Lee *et al.*, 1997), gellan (Wang *et al.*, 2006), scleroglucan (Survase *et al.*, 2007), ganoderan (Tang and Zhong, 2002) and *S. cerevisiae* glucan (Kim *et al.*, 2007).

Temperature and pH also influence these polysaccharide processes. For most bacterial EPS an optimal temperatuture around 28–30°C is chosen

(e.g. in the case of gellan and xanthan) (Giavasis *et al.*, 2000; Casas *et al.*, 2000), while an optimal temperature for fungal polysaccharide synthesis is usually somewhat lower (around 25–28°C) (Cheng *et al.*, 2011; Fosmer and Gibbons, 2011). The chosen process temperature is often a compromise between optimal temperature for cell growth and optimal temperature for polysaccharide synthesis, as in the case of pullulan synthesis where optimum temperature for pullulan formation (27.4 g l^{-1}) was 26°C, whereas 32° C was optimal for cell growth (10.0 g l^{-1}). In such cases a bi-staged process can be adopted, in order to achieve high biomass at a first stage and then stimulate polysaccharide synthesis at a second stage (Wu *et al.*, 2010).

In the production of EPS from LAB, the optimal temperature varies significantly. Mesophiles such as *L. cremoris* and *L. casei* strains produce higher amounts of polysaccharides at 18–20°C, conditions which are sub-optimal for growth, while high process temperatures (37–42°C) are used for EPS production by thermophiles, such as *L. bulgaricus* and *L. helveticus* (Cerning *et al.*, 1992; Mozzi *et al.*, 1995; De Vuyst and Degeest, 1999). Temperature (and other process condition) may also affect the composition of the biopolymer, as in the case of xanthan where a relatively low temperature (25°C) caused an increase in the MW and a decrease in acetate and pyruvate groups (Casas *et al.*, 2000).

For many bacterial EPS such as xanthan and gellan, synthesis as well as growth is optimal at neutral pH, and the potential acidification of the process fluid (if pH is not controlled) owing to the formation of organic acidic (such as acetate, pyruvate) is detrimental to the production process (Giavasis *et al.*, 2000; Palaniraj and Jayaraman, 2011). However, in the curdlan process, a downshift of pH from 7 to 5.5 at the exponential growth phase increased the metabolic flux for the formation of sugar nucleotides, leading to increased curdlan production compared to processes with a stable pH of 7 (Zhan *et al.*, 2012). For *Zymomonas* levan an optimal concentration (27.2 g l^{-1} for a genetically engineered strain and 15.4 g l^{-1} for its natural parent strain) has been achieved at pH 5 and 25°C, while an increase in process temperature up to 35°C stimulates ethanol synthesis instead of levan formation (Senthilkumar and Gunasekaran, 2005).

In fungal fermentation processes pH values are preferably low, for instance pH 3.5–4.5 for scleroglucan and pH 4.5–5.5 for pullulan (Harvey, 1993; Giavasis *et al.*, 2002). The pH, value of the process fluid may also determine cell morphology in fungal polysaccharide processes. At low pH *A. pullulans* cells acquire a yeast-like (unicellular) morphology which is essential for glucan formation, while at higher pH most cells are in mycelial form, which elaborates little or no pullulan (Reeslev *et al.*, 1997). Other fungal polysaccharides are optimally synthesized at low pH, such as grifolan at pH 5.5 (Lee *et al.*, 2004) and ganoderan at pH 4–4.5 (Yang and Liau, 1998) or during an uncontrolled process with a pH drop from 5 to 4 (Kim *et al.*, 2002). Shu *et al.* (2004) tested the effect of different pH levels (from 4 to 7.0) upon polysaccharide formation by *A. blazei*. They reported an

increase in biopolymer yield with increased pH, but at low pH, MW (and biological activity of the glucans) was maximal.

Aeration and agitation are also important factors for microbial polysaccharide production. As almost all microorganisms used are aerobic, oxygen is necessary for cell growth and, in the case of pullulan and xanthan, it is also stimulatory for polysaccharide synthesis (Rho *et al.*, 1988). Nevertheless, it has been reported that low dissolved oxygen (DO) in the bioreactor, and even DO limitation, can enhance scleroglucan and schizophyllan synthesis, in contrast to cell growth (Rau *et al.*, 1992). A possible explanation for this may be the presence of oxygen-sensitive biosynthetic enzymes, or the utilization of the carbon source primarily for glucan synthesis under growth-limiting conditions. Further, high (DO) and oxidative condition in the fermentor may cause a radical-induced degradation of scleroglucan (Hjerde *et al.*, 1998). Low DO levels also favour exopolysaccharide (ganoderan) production by *Ganoderma lucidum,* in contrast to cell growth (Tang and Zhong, 2003). In gellan production, some oxygen limitation may favour EPS synthesis and although good mixing (e.g. 500 rpm) is essential in the viscous gellan fermentation broth, high aeration rate (above 1 vvm, volume per volume minute) reduces gellan yield and MW (Giavasis *et al.*, 2006).

Conversely, the synthesis of cell wall polyssacchrides by *S. cerevisiae* is restricted at low aeration rate and DO (in the range 0–50%), following a decrease in biomass concentration (Aguilar-Uscanga and François, 2003). Process conditions for EPS production by lactic acid bacteria differ compared to the above processes. Here, little or no aeration is needed as most strains are microaerophilic and low agitation (e.g. 100 rpm) is usually applied, since the low EPS content of the fermentation broth (around or below 1 g l^{-1}) does not affect broth viscosity and bulk mixing (De Vuyst and Degeest, 1999). This is also the case for dextran-producing strains of *L. mesenteroides*, which require no aeration (Leathers, 2002a), while the alternan-producing *L. mesentoroides* NRRL B-1355 showed optimal levansucrase activity at a moderate DO level (controlled at 75%), as opposed to the non-aerated processes (Raemaekers and Vandamme, 1997).

Oxygen regulation is critical for the production of bacterial alginate which is optimal under microaerophilic conditions. This has been ascribed to the inactivation of some alginate biosynthetic enzymes through higher oxygenation, inactivation of the metabolically important nitrogenases and the fact that at high DO alginate forms a very rigid capsule which hinders nutrient transfer into the cell (Leitão and Sá-Correia 1997; Sabra *et al.*, 2001). The latter, that is the compromise of mass and oxygen transfer by the high viscosity that gradually develops in most microbial polysaccharide processes is a universal problem and may become more intense in fungal fermentation where the floating mycelia intensify this problem. In this sense, high agitation is usually required for adequate mixing. For example, the amount and quality (molecular weight) of pullulan is improved at high

agitation rates, as this promotes the formation of yeast-type (unicellular) cells (McNeil and Kristiansen, 1987). However, in the case of scleroglucan, high agitation stimulates cell growth at the expense of polysaccharide concentration (Schilling *et al.*, 1999). The same authors suggest that moderate agitation has to be applied for production of high molecular weight scleroglucan.

Apart from the above-mentioned process parameters, variance in polysaccharide concentration is also observed among different microbial strains. For example, levan production by *B. subtilis* and *Z. mobilis* is much higher (40–50 g l^{-1}) than that achieved with *Streptococcus salivarus* (Newbrun and Baker, 1967; Viikari, 1984; Shih *et al.*, 2005). Variations between scleroglucan yields obtained from *S. glucanicum* and *S. rolfsii* have also been reported (Giavasis *et al.*, 2002).

Another step in the polysaccharide production process that is crucial for biopolymer yield and quality is the isolation and purification process, in other words the downstream processing. Downstream processing may affect the molecular size or molecular weight distribution, DB, composition (presence of side groups) and of course the total product yield, and accounts for a large part of the total production costs (Lee *et al.*, 1996; Wasser, 2002; Wang *et al.*, 2010). Therefore optimization of this process is a challenging task for chemical engineers. There is no simple method for extracting all microbial polysaccharide. For each biopolymer an appropriate extraction technique must be chosen and optimized based on the structural and physicochemical characteristics of the molecule to be extracted and the desired purity and intended use. For food applications, biopolymers should be free of biomass (cell debris and other intracellular components) and of the reagents used for extraction.

In the case of extracellular slimy polysaccharides, this processing generally involves sterilization or pasteurization of the fermentation broth to kill cells, inactivate undesirable enzymes (e.g. lyases) and facilitate separation of cells from the EPS, subsequent removal of cells by filtration or centrifugation, alchohol pecipitation of the polysaccharide in the cell-free filtrate or centrifugate, followed by further purification (if required) by ultrafiltration, gel permeation/ion-exchange chromatography or diafiltration. The end product in powder form is obtained after drying with air/inert gas/under vacuum, or spray drying, or lyophilization, and final milling to the desired mesh size (Giavasis and Biliaderis, 2006; Singh *et al.*, 2008; Donot *et al.*, 2012).

When the exopolysaccharide is firmly attached to the cell wall (capsular EPS), an initial step of hot alkali treatment or sonication is usually employed to facilitate disengagement of the biopolymer from the cell (Morin, 1998; Wang *et al.*, 2010). This treatment can also result in decolorization or deacylation of the gum, which is sometimes needed to improve its sensory and rheological characteristics (for example in the case of gellan or pullulan) (Giavasis *et al.*, 2000; Kumar *et al.*, 2007). Moreover, in some capsular EPS

such as xanthan, enzymatic cell lysis for cell removal may be more effective and preferable than hot alkali treatment, as it does not affect the composition and physicochemical properties of the polymer (Shastry and Prasad, 2005).

In the case of viscous biopolymers such as xanthan and gellan, thermal pretreatment reduces the high viscosity of the fermentation broth which facilitates further chemical and physical treatment and improves xanthan removal from the cells. However, care must be taken so that pasteurization/sterilization of the fermentation broth at elevated temperatures does not cause thermal degradation of the polysaccharide (Smith and Pace, 1982). Dilution in water or dilute solutions of salts or alcohols can by used alternatively to reduce the viscosity of the process fluid and facilitate filtration and removal of impurities (Garcia-Ochoa *et al.*, 1993). Fosmer and Gibbons (2011) suggested a 50% dilution for optimal extraction of scleroglucan from the fermentation broth via filtration. Cross-flow filtration is a common technique for membrane separation of fungal EPS, where a series of connected filtration cassettes with different MW cutoffs separated cells, proteins, sugars and salts from the EPS. However, the use of ceramic membranes may prove a better alternative, as they provide sterility, stability (against fouling) and reusability (Schmid *et al.*, 2011).

Precipitation of capsular gums like xanthan and gellan is usually achieved by mixing with double (or triple) volume of alcohol or acetone (which represent a significant part of the total processing cost), or divalent, trivalent or tetravalent salts. For the precipitation of food grade xanthan, FDA prescribes the use of isopropanol. Using a combined alcohol and salt precipitation has led to improved xanthan precipitation and yield and reduced the amount of alcohol required (Garcia-Ochoa *et al.*, 1993). In order to reduce the use of solvents, ultrafiltration can prove useful, as it can concentrate xanthan broth by at least five times before the solvent is added (Palaniraj and Jayaraman, 2011). Lo *et al.* (1996) have suggested ultrafiltration of dilute xanthan broths as a complete alternative to alchohol precipitation, which did not impair the physicochemical properties and molecular weight of xanthan. For charged polysaccharides an ion exchange resin may be well suited for purifying the final product without use of undesirable chemical reagents. However, physical extraction (via sonication and separation in resins) may have a lower yield of extracted polysaccharides compared to chemical methods (treatments with alkali, alcohols and acids) (Donot *et al.*, 2012).

To isolate (fungal) cell-wall polysaccharides, a somewhat different process is followed compared to extracellular biopolymers, which includes extraction of polysaccharides from the cell mass with hot alkali or hot water, followed by filtration, centrifugation or dialysis of the extracted material, in order to remove insoluble impurities and finally drying of the purified polysaccharide (Cutfield *et al.*, 1999; Wasser, 2002; Hromadkova *et al.*, 2003). Insoluble polysaccharides are usually isolated by washing the debris of both

cells and biopolymer with dilute acid solution, solubilization of the polysaccharide with alkali solution, centrifugation and collection of the polysaccharide from the supernatant after addition of a new, denser acid solution (Lee *et al.*, 1997).

16.4 Properties and structure–function relationships

The physicochemical properties of microbial polysaccharides such as solubility, viscosity, gelation, water binding, dispersability, emulsifying and stabilizing ability, film formation, interaction with other molecules, chemical stability and degradation are crucial for their application in food and other materials, as has been illustrated in Sedion 16.2 by several examples of food applications. Similarly, their dietary, immunostimulating and other health effects are important for designing functional foods and nutraceuticals. These properties are influenced principally by the composition, the chemical structure and the conformational order (random coil, single, double or triple helix), the degree of branching of biopolymer, the presence of salts or other molecules, and the processing or treatments that they undergo before being used. Below, the relationship between structure and function of these biopolymers will be highlighted.

16.4.1 Physicochemical properties

Xanthan gum is highly soluble biopolymer in both cold and hot water, thanks to its polyelectrolyte nature (presence of acyl groups) and can produce highly viscous solutions, but does not form gels (Garcia-Ochoa *et al.*, 2000). The xanthan molecule seems to have two conformations, helix and random coil, depending on the dissolution temperature and the presence of cations. The conformation shifts from disordered state (at high dissolution temperature) to an ordered state (at low-dissolution temperature and high ionic strength), thus solutions formed at 40–60°C are more viscous than those formed above 60°C (Garcia-Ochoa and Casas, 1994; Casas *et al.*, 2000). X-ray diffraction showed that in the molecular conformation of a (right-handed) helix the trisaccharide side chain is aligned with the backbone and stabilizes the overall structure, principally by hydrogen bonds. In solutions this side chain folds around the backbone protecting it from enzymic or acidic hydrolysis (Palaniraj and Jayaraman, 2011).

The pyruvyl and acetyl groups of xanthan can be removed at low (pH 3) or high pH (pH 9), but this does not have a significant effect on rheological properties (Garcia-Ochoa *et al.*, 2000). In contrast, the acyl content of gellan (acetate/glycerate) plays a crucial role in the gelling properties of the molecule. Thus, a high-acyl gellan produces a soft gel and can be useful in increasing viscosity and improving body and mouthfeel of

foods, while a low-acyl gellan forms rigid, brittle and stable gels which can withstand high temperatures and low pH (Giavasis *et al.*, 2000; Fialho *et al.*, 2008). This phenomenon has been ascribed primarily to the removal of the glycerate groups during deacylation by hot alkali treatment, which decide the level of rigidity or elasticity of gellan gells, along with the presence of divalent cations (Jay *et al.* 1998; Giavasis *et al.*, 2000). Gellan molecules acquire a disordered coil conformation at high temperatures which transform to a double helix upon cooling. At high concentrations, the double helices become compact rod-like aggregates and form gels (Khan *et al.*, 2007).

The effect of deacylation and the removal of side groups of succinoglycal depends on the molecule that is removed. Deacetylation of succinoglycan gels decreased the transition temperature, while the desuccinylated biopolymer had increased the transition temperature and greatly improved pseudoplasticity (Ridout *et al.*, 1997). In the case of acetan aqueous solutions a thermoreversible helix-coil formation is obtained, where acetylation does not play a crucial role (triple helix is not prevented by the presence of acetyl groups) (Ojinnaka *et al.*, 1996; Ridout *et al.*, 1998).

Dextran physicochemical properties vary significantly, depending mainly on the microbial strain used for production, but even in a single strain there is heterogeneity in the molecular size and the proportion of α-(1,6) linkages of dextrans, thus the dextrans resulting from fermentation are fractionated according to molecular size and the intended application (Alsop, 1983; Naessens *et al.*, 2005). Commercial dextran is a white fine powder that dissolves easily in hot or cold water to produce moderately viscous, clear solutions. It does not form gels and remains dissolved even at high concentrations (50%). However, high molecular weight dextrans tend to have a higher degree of branching which in turn has an adverse effect on dextrans solubility in water (Khalikova *et al.*, 2005; Khan *et al.*, 2007). Dextrans with more than 43% of α-(1,3) linked branches, such as mutan are considered insoluble in water (Mehvar, 2000; Monsan *et al.*, 2001). Interestingly, dextrans of medium and low MW (< 500 kDa) exhibit Newtonian behaviour in water solutions at concentrations below 30% (w/v) (Khan *et al.*, 2007). Purified dextran with a MW of 500,000 Da exhibits Newtonian behaviour below a 30% concentration, while 'native' dextran with a higher MW shows a slight pseudoplasticity above 1.5% concentration (McCurdy *et al.*, 1994). The conformation of the molecule also depends on the molecular mass and polymer concentration. At low concentrations dextran (MW 500,000) appears as a random coil, but high concentrations in solutions lead to a more compact coil. The non-Newtonian native dextran is characterized by inter-chain interactions. In addition, concentration positively affects the melting point of dextran (T_g) (McCurdy *et al.*, 1994).

Alternan acquires an extended and tightly coiled conformation in solutions, owing to its unique alternating structure of α-(1,6) and α-(1,3)

linkages (with approximately 10% DB) (Seymour and Knapp, 1980; Leathers *et al.*, 2003). This structure imparts properties of high solubility and low viscosity, despite its high MW (10^6–10^7 Da), as well as resistance to hydrolysis by most microbial and mammalian enzymes (Cote, 1992). In a test period of seven days, alternan was shown to form stable solutions in a pH range of 3–9, and a temperature range of 4–70°C (Leathers *et al.*, 2003). Despite its relatively low viscosity, dense solutions of native alternan (above 12–15%) are difficult to attain, owing to its high molecular weight and relatively high viscosity at that concentration. Thus, attempts have been made to reduce the MW of native alternan. Sonication of alternan yields a modified alternan of MW below 10^6 Da, which is able to give solutions of 50% concentration or higher, with novel rheological properties resembling those of gum arabic (Cote, 1992). Alternatively, native alternan can be modified after hydrolysis by *Penicillium* sp. to yield a polymer with similar viscosifying and bulking properties to gum arabic (Leathers, 2003). More extensive hydrolysis with isomaltodextranase gives a very low MW polymer (MW around 3500 D), with similar rheological characteristics to maltodextrins (Cote, 1992).

Curdlan hydrogels are comparatively weak when prepared by heating at 55–70°C, followed by cooling. Further heating to 80–100°C increases the gel strength and produces an impressively firm and resilient gel, which will not melt upon reheating below 140°C. Autoclaving at 120°C converts the molecular structure to a triple helix which is resistant to microbial degradation (Sutherland, 1998). The curdlan gels are firmer and less elastic than gelatine gels and less brittle than agar and gellan gels. One drawback is that they are susceptible to shrinkage and syneresis. Gels are also formed when alkaline solutions of curdlan are dialysed against distilled water or are neutralized. Interestingly, curdlan gel strength depends on the level and length of heating and on curdlan concentration, and is stable over a wide pH range (2–10), or in the presence of other sugars, salt and lipids (McIntosh *et al.*, 2005; Laroche and Michaud, 2007).

Bacterial levans exhibit high solubility in water, despite their high molecular weight. This is attributed to the highly branched structure of levan (Newbrun and Baker, 1967; Stivala *et al.*, 1975). On the other hand, levan generally has a much lower intrinsic viscosity compared to other polysaccharides of similar molecular weight (Arvidson *et al.*, 2006), which is assigned to the compact spherical or ellipsoidal conformation of the molecule, from which the branches extend radially (Stivala *et al.*, 1975; Rhee *et al.*, 2002). In fact, the viscosity of levan solutions from *Pseudomonas syringae* pv. *phaseolica* remain Newtonian up to a polymer concentration of 20% (Kasapis *et al.*, 1994). *B. subtilis* levans may be fractionated to low molecular weight levan and high molecular weight levan, the former having a lower viscosity than the latter (Shih *et al.*, 2005). Kasapis *et al.* (1994) indicate that levans from *P. syringae* pv. *phaseolica* exhibit solution properties similar to those of disordered linear polysaccharides and no detectable

conformational change with temperature. The same authors observed thermodynamic incompatibility and subsequent reduction of viscosity in mixed solutions of levan–pectin and levan–locust bean gum, which may be related to the role of levan in microbial phytopathogenesis, where levan may act as a barrier protecting bacterial cells from plant cell defence. In addition, levan is non-gelling and non-swelling in aqueous solutions at ambient temperature (Kasapis and Morris, 1994), biocompatible and may form turbid lyotropic crystalline solutions (liquid crystals) at low concentrations (Huber *et al.*, 1994). Relative structural stability of *Z. mobilis* levan is achieved in purified levan–water solutions at pH 4 to 5 and temperatures between 25 and 70°C (Bekers *et al.*, 2005). Bacterial levans are susceptible to enzymic or acid hydrolysis, thus their use as low-calorie ingredient in food is doubtful, in contrast to plant levans which are resistant to digestion in humans (Bekers *et al.*, 2005; Izydorczyk *et al.*, 2005).

Alginate gels have an adjustable strength. This depends on the number of intermolecular cross-links that can be formed between chains, on the type of ions facilitating cross-linking and on the length of blocks between the links. Alginates with high polyguluronate content can form rigid gels in the presence of calcium, especially after deacetylation, while viscosity is mainly a function of the molecular weight of alginate (Moe *et al.* 1995; Sutherland, 2001).

Pullulan is distinguished by its structural flexibility and high solubility, owing to its unique alternating (1,4) and (1,6) structure (Leathers, 2002b). It is readily soluble in water (except for esterified or etherified pullulan), insoluble in organic solvents and non-hydroscopic (Le Duy *et al.*, 1988; Leathers, 2002b). Pullulan forms solutions that are stable in the presence of cations, but does not form gels, probably owing to its linear structure (Izydorczyk *et al.*, 2005). Upon drying, pullulan solutions can form films, the elasticity of which can be improved by addition of plasticers like glycerol, or sorbitol (Singh *et al.*, 2008).

Elsinan is readily dissolved in hot water and gives stable and very viscous solutions, but at high concentrations (approximately ten times that of pullulan). Unlike the structurally similar pullulan, it forms gels at concentrations of 2% when the temperature is lowered to around 4°C, or 5% at higher temperatures (Tsumuraya *et al.*, 1978). Elsinan solutions are highly pseudoplastic and at high shear rates viscosity falls rapidly. Temperature affects the viscosity of these solutions in the following manner: when a 2% solution is gradually heated from 30–45°C a slight decrease in viscosity is observed, after which viscosity increases to reach a maximum value at 60°C. Beyond this temperature viscosity is again reduced and preheating the solution at 90°C for 30 min results overall in lower viscosity (Sandford, 1982). In contrast, viscosity is unaffected by pH in range from 3 to 11 and by several electrolytes at various concentrations, probably owing to its neutral, non-ionic nature (Misaki, 2004). Salivary and

pancreatic amylases as well as *B. subtilis* amylases are capable of hydrolysing elsinan-releasing maltotriose units, while *Aspergillus oryzae* amylases only cleave maltotetraose units (Tsumuraya *et al.*, 1978; Misaki *et al.*, 1982; Misaki, 2004).

Scleroglucan, in a refined form, is also soluble in water at ambient temperatures, owing to the presence of β-D-glucopyranosyl residues that enhance solubility and reduce gelation, resulting in stable viscous solutions over a temperature range of 10–90°C and a pH range of 1–11 (Wang and McNeil, 1996, Giavasis *et al.*, 2002). Loss of viscosity occurs only above pH 12, or after addition of DMSO (dimethylsulphoxide) which results in the disruption of the triple helix of the polymer (Nardin and Vincendon, 1989). Both refined scleroglucan and crude scleroglucan (60–75% gum) solutions (either hot or cold) are pseudoplastic (shear thinning) in concentrations above 0.2–0.5% and compatible with many salts (for example no change in solubility is observed in a 5% NaCl, 20% $CaCl_2$, 5% $NaSO_4$ or a 10% Na_2HPO_4 solution), as well as other polymers, such as gelatine, gellan, xanthan, carrageenan, guar gum and locust bean gum, although no synergism with other viscosifiers is observed (Brigand, 1993). In aqueous solutions of 1.2–1.5% concentration, purified scleroglucan forms sliceable thermoreversible hydrogels at approximately 25°C, caused by cross-linking of the triple helix. At low temperatures around 7°C, the swollen gels are softer and more diffused (Bluhm *et al.*, 1982). The stability of scleroglucan solutions is distinctively better than that of xanthan and other biopolymers, owing to its high molecular weight (Giavasis *et al.*, 2002; Schmid *et al.*, 2011). Apart from the microbial hydrolysis observed in *S. glucanicum* cultures, scleroglucan is not easily degradable and is considered undigestible for humans (Rapp, 1989).

Like many other glucans, schizophyllan and lentinan acquire a single or triple helix conformation with short side groups in their soluble form, stabilized by hydrogen bonds. The degree of branching (DB) and MW strongly influence the solubility and functionality of these molecules as will be discussed later (Bluhm *et al.*, 1982; Toshifumi and Ogawa, 1998; Falch *et al.*, 2000). Fang *et al.* (2004) studied the ability of schizophyllan to form co-gels with gelatin, where the concentration of schizophyllan affected the elasticity or rigidness of the gels (higher elasticity at low gelatin/schizophyllan ratio). Their findings could be useful in food applications where gelatin is already used as a thickener.

Brewer's yeast glycan (BYG) dissolves readily in water, at low or high temperatures, the latter leading to higher solution viscosity. Measurable viscosity is attained only above a 4.5% concentration of the glycan and it is unaffected by pH over the range 2–7 or by the addition of sodium chloride up to a 5% concentration. Viscous solutions of BYG become thinner upon heating and thicker upon cooling and have good freeze–thaw stability (Sandford, 1982).

16.4.2 Nutritional and health effects

Even though not all of the microbial polysaccharides mentioned above are used in commercial food applications, most of them are potentially useful as indigestible dietary fibres, as described in Section 16.2. Toxocilogical tests for those already used in food have revealed that they impose no danger to human health, while many others come from edible mushrooms, so no concerns over potential toxicity should arise. On the contrary, over the last few years their beneficial effects on human health have been widely researched and are already or expected to be utilized in the design of novel functional foods or nutraceuticals. More specifically, many of these polysaccharides possess antitumour, immunostimulatory, antimicrobial or hypolipidaemic and hypocholesterolaemic properties, which are influenced by the structural properties of the biopolymers.

Pullulan is only partially degraded by human amylases and thus has no nutritional value, neither is it not toxic or mutagenic (Okada *et al.*, 1990). In studies with mice fed with pullulan as a 40% replacement for starch, organ-to-body weight was normal (equivalent to the control group fed with starch only), but hypertrophies in the large intestine were found (Sandford, 1982). Interestingly, pullulan has been proposed as a probiotic for humans, since it proved to promote the growth of bifidobacteria (Mitsuhashi *et al.*, 1990). As for scleroglucan, no toxicity, tissue pathology or blood abnormalities have been observed in studies on rats and dogs, and no eye or skin irritation in pigs, rabbits and humans, while its role as an undigestible dietary fibre for humans has been suggested (Rodgers, 1973). Elsinan is gradually digested by human amylases, but more slowly compared to starch (Sandford, 1982).

Feeding tests with dextran have shown that it is slowly but totally metabolized in humans (Halleck, 1972). Despite this, it has been reported that a diet rich in dextrans with high proportion of α-(1,6) linkages contributed to body weight loss (probably owing to slow absorption in the intestine), as these type of linkages are resistant to enzymic attack in the gastrointestinal tract, as opposed to α-(1,4) glycosidic linkages of starch and glycogen which are readily hydrolysed by human α-amylases (Naessens *et al.*, 2005). Dextran, as well as the non-digestible alternan, are biocompatible and non-toxic for humans and the producer strains are safe for use in food (GRAS), as are all LAB and their products. Nutritionally, the susceptibility of levans to acid and enzymatic hydrolysis, suggests that their use as dietary fibre in food is doubtful, in contrast to their plant equivalents (Bekers *et al.*, 2005; Izydorczyk *et al.*, 2005). However, levan-producing strains of *Z. mobilis, B. subtilis* and some Lactobacilli, concomitantly synthesize fructo-oligosaccharides (FOS). FOS, or the mixture of levan and FOS (referred to as 'fructan syrup') are considered new prebiotic substances, as they induce the growth of *Bifidobacterium* sp. and other beneficial microflora in the intestine, improve intestinal functionality, as well as having a low energetic value and a pleasant honey-like taste (Yun, 1996; Dal Bello *et al.*, 2001; Bekers *et al.*, 2004).

For *S. cerevisiae*, another GRAS microorganism, no adverse toxicological data exists for the polysaccharides it elaborates and the use of the organism and its products in food is considered safe.

The multiple health effects of microbial polysaccharides have been studied extensively in the last few years as a response to consumers' demand for healthier food and the need to develop new, milder and more natural bioactive or pharmaceutical ingredients for 'preventive medicine'. These effects can be summarized as immunostimulating-antitumour, antimicrobial and hypocholesterolaemic–hypoglycaemic. The relationship between structure and function of these biopolymers is of great impotance and has been reviewed previously (Sutherland, 1994; Bohn and BeMiller, 1995; Wasser 2002; Giavasis and Biliaderis, 2006). Generally, the primary characteristics that affect the bioactivity of biopolymers are the MW, the type of glycosidic linkage and DB, the conformational state (random coil or helix) and the chemical composition and potential derivatization (Giavasis and Biliaderis, 2006). It appears that high MW and medium or high DB and a (triple) helix conformation, as well as the presence of β-(1,3) linkages play an important role in the expression of antitumour or immunomodulating activity of glucans, possibly owing to the presence of a β-(1,3)-glucan receptor in macrophages, which enables macrophage activation by β-(1,3)-glucans (however there are also reports on immunopotentiating β-(1,6) glucans or bioactive glucans lacking a triple helix, which probably have a distinct immunostimulatory mechanism).

Anti-cholesterol and anti-glycaemic effects are also linked to medium or high MW of the biopolymer, even though some controversial reports are available in the literature (Wasser 2002; Giavasis and Biliaderis, 2006; Laroche and Michaud, 2007; Zhang *et al.*, 2011). Trying to shed light on the impact of structural conformation on the immunostimulating effects of several β-glucans, Suzuki *et al.* (1992) concluded that the triple helix can enhance the alternative pathways of complement (APC) in the immune system more effectively than the single helix, whereas a single helix is a better stimulant of the classical pathways of complement (CPS), which explains some controversial reports on structure–function relationships, since different modes of bioactivity (all contributing to the overall performance of the immune system) are triggered by structurally different biopolymers.

Many fungal β-glucans are associated with a fortified immune system and treatment of cancer, especially in traditional oriental medicine. They act either directly against the tumour cells, or indirectly by boosting the immune system. For example, scleroglucan has very important attributes as a biological response modifier. Sinofilan, the immunopharmacological form of scleroglucan, is used effectively in the clinical treatment of cancer. Scleroglucan has a reportedly high affinity for human monocytes and stimulates phagocytic cells and monocyte, neutrophil and platelet haemopoietic activity (Jamas *et al.*, 1996; Giavasis *et al.*, 2002). In addition, *Sclerotinia*

sclerotiorum glucan (SSG), the pharmacological form of a similar glucan from *Sclerotinia sclerotiorum*, exhibits antitumour and immunostimulating properties when administered parenterally, or orally (Suzuki *et al.*, 1991; Bohn and BeMiller, 1995). Interestingly, oral administration which is important for avoiding pain and side effects and indicative of the potential for incorporation of the biopolymer in nutraceuticals, is a distinct advantage of scleroglucan compared to other immunoactive glucans.

Schizophyllan (a biopolymer chemically and structurally similar to scleroglucan) and lentinan are another two fungal immunotherapeutic glucans used clinically for cancer treatment since 1986, usually in combination with conventional cancer therapies. Schizophyllan, administered along with antineoplastic drugs, seemed to prolong the life of patients with lung, gastric or cervical cancer (Furue, 1987; Wasser, 2002). Further, it can restore mitosis in bone marrow cells suppressed by antitumour drugs (Zhu, 1987). Lentinan from the mushroom *L. edodes* is also effective against gastric, colorectal or breast cancer and in the prevention of metastasis. Notably, both lentinan and schizophyllan, as well as scleroglucan, have low or no toxicity even at high doses and are more effective when administered at the early stages of a treatment (Bohn and BeMiller, 1995; Ikekawa, 2001). In terms of structure–function relationships, it has been clarified that high MW schizophyllan (100,000–200,000 Da or higher) which acquires a triple helix, exhibits significant antitumour activity, while lower MW fractions (5,000–50,000 Da) or denatured polymers lacking a triple helix are biologically inactive (Kojima *et al.*, 1986). Similarly, high molecular weight lentinan with a triple helix conformation had the highest bioactivity compared to low MW or partially denatured polymers (Zhang *et al.*, 2011). In contrast, Kulicke *et al.* (1997) concluded that bioactivity of scleroglucan was not dependent on or favoured by an ordered helix structure, in fact random coil conformations of scleroglucan were better activators of human blood monocytes.

Other immunomodulating glucans include Krestin or PSK (a commercialized antitumour glucan with broad immunostimulating and antineoplastic activity) and the proteglucans from *A. blazei* fruit bodies (Zhu, 1987; Hobbs, 1995; Fujimiya *et al.*, 1998).

The antitumour activity of fungal β-D-glucans is probably due to the mitogenic activity of soluble glucan molecules (especially when they adopt a triple helix conformation), which provoke a number of immune responses, such as activation of natural killer (NK) cell and T-cell mediated cytotoxicity, stimulation of monocytes, increased synthesis of immunoglobulins and cytokines, for example interferons (IFN), interleukines (IL) and tumour necrosis factor-α (TNF-α), activation of peripheral mononuclear cells (PMNC), and enhancement of phagocytosis by neutrophils and macrophages, which can destroy malignant cells (Reizenstein and Mathe, 1984; Arinaga *et al.*, 1992; Falch *et al.*, 2000; Giavasis *et al.*, 2002; Wasser, 2002). It is interesting that interactions of lentinan and other bioactive polymers with

carrageenans may restrict their antitumour activity (Hamuro and Chihara, 1985). This underlines the need for *in vivo* studies to be carried out in a realistic food matrix, rather than polysaccharides in pure form, before these polysaccharides are applied in potential functional foods and nutraceuticals.

The insoluble glucans synthesized by *S. cerevisiae* exhibit various immunostimulating effects. PGG and Zymosan have mitogenic activity and increase the production of cytokines and monocyte and neutrophil phagocytosis. Also, solubilized *S. cerevisiae* glucans after chemical derivatization (e.g. glucan sulphate, sulphoethyl glucan, carboxymethyl glucan and oxidized glucan) show equal or higher antitumour activity compared to the native insoluble glucans (Jamas *et al.*, 1991; Bohn and BeMiller, 1995; Sandula *et al.*, 1999). With regard to structure, *S. cerevisiae* β-D-glucans with a DB of 0.2 had higher immunostimulating activity compared to glucans with a DB of 0.05, owing to the higher affinity of the branched glucan for the β-glucan receptor of human macrophages. Also, immunomodulatory activity of *S. cerevisiae* particulate β-D-glucans was associated with high molecular weight among glucans of 500,000–4000,000 Da (Cleary *et al.*, 1999).

Levans from *Z. mobilis, B. subtilis* (natto), *Aerobacter* sp., *Microbacterium laevaniformans* and *Rahnella aquatilis* also exhibit anticarcinogenic, radioprotective and immunomodulating properties, including the prevention of allergic disorders. These are mediated by generation of mononuclear cells, increase in peripheral leucocytes and spleen cell antibodies, and the stimulation of macrophages, the induction of interleukin and the control of immunoglobulin levels in the serum (Calazans *et al.*, 2000; Yoo *et al.*, 2004; Yoon *et al.*, 2004; Xu *et al.*, 2006). The high molecular weight and increased degree of branching is reported to play a decisive role in the expression of such properties by levans (Yoo *et al.*, 2004; Yoon *et al.*, 2004).

Although native, unbranched and insoluble curdlan does not posses immunostimulating properties, chemically modified and branched curdlans are biological response modifiers and exhibited significant antitumor activity, which may not involve the typical stimulation of macrophages and phagocytosis observed in other bioactive glucans (Bohn and BeMiller, 1995; McIntosh *et al.*, 2005).

Moreover, exopolysaccharides from lactic acid bacteria (other than dextran or alternan) have been isolated, which have antitumor or immunostimulatory activity *in vitro* and *in vivo*. Specifically, a phosphopolysaccharide from *L. lactis* ssp. *cremoris* stimulates lymphocyte mitogenicity, macrophage cytostaticity, cytokine synthesis in macrophages and antigen-specific antibody production (Nakajima *et al.*, 1995). A similar phosphopolysaccharide from *Lactobacillus delbrueckii* spp. *bulgaricus* injected intraperitoneally (solutions of 10–100 μg ml^{-1} administered in a 100 mg kg^{-1} dose in mice), caused an increase in the number and the tumouricidal activity of intraperitoneal macrophages (Kitazawa *et al.*, 2000). The phosphate

group of these polymers seems to be essential for the expression of these properties (Kitazawa *et al.*, 1998, 2000).

Apart from the anticarcinogenic–immunomodulatory effects, the antimicrobial activity of several microbial glucans may find novel applications in food. Sizofiran (a commercialized pharmacological schizophyllan product) was used successfully in order to stimulate immune responses of patients with hepatitis B virus, via the increased excretion of interferon-gamma and the proliferation of peripheral blood mononuclear cells (PBMC) (Kakumu *et al.*, 1991).

Lentinan is also active against bacterial infections, such as tuberculosis and *Listeria monocytogenes* infection. The antimicrobial activity of lentinan is reportedly accomplished by an improvement in phagocytosis of microbial cells by neutrophils and macrophages (Furue, 1987). The immunomodulating and microbiocidal activity of lentinan against *Salmonella enteritis* and *Staphylococcus aureus* was also shown in immunological studies (Mattila *et al.*, 2000).

Insoluble glucan from baker's yeast, as well as SSG glucan from *S. sclerotiorum* helped control the growth of *Mycobacterium tuberculosis* (*in vitro*) (Hetland and Sandven, 2002), while the supply of a relatively low dose of PGG glucan from *S. cerevisiae* inhibited the growth of antibiotic-resistant *S. aureus* in the blood of contaminated rats, which was linked to elevated activity of monocytes and neutrophils (Liang *et al.*, 1998). In addition, oral immunization with levan from *A. levanicum* was tested successfully against pneumonia caused by *P. aeruginosa* and proved to induce levan-specific titres of serum immunoglobulin A, especially when supplied at the beginning of the infection (Abraham and Robinson, 1991).

The indigestible or slowly degraded biopolymers also have potentially hypocholesterolaemic and hypoglycaemic properties, although these have not been extensively studied in clinical experiments. Few reports are available with regard to the potential anticholesterol and antiglycaemic effects of commercial microbial polysaccharides, with some exceptions, as in the case of alginates (Khotimchenko *et al.*, 2001). Elsinan and levan, for example, have exhibited remarkable cholesterol-lowering effects in hypercholesterolaemic rats (Yamamoto *et al.*, 1999; Misaki, 2004).

Additionally, the documented contribution of dextrans to body weight loss is probably due to the slow and gradual hydrolysis of the molecule, which suppresses blood glucose levels (Naessens *et al.*, 2005). The ability of sodium alginate to lower blood glucose and increase faecal excretion of cholesterol has been reported to depend on the MW, with polymers of 100,000 Da being more effective than polymers of 50,000 or 10,000 Da (Kimura *et al.*, 1996) Although there are no reports on biological activity of native pullunan, chemical derivatization of pullulan may infer anticholesterol activity, as has been achieved with diethyl-amino-ethyl (DEAE) derivatized gellan which obtained negative and positive charges and acquired novel bile acid binding and anticholesterolaemic capacity (Yoo

et al., 2005). Lentinan from shiitake mushrooms can also be used for the treatment of cholesterol in humans. It works by reducing overall levels of lipoproteins (both high dersity lipoprotein (HDL) and low density lipoprotein (LDL) in blood (Breene, 1990). It is generally proposed that the reduction of cholesterol levels is due to the interruption of enterohepatic circulation of bile acids, which leads to higher liver cholesterol and bile acid excretion in the feces (Seal and Mathers, 2001), while the regulation in blood glucose levels results from the attachment of undigestible polysaccharides to the intestinal surface, which decelerating glucose absorption (Hikino *et al.*, 1985; Kimura *et al.*, 1996). The incorporation of these biolymers in novel foods could lead to the production of innovative products which might help regulate the cholesterol blood levels of consumers.

16.5 Future trends

Microbial polysaccharides are complex molecules with versatile properties and numerous applications in foods; the search for new biopolymers with attractive properties continues. However, only a handful of experimentally studied polysaccharides have been commercialized owing to problems related to low production yields, costly manufacture or regulatory restrains, which may be overcome in the future. Future research at a biosynthetic and genetic level and optimization of bioprocesses and extraction methods is necessary and is expected to broaden their use in the food industry and allow the commercialization of novel biopolymers. For instance, the regulation of genes and the overexpression or downregulation of key biosynthetic enzymes involved in the synthesis of LAB polysaccharides or mushroom biopolymers (where genetic and metabolic engineering studies are still scarce), coupled with cost-effective downstream processing (e.g. using robust filtration systems and a single-step extraction of exopolysaccharides) may lead to economically viable production of food grade biopolymers from GRAS microorganisms, which could be readily adopted by the food industry. More research is needed with regard to the nutritional and health effects of microbial polysaccharides, especially at the level of clinical trials, in order to test their performance as ingredients in functional foods and consolidate potential health claims of novel food products. The latter is expected to boost the adoption of several biopolymers as bioactive molecules in novel foods and nutraceuticals, as many of them have shown impressive medicinal properties *in vitro* or in clinical trials in their purified form. Since these biopolymers are to be incorporated in a complex food matrix, the interactions with other food components and the impact of food processing (i.e. effect of high/low temperature, high pressure or vacuum, drying, acidic environment and presence of cations, presence of other polysaccharides or proteins which may alter their functional properties) on these molecules should be thoroughly studied. For established food

polysaccharides, novel uses, for example in edible films or coatings that improve shelf life and stability of food (alone or in combination with other ingredients) and modification of their structure, composition and properties are other interesting areas of research. All the above ideas are expected to bring about innovative food applications with high consumer acceptance and commercial success for these exciting products of microbial metabolism.

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