MICROBIAL POLYSACCHARIDES. PRODUCTION, CHARACTERISATION AND PROPERTIES

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Abstract: This paper presents the main microbial polysaccharides produced at industrial and technical level, their functions in the natural environment and in industry and the methods used for the characterisation and analysis of functional properties.

Keywords: extracellular polysaccharides, microorganisms, biological functions, characterisation, functional properties

The industrial use of polysaccharides based until recently on materials extracted from plants (starch, cellulose, pectins, galactomanans, gums) or algae (carragenan, alginates, agar). In the last 30 years, biotechnologies for the microbial production of extracellular polysaccharides were developed (Dumitriu, 2002).
The polysaccharides of microbial origin are largely used in food industry, biotechnology, medicine and pharmacy (Sutherland, 2002). The success is due to their properties and to the diversity of producing microorganisms and synthesised polysaccharides.

1. MICROORGANISMS USED AND INDUSTRIAL PRODUCTION

The microorganisms used as industrial or technical producers of extracellular polysaccharides are bacteria. Species of *Xanthomonas, Leuconostoc, Pseudomonas, Alcaligenes* which produce xanthan, dextran, gellan, curdlan are the most known and industrially used (Sutherland, 1990). Actually, high attention is accorded to the exopolysaccharides produced by lactic bacteria (Sutherland, 2002) (van Casteran et al., 1998) which are already accepted as GRAS (Generally Recognised As Safe) and the most adequate for the food industry. Another group of microorganisms producing of exopolysaccharides are the cianobacteria (Moreno et al., 2000) (Bejar et al., 1998).

The fungal polysaccharides are limited, pullulan produced by *Aureobasidium pullulans* (Wecker, 1992) and scleroglucan synthesized by *Sclerotium glucanicum* being the most known and already obtained at technical scale.
The newest type of microorganisms, the archeae, are also presented in the literature as polysaccharides producers (Thediek and Rausch, 2000), as the specie *Haloferax mediterranei* (Oren, 2002a) (Anton et al., 1988).
The main microbial polysaccharides produced at industrial and technical level as presented in Table 1.

Table 1. Principal microbial polysaccharides produced at industrial and technical level

Exopoly-saccharide	Microorganism	Monomers	Principal uses	Literature
Alginates	*Azotobacter vinelandii*	Acid D–mannuronic Acid L–guluronic	Gelling agent	(Vermani et al., 1995)
Dextrans	*Leuconostoc mesenteroides* *Klebsiella*	D–glucose	Gelling agent Sanguine plasma	(Ionescu, 2001)
Xanthan	*Xanthomonas campestris*	D–glucose, 6-acetyl mannose, acid D-glucuronic, D–manose-4,6 piruvate	Thickening agent	(Schuster, 1996)
Pullulan	*Aureobasidium pullulans*	α1-4, α1-6 D–glucose	Fibres, Adhesive	(Wecker, 1992)
Scleroglu-can	*Sclerotium glucanicum*	β-1,3-D-glucose β-1,6-D-glucose	Covering agent	(Sutherland, 1990)

The most used cultivation systems are batch, fed-batch and continuous (Muttzall, 1993). Some examples of industrial cultivation systems for polysaccharide production are shown in Table 2.

Table 2. Industrial production of principal exopolysaccharides (Ionescu, 2001)

Exopoly-saccharide	Cultivation system	Cultivation conditions	Cultivation medium
Alginate	Continuous	30°C; pH 7.2; Aeration (oxygen in excess doesn't stimulates the synthesis of EPS); Agitation.	Carbon source: sucrose (20 g/l); Higher production speed through molybdenum and phosphorus limitation or through smaller dilution rates.
Dextran	Batch or fed-batch	25°C (30°C optimal for microorganism, 23°C optimal for EPS); pH 6.0 (optimal for EPS production); No aeration in the phase of EPS stimulation.	Carbon source: sucrose (5-10 g/l): sucrose conversion yield 90% High levels of nitrogen and $CaCl_2$ stimulate the EPS production.
Xanthan	Batch	28°C; pH 7.0; Aeration (0.5-0.75 l/min. at the beginning, 0.75-1.5 l/min. after 2 days because of the increase in viscosity). Agitation.	Carbon source: glucose (2-3.5 g/l);
	Continuous		Dilution rate control. Nitrogen, sulphur or potassium limitation.

2. BIOLOGICAL FUNCTIONS OF EPS

Extracellular polysaccharides are found in nature in two forms (Dan, 2000):
- attached to the synthesising microbial cell as discrete structure called capsule

- as soluble mucilage excreted by cell in the environment. They are important for the industry because of the easy recovery from cultivation broths.

In this paper, the extracellular polysaccharides are abbreviated as EPS. They contributes at the gum structure of bacterial colonies on solid media (biofilms) and at the increase of viscosity in liquids (Wecker, 1992) (Schuster, 1996). Normally, EPS don't contribute to the microbial structure, the intracellular functions being not affected if they are absent (Sutherland, 1990). The extracellular polysaccharides have specific functions (Table 3). In many cases these functions are not fully understood.

Table 3. Biological functions of EPS (Wingender, et al., 1999) (Wolfraardt et al., 1999)

Function	Relevance
Adhesion to surfaces	Initial step in surfaces colonisation, accumulation of bacteria on nutrient-rich surfaces
Protective barrier	Resistance to non-specific and specific host defence, resistance to certain biocides including disinfectants and antibiotics
Cell-to-cell recognition	Symbiotic relationships with plants and animals, initiation of pathogenic processes
Structural elements of biofilms	Mediation of biofilms mechanical stability, determination of the shape of EPS structure (capsule, slime, sheath)
Retention of water	Prevention of desiccation under water-deficient conditions
Sorption of exogenous organic and inorganic compounds	Scavenging and accumulation of nutrients from the environment, sorption of xenobiotics and toxic metal ions (detoxification). Promotion of polysaccharide gel formation
Interaction with enzymes and enzymatic activities	Accumulation/retention and stabilisation of secreted enzymes. Digestion of exogenous macromolecules for nutrient acquisition

3. CHARACTERISATION OF EPS

Polysaccharides are defined in terms of composition (type and relative abundance of monomers), structure (relative distribution of monomers and type of chemical bounds between them), conformation (arrangement of monomers chains and bounds between them), relative molecular mass and type and arrangement of substitutes (Morin, 1998). These informations are used to analyse the functional properties of polysaccharides, like solubility in water, relative viscosity and rheological behaviour (Stokke et al., 1998), ions binding capacity (DePhilippis and Vincenzini, 1998).

The first step in structure analysis is the determination of chemical composition, especially the identification of monomers. Polysaccharides are composed of monosugars bounded through glycosidic bounds. The type of monomers is limited (neutral hexoses, metilpentoses, oxisugars, aminosugars, uronic acids), the great variety of EPS being due to the huge possibility to create bounds (Sutherland, 1994). Most monosugars components of microbial polysaccharides are commune with plants. So, D-glucose, D-galactose and D-manose in the piranosic form are the most frequent (Lindberg, 1998). Some polysaccharides contain the L-forms of D-glucose, D-galactose and D-manose, together with L-fucose and L-rhamnose (Thedieck and Rausch, 2000).

Some microorganisms produce EPS containing aminosugars (glucosamine, galactosamine (Robijn et al., 1996)), in the piranosic form. Most of polysaccharides are polyanionic, glucuronic acid and galacturonic acid being usual (Lindberg, 1998).

Polysaccharides have one structural unity (homopolysaccharides) or more structural unities (heteropolysaccharides) (Lindberg, 1990). Monosugars are bound to form linear chains (bacterial cellulose, curdlan or pulullan) or ramified (xanthan, dextrans) (Ionescu, 2001).

In solution, polysaccharides are found as hydrogels. A gel is a soft material, solid or solidified having two or more components (one of them being a solvent in high quantity) (Picout and Ross-Murphy, 2002); when water is the liquid, a hydrogel is formed. Hydrogels can have four types of structures:

- Well ordered lamellar structures;
- Completely disordered covalent polymeric networks (especially the synthetic polymers);
- Polymeric networks formed through physical aggregation, disordered with ordered regions;
- Disordered particulate structures (Picout and Ross-Murphy, 2002).

Most structures of known olygo- and polysaccharides are registered in the database CarbBank (Internet server adress:
ncbi.nlm.nih.gov subdirectory/repository/carbbank).
Conformation refers at the form of polysaccharide chains; it depends on the monomers and their position and bound types in the polymeric chains (Belitz and Grosch, 1999). The main conformations adopted by EPS are:
- ribbon-type chains, like cellulose or alginate;
- helix conformation, founded to lichenin;
- combined conformation, as the majority of heteroglycans (Belitz and Grosch, 1999).

Together with the structure, the conformation offer valuable informations on the properties of polysaccharides and of practical applications.
As substitutes, EPS contain variable compounds, the most usual being pyruvate, sulphate and phosphate attached to a neutral hexose (Sutherland, 1990). Sulphated polysaccharides are often found to animals (heparins, condroitinsulphates, dermatansulphates) (Medcalf, 1978) and algae (Arad, 1988). Only few microorganisms were identified to produce sulphated EPS: *Bacillus* species (Manca et al., 1996), *Halomonas* species (Bejar et al., 1998), the archeon *Haloferax mediterranei* (Sutherland, 1994).

The modern methods used for the characterisation of microbial polysaccharides are presented in Table 3.

Table 3. Principal characteristics of microbial polysaccharides and analysis methods

Characteristics	Analysis methods	Literature
Quantitative analysis of polysaccharides	Gravimetric methods Colorimetric methods	(Bergmaier et al., 2001) (Ramus, 1977)
Quantitative and qualitative analysis of monosaccharides components of EPS	High-Performance Liquid Chromatography (HPLC) - Reverse-phase HPLC - Ion-exchange HPLC	(Unger and Weber, 1999) (Meyer et al., 2001) (Kaiser and Benner, 2000)
EPS structure and conformation	Nuclear magnetic resonance NMR	(Guetta et al., 2003) (Cowman et al., 2001)

	Differential Scanning Calorimetry DSC	(Spigno and de Faveri, 2003)
	X-ray diffraction	(Allen Busch et al., 1999)
	Rheological analysis	(Picout and Ross-Murphy, 2002) (Marques et al, 2002)
Quantitative and qualitative analysis of substitutes	Ion-Exchange HPLC	(Thomas et al., 2003)
	NMR	(Pereira et al., 2002)
	Infrared Spectroscopy IR	(Lijour et al., 1994)
EPS form and dimension	Dynamic Light Scattering or Static Light Scattering	(Ioan et al., 2001) (Santiago et al., 2002)

4. FUNCTIONAL PROPERTIES OF EPS

Biopolymeric systems are used in the food industry to create products with specific characteristics, based on microstructure. In such systems is very important to understand the relation between structure and functional properties (Cesaro et al. 1992), to know the kinetic of structure formation and destruction and how this can be influenced by the working conditions and composition (Aguilera and Stanley, 1999).
The functional properties are defined as the properties measurable using instruments and correlated with the final product characteristics through these measurements.The functional properties of a compound can be identified following the characteristics:

- Rheological behaviour (flow and viscosity functions, viscoelasticity);
- Behaviour towards water, depending on hydrogen and van der Waals interactions;
- Interactions of macromolecules with other macromolecules (properties of polymerisation through intermolecular ionic, hydrophobic or covalent associations);
- Interactions with small molecules, with molecules having little polarity (properties at the interfaces, formation of polydispersed systems) (Linden and Lorient, 1999).

Rheological properties of polysaccharides

The rhelogical behaviour of polysaccharides solutions and the influence of physical or chemical factors on the rheological properties are important because they offer informations on the bioprocess, the biopolymer quality, the relations between microstructure and physical properties (Pelletier et al., 2001), textural analysis (Moreno et al., 2000).
Rheological characteristics depend on a large number of factors, as observed in Figure 1.

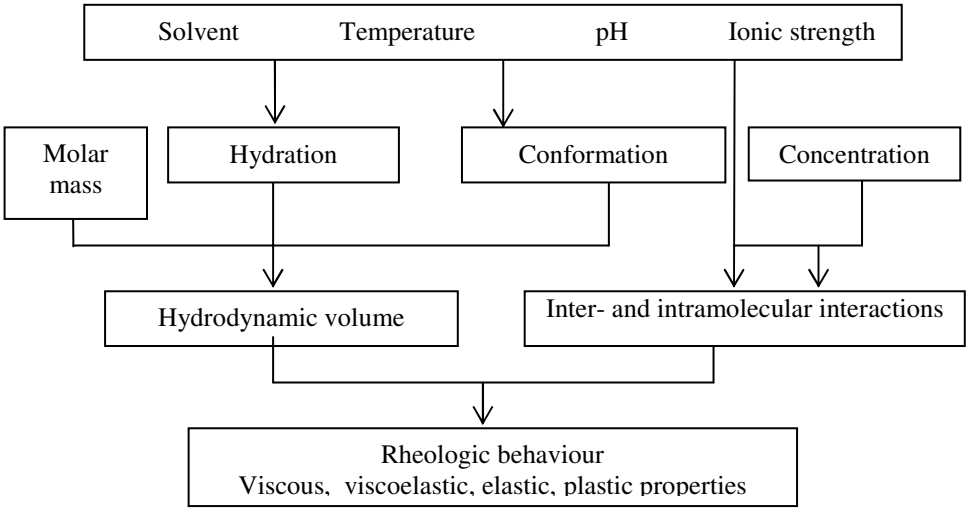

Figure 1. Factors influencing the rheological characteristics of biopolymeric aqueous solutions (Steffe, 1996)

For example, if the polysaccharide is a polyelectrolyte, viscosity can be controlled through electrostatic repulsion, ionic strength or addition of di- and polyvalent cations (making possible the formations of gel through ionic bounds) (Mironescu, 2005).
The molecules size influences the rheological behaviour at various shear stress. For example, due to the length and rigidity of hydrated alginate molecules, the aqueous solutions of this polysaccharide are shear-thinning (or pseudoplastic\ (Imeson, 2002); the alginate molecules are disordered at small shear rates, whereas at high shear rates the parallel orientation of polymeric chains occurs and the apparent viscosity decreases (Aguilera and Stanley, 1999).
For the rheological analysis of EPS, two measuring systems are recommended: parallel plate and cone-plate geometries (Steffe, 1996). Using

these geometries, steady-state and oscillatory (or dynamic) measurements can be performed; the first one type of analyses gives informations on the flow behaviour of polysaccharides and the second one characterises the viscoelastic behaviour (Hochstein, 2005).

Association properties of polysaccharides

The tendency of molecules to associate when the solutions are destabilised is due to the destruction of the equilibrium between the attraction and the rejection forces given by changes in medium (pH, ionic strength, temperature) and to the formation of new bounds (Mironescu, 2005). For example, polysaccharides form gels in the presence of water and under temperature influence; the gelling process implies interactions between polymer chains with formation of complex structures (double helix) where the solvent molecules are entrapped (Figure 2).
Some EPS form gels in the presence of ions, like alginates at the interaction with calcium ions (Braccini et al., 1999) (Figure 3).

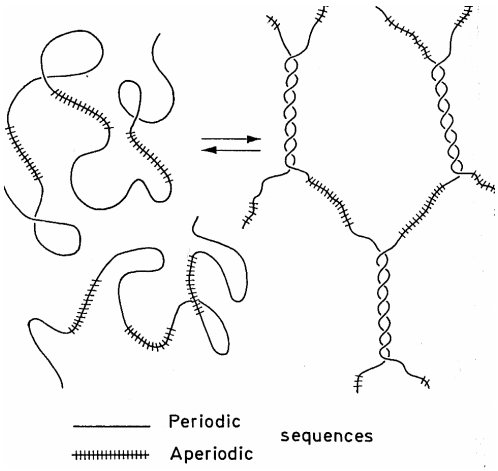

Figure 2. Schematic representation of gel formation process [Belitz and Grosch, 1999]

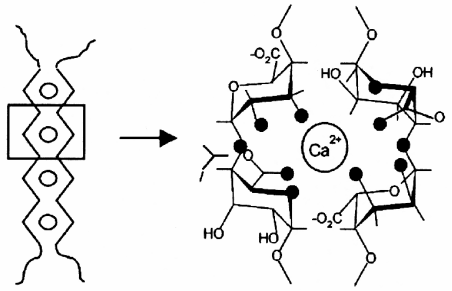

Figure 3. Representation of coordinative bounds between Ca^{2+} and alginate [Bracccini and Perez, 2001]

Salts ions added in solutions act as flocculants by neutralising the repulsive charge at the surface. The flocculation efficiency is given by the critical coagulation concentration of counter-ions, but is also dependent from the atomic number (Belitz and Grosch, 1999).

5. CONCLUSIONS

Microbial polysaccharides are found elsewhere in nature, produced especially by bacteria. Their composition and structure are various, giving different properties which make it valuable for various applications in the food industry, in medicine or pharmacy.
The functional role of EPS is various and not clearly explained until yet.
The development of analysis methods allows the characterisation of microbial polysaccharides in terms of composition, functional groups, structure, conformation, molecular mass, form and dimension.
The main functional properties of EPS are the rheological properties and the association properties.

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