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Advances in bacterial exopolysaccharides: from production to biotechnological applications

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A vast number of bacterial extracellular polysaccharides (EPSs) have been reported over recent decades, and their composition, structure, biosynthesis and functional properties have been extensively studied. Despite the great diversity of molecular structures already described for bacterial EPSs, only a few have been industrially developed. The main constraints to full commercialization are their production costs, mostly related to substrate cost and downstream processing. In this article, we review EPS biosynthetic and fermentative processes, along with current downstream strategies. Limitations and constraints of bacterial EPS development are stressed and correlation of bacterial EPS properties with polymer applications is emphasized.

Bacterial exopolysaccharides (EPSs)

Only a few of the huge number of new bacterial EPSs reported over recent decades have emerged as industrially important biopolymers with significant commercial value, particularly with regard to their use as biomaterials (e.g. bacterial cellulose) [1,2] or as rheology modifiers of aqueous systems (e.g. xanthan gum) [3] (Box 1).

Some bacterial EPSs can directly replace polysaccharides extracted from plants (e.g. guar gum or pectin) or algae (e.g. carrageenan or alginate) in traditional applications, because of their improved physical properties (e.g. xanthan gum or gellan gum) (Table 1) [4,5]. Conversely, other bacterial EPSs possess unique properties that can launch a range of new commercial opportunities (e.g. bacterial cellulose or levan) [6,7]. Examples of two newly reported bacterial EPSs with great potential include GalactoPol, synthesized by *Pseudomonas oleovorans*, which is composed mainly of galactose [8], and FucoPol, a fucose-containing EPS that is synthesized by *Enterobacter* A47 [9] (Table 1). Several microorganisms isolated from extreme environments, such as deep-sea hydrothermal vents, Antarctic ecosystems, saline lakes and geothermal springs have recently started to be studied as potential sources of valuable biopolymers, including EPSs [10,11].

The global market for hydrocolloids, which includes many polysaccharides, is still dominated by plant and algal polysaccharides (e.g. starch, galactomannans, pectin, carrageenan and alginate). This market valued at >4 million US$ in 2008, with xanthan gum being the only significant bacterial EPS, which accounted for 6% of the total market value [3].

For new polymers to arise as commodity products in large markets, such as the hydrocolloids, it is crucial to lower their production costs [12]. Approaches for the reduction of production costs might involve using cheaper substrates, improving product yield by optimizing fermentation conditions, or developing higher yielding strains (e.g. by mutagenesis or genetic manipulation), and optimizing downstream processing [2,13]. Nevertheless, the greatest potential of bacterial EPSs is related to their use in high-value market niches, such as cosmetics, pharmaceuticals and biomedicine, in which traditional polymers fail to comply with the required degree of purity or lack some specific functional properties. Such markets will provide opportunities for the development of up-and-coming bacterial EPSs; providing that they have unique desirable physicochemical properties. In such high-value applications, product quality wholly surpasses cost production and product yield issues. In particular, downstream processing requirements are highly demanding in those application fields. Separation processes become especially challenging when polymers are considered for medical applications because a high-purity product is envisaged.

This article reviews EPS production by bacteria, including recent advances in the elucidation of the biosynthetic pathways involved and the fermentative bioprocesses developed, as well as the main constraints to full commercialization of these biopolymers. The techniques used to study and characterize bacterial EPS physicochemical properties are described, followed by their correlation with actual and potential applications.

Processes for EPS biosynthesis

Biosynthetic pathways

Most bacterial EPSs are synthesized intracellularly and exported to the extracellular environment as macromolecules [2,6]. There are a few known exceptions (e.g. levans and dextrans) whose synthesis and polymerization occur outside the cells by the action of secreted enzymes that convert the substrate into the polymer in the extracellular environment [2].

Bacterial biosynthetic pathways comprise substrate uptake, central metabolite pathway and polysaccharide

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Box 1. Extensively studied and commercially exploited bacterial EPSs

Xanthan gum: the first industrially produced biopolymer [44] and the most extensively studied and widely accepted commercial microbial polymer [45]. It is a heteropolysaccharide secreted by bacteria of the genus *Xanthomonas*, and consists of a glucose backbone with trisaccharide side chains that contain glucuronic acid, mannose, pyruvil and acetyl residues.

Gellan and related polymers (**sphingans**): heteropolysaccharides produced by members of the genus *Sphingomonas*, which are characterized by a common tetrasaccharide backbone that contains rhamnose, glucose and glucuronic acid. Gellan, welan, rhamsan and diutan are differentiated by variation in composition and linkage of the side chains (e.g. gellan contains acetyl and glyceryl substituents, whereas welan has a rhamnose or mannose side group branching) [46].

Alginate: linear polysaccharide that is composed of mannuronic and guluronic acids that form block structures of poly-mannuronic acid sequences, poly-guluronic acid sequences and mixed sequences, secreted by bacteria from the genera *Azotobater* and *Pseudomonas*. Algal and bacterial alginates differ mainly by the later being acetylated [5].

Glucans: glucose homopolysaccharides that differ in the type of glycosidic linkage, degree and type of branching, chain length, molecular mass, and polymer conformation. There are two major types of glucans, namely, β-glucans (e.g. **cellulose** [1] and **curdlan** [24]) and α-glucans (**dextran**, mutan, alternan and reuteran [47,48]) Several bacteria of the genera *Gluconacetobacter*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina* and *Salmonella* have shown the ability to produce cellulose [1]. α-Glucans are produced from sucrose by the action of dextransucrase, which is an extracellular enzyme that is synthesized by several bacteria of the genera *Leuconostoc*, *Streptococcus* and *Lactobacillus* [49].

Hyaluronan: linear polymer of a repeating disaccharide unit that is composed of glucuronic acid and *N*-acetylglucosamine [15], produced by several bacterial strains (e.g. *Pseudomonas aeruginosa* and group A and C *Streptococci* attenuated strains) [2].

Succinoglycan: branched EPS with glucose and galactose backbone and tetrasaccharide side chains composed of glucose residues. Succinate, pyruvate and acetate are commonly found as nonsaccharide substituents. It is produced by several soil bacteria, such as *Rhizobium*, *Alcaligenes*, *Pseudomonas* and *Agrobacterium* [50].

Levan: highly branched fructose homopolysaccharide synthesized from sucrose by the action of the enzyme levansucrase [2], an extracellular enzyme synthesized by several bacteria, including species of the genera *Bacillus*, *Rahnella*, *Aerobacter*, *Erwinia*, *Streptococcus*, *Pseudomonas* and *Zymomonas* [54].

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synthesis (Figure 1). Depending on substrate type, it can be taken up by the cell either through a passive or an active transport system (Figure 1a), following which, it is catabolized by intracellular phosphorylation, or it can be transported and oxidized through a direct oxidative periplasmic pathway. The periplasmic oxidative pathway exists only in certain bacteria, whereas the intracellular phosphorylative pathway is ubiquitous amongst bacteria. Both these systems have been reported in several EPS-producing strains and they can function simultaneously if there is substrate availability [14].

In the cytoplasm, the substrate is catabolized through glycolysis (Figure 1b) and the primary metabolites formed are used as precursors for the synthesis of small biomolecules (e.g. amino acids or monosaccharides). Polysaccharides synthesis requires the biosynthesis of activated precursors that are energy-rich monosaccharides, mainly nucleoside diphosphate sugars (NDP-sugars), which are derived from phosphorylated sugars (Figure 1c) [6,15].

The secretion of EPS is a challenging process for the bacterium, in which hydrophilic, high molecular weight polymers assembled in the cytoplasm must traverse the cell envelope, without compromising the critical barrier properties [16]. In contrast with the wide diversity of the molecular structures found in EPSs, the pathways for their biosynthesis and export in most Gram-negative bacteria have been reported to follow one of two mechanisms: the Wzx–Wzy-dependent pathway, in which the polymer repeat unit is assembled at the inner face of the cytoplasmic membrane and polymerized at the periplasm, and the ABC transporter-dependent pathway, in which polymerization occurs at the cytoplasmic face of the inner membrane (Figure 1d) [16].

Fermentative production
Although the composition and amount of EPS produced by a bacterium are genetically determined traits, they are highly influenced by media components and cultivation conditions. EPS synthesis is generally favored by the presence of a carbon source in excess, concomitant with limitation by another nutrient (e.g. nitrogen or oxygen) [1,2,7,17]. The production of most bacterial EPS occurs under aerobic conditions. For optimal production of some EPSs, maximal aeration is required (e.g. xanthan gum) [2], whereas for others, synthesis is maximized under microaerophilic conditions (e.g. bacterial alginate) [5]. GalactoPol and FucoPol are examples of bacterial EPSs whose production occurs under conditions of carbon availability concomitant with nitrogen and oxygen limitation [8,9,18,19]. Supplementation with vitamins, aminoacids and precursors has also been reported to stimulate bacterial growth and synthesis of several bacterial EPSs, including gellan gum [20], welan [21], bacterial cellulose [22] and curdlan [23].

For most EPSs, the basic carbohydrate structure does not change significantly with growth conditions, but its content in substituent groups can vary extensively, thus changing polymer properties [2,5]. Exceptions to this behavior have been reported for some EPS-producing strains, such as *Rhizobium* and *Pseudomonas* [2,13], and it allows for the tailoring of polymer composition. Many EPS producers are also able to accumulate variable amounts of intracellular storage products (e.g. glycogen or polyhydroxyalkanoates), thus reducing the full potential for EPS production. *Xanthomonas* sp. is exceptional in that it does not produce significant amounts of other polymers, thus achieving very high substrate conversion into EPS [2].

FucoPol shows a partially growth-associated EPS production trend, with polymer synthesis being initiated during the exponential growth phase but proceeding with identical production rate when cell growth is restricted [19]. This production trend is typical of other EPS-producing strains, including *Xanthomonas* sp. during xanthan production [13]. Nonetheless, several examples exist of polymer synthesis occurring only during the exponential growth phase (growth-associated synthesis) (e.g. gellan

Table 1. Extensively studied bacterial EPS: overview of the most relevant physicochemical and functional properties, main areas of application and market assessment.

EPS	Components	Charge	Molecular weight	Main properties	Main applications	Market (metric tons)	Market value (US$)	Price (US$)/kg	Refs.
Xanthan	Glucose, Mannose, Glucuronic acid, Acetate, Pyruvate	Anionic	$(2.0\text{-}50) \times 10^6$	• Hydrocolloid - High viscosity yield at low shear rates even at low concentrations; - Stability over wide temperature, pH and salt concentrations ranges	• Foods • Petroleum industry • Pharmaceuticals • Cosmetics and personal care products • Agriculture	96 000	235 millions	3 - 5	[2,3,6,13]
Gellan	Glucose, Rhamnose, Glucuronic acid, Acetate, Glycerate	Anionic	5.0×10^5	• Hydrocolloid -Stability over wide pH range • Gelling capacity • Thermoreversible gels	• Foods • Pet food • Pharmaceuticals • Research: agar substitute and gel electrophoresis	N.A.	15 millions	55-66	[2–4,6,17]
Alginate	Guluronic acid, Mannuronic acid, Acetate	Anionic	$(0.3\text{-}1.3) \times 10^6$	• Hydrocolloid • Gellling capacity • Film-forming	• Food hydrocolloid • Medicine - Surgical dressings - Wound management -Controlled drug release	30 000	N.A.	5-20	[2,3,5,13,36]
Cellulose	Glucose	Neutral	$\sim 10^6$	• High crystallinity • Insolubility in most solvents • High tensile strength • Moldability	• Foods (indigestible fiber) • Biomedical -Wound healing -Tissue engineered blood vessels -Audio speaker diaphragms	N.A.	N.A.	5.8-12	[1,2,13,32,35]
Dextran	Glucose	Neutral	$10^6 - 10^9$	• Non-ionic • Good stability • Newtonian fluid behavior	• Foods • Pharmaceutical industry: Blood volume expander • Chromatographic media	2 000	N.A.	N.A.	[2,12,13]
Curdlan	Glucose	Neutral	$5 \times 10^4 - 2 \times 10^6$	• Gel-forming ability • Water insolubility • Edible and non-toxic • Biological activity	• Foods • Pharmaceutical industry • Heavy metal removal • Concrete additive	N.A.	N.A.	55	[2,13,24]
Hyaluronan	Glucuronic acid, Acetylglucosmine	Anionic	2.0×10^6	• Biological activity • Highly hydrophilic • Biocompatible	• Medicine • Solid culture media	N.A.	1 billion	100 000	[2]
Succinoglycan	Glucose, Galactose, Acetate, Pyruvate, Succinate, 3-hydroxybutyrate	Anionic	LMW $<5 \times 10^3$ HMW $>1 \times 10^6$	• Viscous shear thinning aqueous solutions • Acid stability	• Food • Oil recovery	N.A.	N.A.	N.A.	[2,38]
Levan	Fructose	Neutral	3.0×10^6	• Low viscosity • High water solubility • Biological activity: Anti-tumor activity Anti-inflammatory • Adhesive strength • Film-forming capacity	• Food (prebiotic) • Feed • Medicines • Cosmetics • Industry	N.A.	N.A.	N.A.	[2,24,39]

| GalactoPol | Galactose Mannose Glucose Rhamnose Acetate Succinate Pyruvate | Anionic | (1.0–5.0) × 10^6 | • Viscous shear thinning solutions in aqueous media • Film-forming • Emulsifying capacity • Flocculating capacity | – | Hydrocolloid for use in: (1) -Food and feed -Cosmetics -Pharmaceuticals and medicine -Oil recovery • Coatings • Packages | – | – | [8,18,40–43] |
| FucoPol | Fucose Galactose Glucose Acetate Succinate Pyruvate | Anionic | (2.0–10.0) × 10^6 | • Viscous shear thinning solutions in aqueous media • Film-forming • Emulsifying capacity • Flocculating capacity • Biological activity due to fucose content | – | • Hydrocolloid for use in: (1) -Food and feed -Cosmetics -Pharmaceuticals and medicine -Oil recovery • Source of fucose and fuco-oligosaccharides | – | – | [9,19] |

(1) Potential applications; N.A.: data not available.
This table is not intended to be exhaustive.

gum or bacterial cellulose) [1,4], as well as polymer production only in the post-stationary growth phase (growth-dissociated synthesis) (e.g. curdlan) [23].

Sugars are the most commonly used carbon sources for the production of bacterial EPS [7]. However, cheaper substrates, such as agro-food or industrial wastes and byproducts, have been demonstrated to be adequate for the production of several bacterial EPSs (e.g. molasses, cheese whey or glycerol byproduct) [13,22,24–31]. The recently reported EPS GalactoPol and FucoPol have been synthesized by bacteria grown on glycerol byproduct from the biodiesel industry [8,13,18,19]. This low-cost substrate is a suitable carbon source for the production of both polymers.

Although many of the referred low-cost wastes or byproducts are apparently promising for production of many bacterial EPSs, there are some possible problems associated with their use as substrates. Different metabolic pathways might be followed because of the different nutrient composition and presence of contaminants [13]. Thus, different polymers and/or (unwanted) byproducts might be synthesized by the bacteria. Non-reacted components might accumulate in the broth and eventually become inhibitors, which lowers product yield. For specific high-value applications, in which high purity and high quality products are needed, usually good quality substrates must be used to reduce the risk of impurity carryover to the final product. Therefore, in such cases, the use of wastes or byproducts might not be an option or, otherwise, higher investment must be put in downstream procedures.

Bioprocess constraints and improvements
Traditional strategies to improve the fermentative production of bacterial EPS include improved strain selection and optimization of cultivation conditions. However, these strategies allow only marginal bioprocess improvement due to the physiological limits for each given bacterium.

The most interesting prospects for increasing bacterial EPS production are through metabolic engineering either by manipulation of the genes that encode the enzymes that catalyze the reactions in the pathways, or by altering the regulatory pathways that affect gene expression and enzyme activity [13]. Biosynthetic processes can be controlled at three different levels: synthesis of sugar nucleotide precursors; assembly of the repeating unit; and polymerization and export. The modification of the expression of single genes or groups of genes can be used to increase the conversion efficiency of the chemical entities involved, and therefore, enhance EPS yield. However, it might also provide a means of altering the polymer composition [17]. Metabolic engineering of EPS-producing strains has been attempted, for example, for the production of xanthan [2], gellan [17], bacterial cellulose [32] and levan [2]. For some of these polymers (e.g. bacterial cellulose), this approach has been successful and has led to higher EPS production [2,32]. However, for others, the results have not shown significant improvements, mostly because the steps and the regulation of the pathways are still poorly understood [2,17].

Bacterial cell growth and EPS production might be monitored by Raman microspectroscopy, which provides
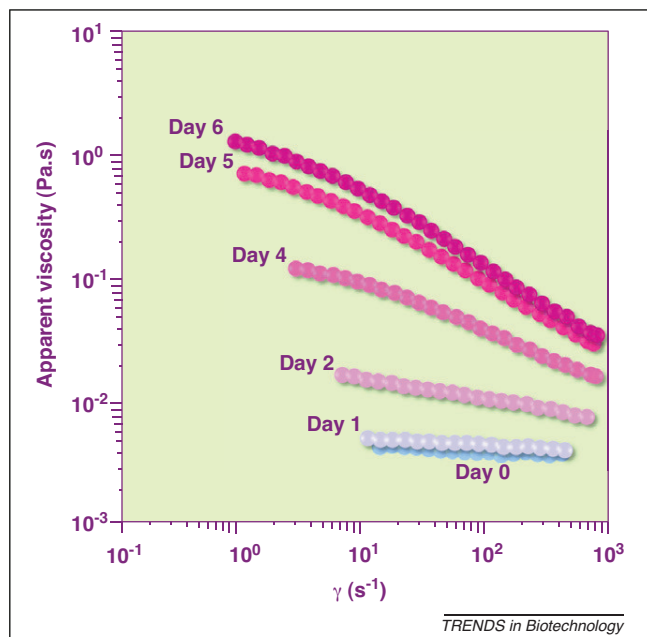
TRENDS in Biotechnology
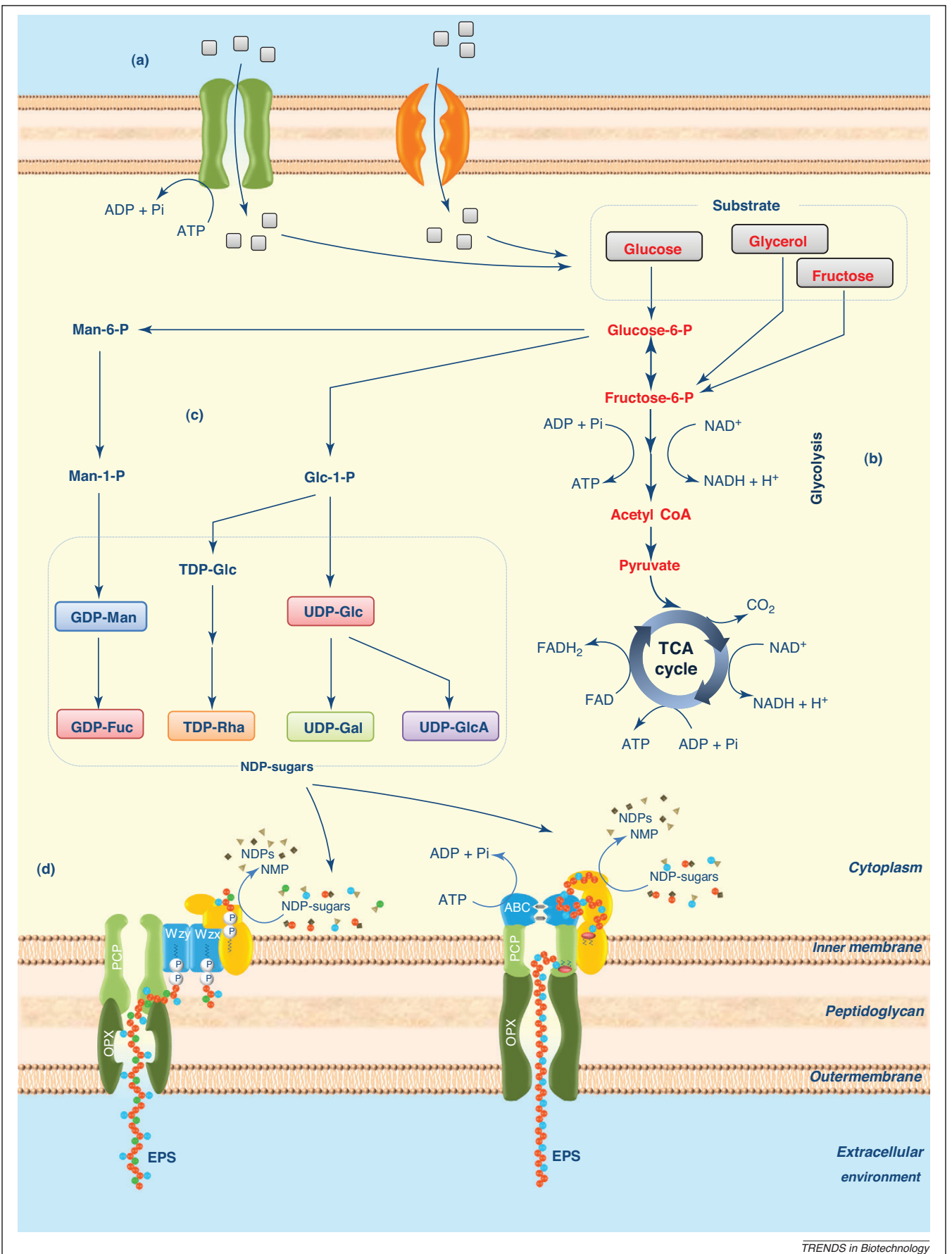
Figure 2. Flow curves of *Enterobacter* A47 culture broth samples at different times of the cultivation run for the production of FucoPol, measured at 30 °C (adapted from [19]).

a nondestructive, *in situ*, real-time evaluation of bacterial cell surface components at the single cell level or on bacterial colonies. Also, by coupling this technique with high-resolution imaging techniques such as atomic force microscopy (AFM), one would be able to quantify biophysical properties of a single bacterial cell surface, or cell colonies that develop in dynamic cultivation processes [34]. Eventually, this monitoring strategy could enable control of EPS synthesis and characteristics upon production.

During most EPS production, the rheology of the fermentation broth changes drastically from an initial Newtonian fluid behavior, with a viscosity near that of water, to a highly viscous fluid with shear thinning behavior (Figure 2) [8,13]. This increase in viscosity frequently causes a loss of bulk homogeneity, which makes it very difficult to maintain appropriate mixing, aeration or control of bioreactor parameters.

One strategy to improve the hydrodynamics of fermentation broths is the optimization of mechanical mixing using different paddle configurations or increasing stirring rate. However, the use of these devices can result in cell rupture due to the increased mechanical stress [33] or altered polymer properties [35,36].

Water-in-oil cultivation technology has been developed to enhance the performance of highly viscous fermentation processes. In this technology, the aqueous broth is dispersed as fine droplets in a continuous phase of organic solvent (oil). The biopolymers produced are confined within the dispersed droplets, therefore, the overall system viscosity can be kept manageable [13]. The feasibility of this new cultivation technology has been demonstrated for xanthan fermentation [38]. Surfactants have been reported to enhance oxygen and mass transfer during fermentation for some bacterial EPS production, such as xanthan [13] and gellan [37]. The mechanisms that underlie such effects might be related to the increase in membrane permeability, changes in lipid metabolism, and stimulation of polymer release [37]. Although promising, the impact of these approaches upon polymer yield and quality must be evaluated.

Extraction and purification

Recovery of extracellular microbial polysaccharides from the culture broth is commonly achieved by procedures that involve: (i) cell removal, usually achieved by centrifugation or filtration; (ii) polymer precipitation from the cell-free supernatant by the addition of a precipitating agent that consists of a water-miscible solvent in which the polymer is insoluble (e.g. methanol, ethanol, isopropanol or acetone); and (iii) drying of the precipitated polymer, namely by freeze drying (laboratory scale) or drum drying (industrial scale) [8,17,36,44].

In many procedures, the broth is subjected to heat treatment (up to 90–95 °C) at the end of the fermentation process, before cell removal [17]. This heat step aims at killing the bacterial cells and inactivation of enzymes that could cause polymer degradation in subsequent steps. Moreover, it also gently reduces broth viscosity. Cell removal is facilitated by dilution of the culture broth by addition of deionized water before centrifugation/filtration. However, this approach increases operating costs, because considerably higher volumes of cell-free supernatant are generated, and consequently, higher volumes of precipitating agent are required [8,18].

There is a large range of low-molecular-weight compounds, co-produced or added during the production processes, which end up as impurities in the final product (e.g. cell debris, salts and proteins [7,40,52]). To obtain a higher

Figure 1. Simplified schematic diagram summarizing the biosynthetic pathways involved in bacterial EPS synthesis by Gram-negative bacteria. **(a)** The substrate enters the cell through specific transport mechanisms. For example, glycerol crosses the membrane, through facilitated diffusion, down its concentration gradient, whereas movement of most sugars is coupled to the proton motive force through ATP-driven transport systems (e.g. sugar porter families), wherein ATP hydrolysis is used to gain energy to drive the substrate against its concentration gradient [14,51]. **(b)** The substrate entering the cell is first catabolized by the glycolysis pathways into pyruvate, which under aerobic conditions, is converted to acetyl-CoA and enters the tricarboxylic acid cycle. The primary metabolites produced in these pathways are used as precursors for the biosynthesis of small biomolecules (e.g. aminoacids, ribonucleotides and hexosamines) [6,13]. **(c)** Phosphorylated sugars are converted into energy-rich monosaccharides, mainly nucleoside diphosphate sugars (NDP-sugars). These precursors (e.g. UDP-Glc, UDP-Gal and GDP-Man) are interconverted through reactions of epimerization, oxidation, decarboxylation, reduction and rearrangement [2,6]. **(d)** Polysaccharide synthesis and polymerization occurs through one of two mechanisms. (i) In the Wzx–Wzy-dependent system (left), the repeat unit is synthesized by the sequential transfer of monosaccharides from NDP-sugars to a polyprenylphosphate lipid carrier. Mature repeat units are transported across the inner membrane by a presumed flippase (Wzx) to the periplasmic face, where polymerization occurs by the action of a polymerase (presumed Wzy). In many bacteria, the translocation pathway that spans the cell envelope is formed by a polysaccharide copolymerase (PCP) that determines polymer chains length, and an outer membrane polysaccharide export protein (OPX) that forms a channel [2,16,44]. (ii) In the ABC-transporter-dependent system (right), the polysaccharide is polymerized at the cytoplasmic face of the inner membrane through the sequential addition of sugar residues to the nonreducing end of the polymer chain. The polymer is exported across the inner membrane through an ABC transporter, followed by its translocation across the periplasm and the outer membrane, through PCP and OPX proteins [16]. Abbreviations: Fuc, fucose; Gal, galactose; Glc, glucose; GlcA, glucuronic acid; Man, mannose; Rha, rhamnose; GDP, guanosine diphosphate; TDP, tyrosine diphosphate; UDP, uridine diphosphate; NDP, nucleoside diphosphate; NMP, nucleoside monophosphate.

purity grade polysaccharide, the polymer is additionally subjected to one or several of the following processes: re-precipitation of the polymer from diluted aqueous solution (<1.0 g/l); chemical deproteinization (e.g. salting out or protein precipitation with trichloroacetic acid [53]) or enzymatic methods (e.g. proteases [52]); and membrane processes (e.g. ultrafiltration and diafiltration [7,9,54]). Some of these purification procedures can decrease product recovery or have a negative impact on polymer properties, namely, when protein removal is carried out with the addition of chemicals that might react with EPS components. Therefore, the choice of the most appropriate procedure must be made carefully as a compromise between product recovery, product purity, and its impact on polymer properties. As such, research is still needed, either for the improvement of the existing extraction and purification processes, or for the development of new approaches that are focused on the specifications required for the final product.

Physicochemical characterization

An overall characterization of biopolymers involves the evaluation of their chemical, physical and biological properties, which are key factors to understand their behavior in different environments.

Chemical composition

The evaluation of EPS chemical composition concerns the identification of sugar residues, repeating units (which may be formed by more than one sugar/sugar-based molecule) and chain groups constituents (e.g. acyl and phosphate groups) (Box 2). The traditional method, which consists of acid hydrolysis followed by derivatization to alditol acetates assayed by gas chromatography [8], has been gradually replaced by high pressure anion exchange chromatography with pulsed amperometric detection [55], which is more straightforward because it avoids the derivatization step. Characterization of single carbohydrates using capillary electrophoresis, without significant carbohydrate modification after hydrolysis, has also been well documented [56].

Chemical structure

The possible multiple combinations of monomeric units, along with the stereospecificity of glycosidic linkages (α or β anomers), leads to very complex chemical structures that are difficult to resolve, which range from linear homopolysaccharides to highly branched heteropolysaccharides (Box 2). According to the most used methods, the linkage pattern of the monomers is evaluated by methylation of all free hydroxyl groups, followed by polysaccharide hydrolysis to monosaccharides, reduction to alditols and acetylation to give partially methylated alditol acetates, which are eventually analyzed by GC-MS [57]. Smith degradation can be used to fragment selectively the polysaccharides. It is based on three consecutive steps: oxidation with periodate, followed by a reduction to a polyalcohol with borohydride, and finally, mild acid hydrolysis [57]. Furthermore, the decrease in molecular weight and branching might also be achieved, either by nonspecific partial acid hydrolysis, or by using enzymes that are specific for different glycosidic linkages. The low-molecular-weight fragments obtained can be analyzed by several techniques, namely, GC-MS [57], MALDI-TOF-MS [58] and NMR [57].

With regard to NMR, both solid state [8] and improved liquid state 2D NMR methodologies (such as 2D correlated spectroscopy, 2D nuclear Overhauser effect-enhancement spectroscopy, 2D total correlation spectroscopy and 2D heteronuclear single quantum coherence), have been applied [57,59]. These techniques allow the evaluation of the interactions of each carbon/hydrogen with adjacent atoms and chemical groups, and eventually determine their relative position in the structure.

Average molecular weight

Various techniques have been used for the determination of average polymer molecular weight and polydispersity index. High-performance size-exclusion chromatography with multi-angle laser light scatter detection is a recent efficient method for the evaluation of polysaccharide absolute molecular weight, which provides greater resolution than traditional gel permeation chromatography [41,60].

Properties in solution

The molecular weight, chemical structure and composition of polysaccharides, namely the presence of ionizable groups that confer the polymer a polyelectrolyte behavior, greatly affect their properties in aqueous solutions. Such properties are related to the molecular characteristics of biopolymers, such as intrinsic viscosity (hydrodynamic volume of a single molecule), critical overlap concentration (concentration above which coil overlapping starts to occur), molecular conformation and flexibility, as well as the nature and number of intra/intermolecular interactions [41,61,62]. Dilute solution viscometry is the first approach that is normally used for probing the referred fundamental molecular properties, which support the whole of the viscosity behavior of polysaccharide systems [41,61,62]. In addition, AFM is a rather versatile technique that is able to assess the complex and intact 3D molecular structure of
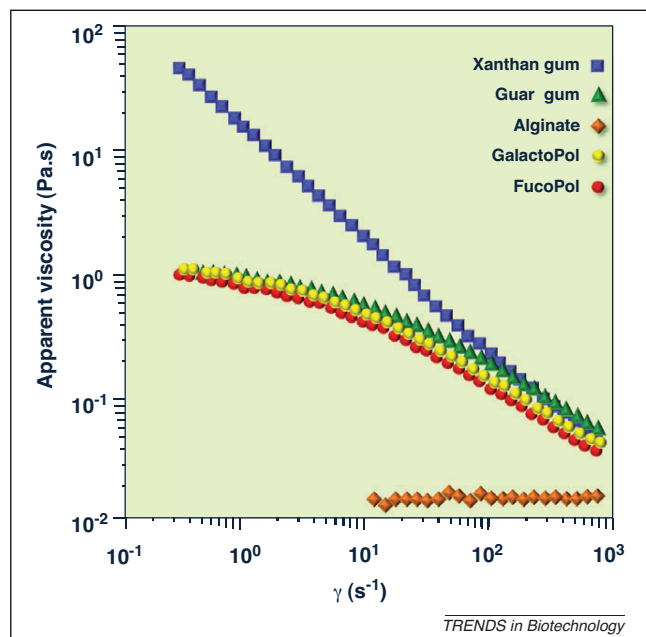

Box 2. Bacterial EPSs

Bacterial EPSs are biopolymers that are secreted by the cells, and form a capsule that remains associated with the cell surface, or a slime that is loosely bound to the cell surface [2]. These biopolymers are mainly composed of carbohydrates, with glucose, galactose and mannose being the most common monomers. Other neutral sugars (e.g. rhamnose and fucose), some uronic acids (mainly glucuronic and galacturonic acids) and aminosugars (N-acetylamino sugars) are also frequently present. Although most bacterial EPSs are composed of repeating units with varying sizes and degrees of ramification, some have irregular structures (e.g. bacterial alginates). Given the diversity of sugar components, there is a wide range of possible molecular structures. Moreover, this variability is increased by the different glycosyl linkages and configurations that are also possible [6,7]. In addition to carbohydrates, bacterial EPS might contain several organic ester-linked substituents and pyruvate ketals. The presence of some of these acyl groups confers on the EPS an anionic character, increases its lipophilicity, and affects its capacity to interact with other polysaccharides and cations. The properties of bacterial EPS are determined by its chemical composition, molecular structure, average molecular weight, and distribution.

Figure 3. Comparative flow curves of two new bacterial EPS (GalactoPol and FucoPol) and some commercial polysaccharides (xanthan gum, guar gum and algal alginate), with the same polymer concentration (1.0%) and solvent (0.1 M NaCl), at 25 °C (adapted from [9]).

polysaccharides. It can identify the conformation of individual molecules and their assemblies (e.g. aggregation phenomena and gel systems) [64,65]. When dealing with concentrated polymer solutions, rheometry is a fundamental tool to assess viscosity and viscoelastic properties of polysaccharide systems (Figure 3) [25,38,41,42].

Functional properties and applications
Knowledge of how molecular arrangements influence polymer properties in aqueous systems is of utmost importance for screening industrial applications. Good examples of the correlation between chemical properties and functionality are gellan and xanthan gums dispersions. Both are negatively charged heteropolysaccharides but with different polymer composition and structure (Box 1). Gellan linear molecules are in disordered coil states at high temperatures, which turn into double helical forms upon cooling. At high concentrations, the double helices change to thicker rod-like aggregates and form a macroscopic gel [63]. However, the final gel properties are dependent on the content of the acyl groups: the acylated form produces flexible and thermoreversible gels, whereas the deacylated type forms hard, nonelastic brittle gels [63]. As such, it is used as a gelling agent in several industries (Table 1).

Xanthan branched chains [63], although forming double helices, do not create gel structures. Instead, xanthan produces highly viscous solutions with entangled polymer chains. As a consequence, it is mainly applied as a viscosity enhancer (Table 1).

Properties of EPS can be changed dramatically using mixtures with other biopolymers. The mixture of galactomanans (e.g. locust bean gum) with xanthan is a well-known example of synergism that results in the formation of a gel structure [66]. In addition, chemical modifications that

change the nature of polymer chain constituents [66,67], and chemical reactions with other biopolymers and/or cross-linking agents [68], have also been explored to achieve polymeric structures with enhanced functionality.

Bacterial EPSs have been extensively used in high-value applications, such as food, pharmaceutical, medical and cosmetic products or processes (Figure 4), wherein they are mostly used as thickening, stabilizing, binding and structure creation agents (Table 1), as a result of their non-Newtonian behavior and high viscosity in aqueous media (Figure 3). When applied in food products, they must be able to maintain their properties when incorporated into formulations, in which they might experience significant variability of pH and ionic strength, along with the influence of other food components.

More recently, research has turned to the creation of novel structures (micro/nanospheres, polymer beads and capsules) with edible safe materials, wherein bioactive compounds (e.g. antioxidants, vitamins, probiotics or prebiotics) are encapsulated [69]. Encapsulation techniques are designed to protect the bioactive substances and to promote their controlled release.

Some polysaccharides (e.g. bacterial alginate, gellan FucoPol or GalactoPol) possess the capacity to establish physical and/or chemical intermolecular interactions, which result in a cohesive polymeric matrix that is able to form a film (Figure 4, Table 1). Recent research has focused on developing polymeric matrices with tuned properties (e.g. transparency, barrier and mechanical properties, biocompatibility or bioactivity) for several applications, namely edible coatings for food products [70,71] and packaging purposes [43,72] (Table 1, Figure 4).

Furthermore, the ability of EPSs to establish polymeric matrices enables their *in vitro* manipulation to shape them as structured materials (e.g. nanoparticles, scaffolds or hydrogels) that are adapted to specific biomedical applications including drug delivery, imaging, tissue engineering [75] and wound dressings [71].

As a result of their unique and tunable properties, several bacterial EPSs play an important role in the development of new pharmaceuticals, not only because of their ability of forming polymeric matrices, but also due to their inherent biological activity. For example, xanthan, sulfated dextran and sulfated curdlan, are used as antiviral [73] and anticancer agents [74]. As a result of its high fucose content, Fucopol [9] is seen as a product with potential to be used in antitumor, anti-inflammatory and immune-enhancer drugs (Table 1, Figure 4).

EPS can also be used as a source of oligosaccharides and sugar monomers, which constitute added value applications. Fucose is an example of a rare sugar that is difficult to obtain, for which supply falls short of demand in the international market (www.1biw.com/pj_view.aspx?id= 13772). Formulations that contain fucose, as well as fucose-containing oligosaccharides, have been reported to have properties that potentiate their use, either in pharmaceuticals (e.g. as anticarcinogenic or anti-inflammatory agents), or in cosmetic products (as anti-aging agents) [7]. Therefore, polysaccharides with a high content of fucose (or other added value components), such as FucoPol, can be seen as sources of valuable chemicals.
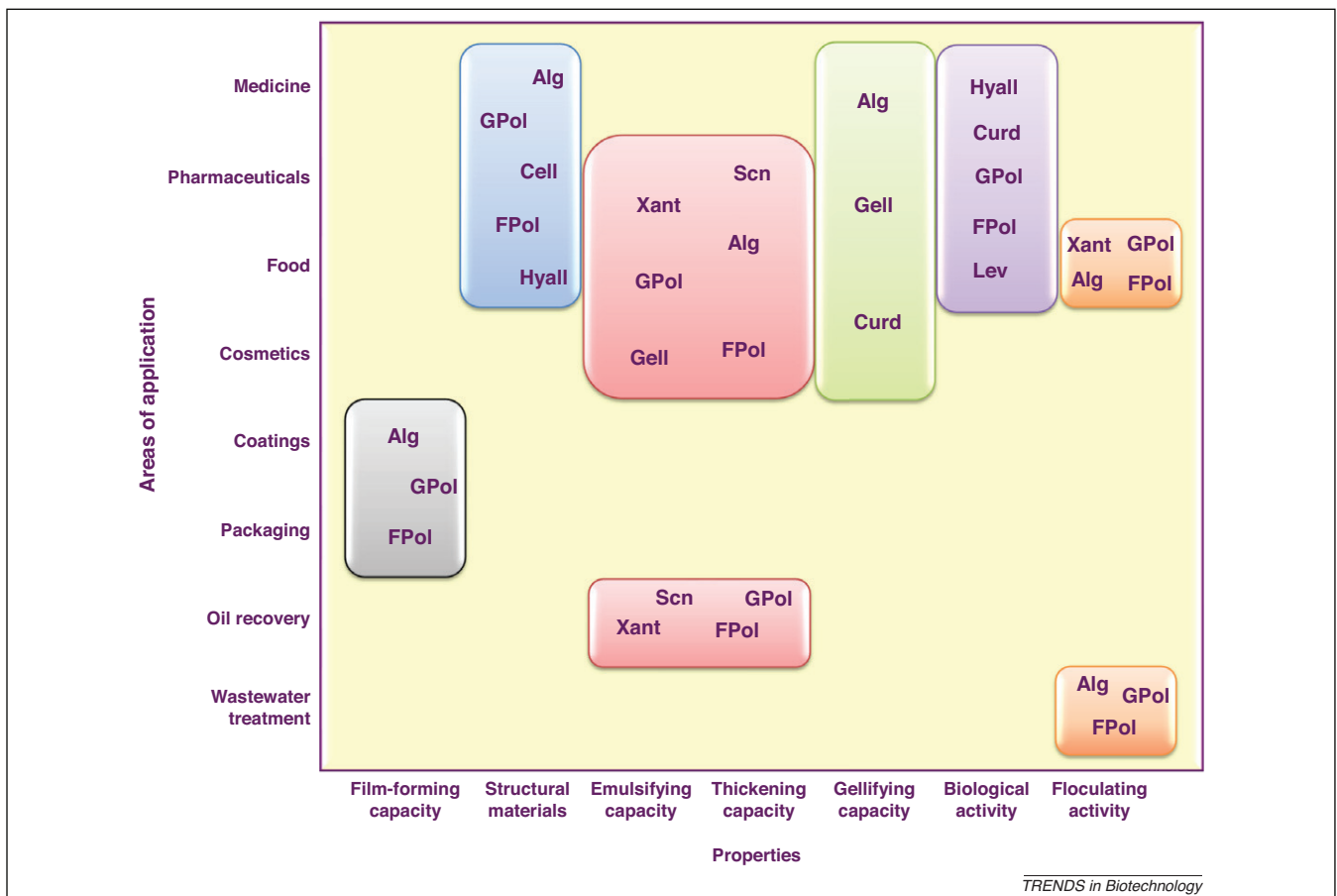
Figure 4. Diagram representing the correlation between the most relevant polysaccharide properties with their main areas of application. Abbreviations: Alg, bacterial alginate; Curd, curdlan; FPol, FucoPol; Gell, gellan gum; GPol, GalactoPol; Hyall, hyaluronan; Lev, levan; Scn, succinoglycan; Xant, xanthan gum.

The range of bacterial EPS applications includes areas in which larger amounts of polymer are used but that do not require a higher purity grade. One of the most important applications is in drilling fluids for oil recovery, in which xanthan gum is the most used bacterial polysaccharide due to its high viscosity-enhancing ability at low concentrations [76] (Table 1, Figure 4). Furthermore, novel/improved applications of extracellular polysaccharides are under study, namely for toxic compound sequestration [77], activated sludge settling [78], incorporation of clay nanoparticles in aqueous foams for suppressing gasoline vaporization [79], and production of elastic absorbents for chemical absorption of carbon dioxide [80].

Outlook and perspectives

To date, polysaccharides recovered from plant, algae and animal sources are still the major contributors to the overall hydrocolloid market. This is mainly because of the higher prices of bacterial polysaccharides, which are a consequence of the high value of the carbon sources commonly used and of the associated downstream costs.

Nevertheless, the research interest in bacterial production of polysaccharides is continuously growing, and is focused on using low-cost substrates and improving downstream processing, and on metabolic engineering that aims for production of polymers with fine-tuned properties. In

fact, the main advantage of bacterial polysaccharides relies on the possibility of tailoring their chemical composition and structure, which foresees rather specific applications in pharmaceutical products, medical devices and cosmetics. In the next few years, we can expect a significant increase in added-value products/technologies based on bacterial polysaccharides, especially developed for a market niche, in which the traditional hydrocolloids are not able to compete.

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