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Biotechnological challenges and perspectives of using exopolysaccharides

Abstract

Exopolysaccharides have several biotechnological applications fields including food industry, cosmetic, agricultural, pharmaceutical, medical, chemical and others. The main molecules come from plants, bacteria, fungi and algae. Although these substances are already used industrially, only a small portion of the biopolymers market is represented by them. In order to have a greater market share of these molecules, it is necessary to optimize several technological stages of EPS production on a large scale. Considering that the processes of extraction, isolation and purification of these polysaccharides can affect their physicochemical, structural and techno-functional properties, their gradual yield with maximum efficiency becomes a challenging objective. In this paper, the EPSs will be concisely addressed as regards their functional biotechnological applications and their productive aspects.

Keywords: polysaccharides, carbohydrate, polymers, natural compounds and BAL

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Introduction

In recent decades, natural polysaccharides have received special attention from researchers due to their possible physical-chemical, functional and industrial applications. Some of its advantages include biodegradability, abundance in nature, versatility in product development and health benefits. The major molecules are originated from plants, bacteria, fungi and algae.[1] The food industry widely uses vegetable polysaccharides, however they are not accepted in certain dairy products because of the labelling rules that allow only the inclusion of other sub-products of dairy origin. In addition, the usage of vegetable polymers in dairy products is banned in many European countries.[2] These restrictions forced the dairy industries to look for other possible polymers from dairy sources. In this way, the extracellular polysaccharides of lactic acid bacteria (BAL) have become the best option either because of the milk origin but also their GRAS (Generally Recognized as Safe) status.

Currently the food industry has extensively been using microbial exopolysaccharides (EPS) due to its application in improving the texture of fermented dairy products (xanthan gum), the production of films for biodegradable and/or edible packaging (chitosan) and also as a substitute for fat (Inulin).[3–6] Although these molecules have already been effectively used in the cosmetic (rheological stabilizer, fragrance carrier), pharmaceutical (nanoparticles for controlled drug release), agricultural (increases root water retention, improves salt tolerance), medical (adjuvant for vaccines, biofilm matrix, biodegradable food packaging, thickeners, emulsifiers, gelling agents), its representativeness in the world market for biopolymers consumption is still insignificant.[7,8] In this context, it is necessary to understand the possible actions that can increase consumption and reduce technological bottlenecks.

Biotechnological profile of the EPS

EPS are defined as carbohydrate polymers that can be produced by different microorganisms and are secreted in the extracellular environment or bound to the cell surface, forming capsules, which play an important role in cellular metabolism.[9] Bacteria and microalgae produce an amount of EPS suitable for an industrial scale based usage, while molds and yeasts produce fewer amounts.[10] These molecules are water soluble and have long, branched chains. They are classified into two groups: homo-EPS, consisting of a single type of monosaccharide (α-D-glucose, β-D-glucose, fructose and other representatives of polygalactans) and hetero-EPS, composed of different types of monosaccharides, especially D-glucose, D-galactose, L-rhamnose and its derivatives.[1]

Technological processing for EPS obtention

The processes of extraction of polysaccharides can affect their physical-chemical and structural properties. Therefore, the characterization of these molecules after their extraction is essential for their use in the industrial sector and dependant on the chemical structure extracted, especially for functional products.[11]

Previous studies have reported that the isolation of EPS from the fermentation medium and its subsequent purification step are challenging issues.[1] Different extraction procedures can result in different percentages of pure EPS. Considering that the determination of the physicochemical and/or techno-functional properties requires an EPS as pure as possible, the purity required for the quantification of EPS, the integrity of its bonds, branching and its sugar monomers will greatly depend on the adopted analytical approach.[12]

There are several EPS isolation procedures reported in the literature. Many of them use trichloroacetic acid and/or proteases for protein removal, dialysis, filtration or size exclusion chromatography (SEC) for the removal of minerals, monomeric/dimeric sugars and organic solvents for precipitation of EPS.[13] One of the most arduous and laborious hurdles ever reported for the isolation and obtaining of highly purified EPS is avoiding reactions of the molecule with components of the medium or solvents. Also the removal of proteins to avoid the co-precipitation of EPS is appropriate. The molecule can interact particularly with proteins (electrostatic or hydrophobic forces), and can also be trapped in pores of the protein network.

Depending on the adopted isolation steps, differences in EPS recovery are observed in several studies.

On the other hand, heat treatment or severe acid can cause cleavage and structural changes of EPS. An appropriate protocol for the isolation, quantification and characterization of EPS is essential so that each step of the process, fermentation medium, isolation purpose and characterization method is carefully adjusted for each type of previously planned EPS.[14,15]

Bacterial production of EPS

Although researchers have shown several important functional effects of EPS, their physiological role in the bacterium has not yet been fully elucidated.[10] Studies have stated that these molecules are generally not useful as storage compounds because many bacteria are not able to metabolize them.[4] However, these carbohydrates appear to be important for protection and cell to cell communication. The polymer layer around the bacteria bound to water increases the viscosity and affects the diffusion properties inside and outside the cells. They are also important for protecting cells from osmotic stress, antibiotics, toxic compounds or phage attacks.[8]

The amounts of hetero-EPS produced by the bacteria are quite varied. For example, *Streptococcus thermophilus* produces 50-350mg/l; *Lactococcus lactis subsp. cremoris* 80-600mg/l; *Lactobacillus delbrueckii subsp. bulgaricus* 60-150mg/l; *Lactobacillus casei* 50-60mg/l; and *Lactobacillus plantarum* approximately 140mg/l. The highest hetero-EPS yields were verified for *Lactobacillus rhamnosus* RW-9595M and *Lactobacillus kefiranofaciens* WT-2B, 2775mg/l and 2500mg/l, respectively.[16] It is seen that the amount of EPS produced by *Lactobacillus sp*. is very low when compared to the production of other industrially important microorganisms, such as *Xanthomonas campestres*, which produces 30-50g/l xanthan gum. However, the production of EPS produced by LABs must be stimulated since they are GRAS microorganisms, especially those with bioactive properties.

It is well known that monosaccharide subunits of Hetero-EPS are not species-specific. However, in the case of microorganisms contained in the kefir grain, particularly *L. kefiranofaciens subsp. kefiranofaciens* there is a molecule with a remarkable chain of Monosaccharide subunits of Hetero-EPS namely kefiran.

Biotechnological applications of promising EPSs

One of the most auspicious bioactive Hetero-EPSs, candidate for pharmaceutical and food industry usage is known as kefiran.[17] Although it was discovered in 1967, only recent studies have identified and characterized important biological activities for kefiran, such as immunomodulatory action;[18] anti-inflammatory effect;[19] antioxidant;[20] anticancer;[21,22] antimicrobial activities[23,24] and modulation of lipids, blood pressure and blood glucose.[25] Additionally, pro-apoptotic and anti-proliferative effects for human colon adenocarcinoma cell lines (Caco-2 and HT-29) suggest that kefiran can be used as a functional food to prevent oncological diseases of relevant prevalence.[26]

Kefiran is a water-soluble, branched molecule containing approximately equal proportions of D-glucose and D-galactose, which are synthesized by the microorganisms present in kefir grains.[27] Research has shown applicability of this EPS in the food industry: a) increase in the viscosity of aqueous solutions and acid milk gels;[17] b) production of edible and transparent EPS similar to most films based on polysaccharides, applicable to regulate transferring of gas, aroma, flavour and oils in a food system; c) increase in the shelf-life and quality of food products, among others.[28]

Levan is a natural (*Bacillus subtilis and other microorganisms)* Homo-EPS composed of fructose monomers. It has many different properties, such as high solubility in oil and water, adhesiveness, good biocompatibility and film forming capacity, great potential as a new functional biopolymer in food, animal food, cosmetic, pharmaceutical and chemical industries. Other studies have also been reported using it as a bioflocculation agent, prebiotic, cryoprotectant, peptide-based drug nano carrier system and multi-layer adhesive film former.[5,8] Due to its exceptionally high production costs, Levan has never been able to find its right place in the polymer market, and therefore, microbial systems producing high-grade levan have gained increasing industrial importance.[8]

Gellan gum is a natural, water-soluble, anionic EPS (*Sphingomonas elodea*) which in the food industry is commonly used as an additive (stabilizer, thickening agent and gelling agent). However, gellan gum applications can also be extended to membranes and barrier coatings for the food industry, such as breading and batters for chicken, fish, cheese, vegetables and potatoes.[29,6]

Alternan is a natural (*Leuconostoc mesenteroides*), Hetero-EPS produced by alternansucrase that due to its unique structure, has high solubility, low viscosity and remarkable resistance to enzymatic hydrolysis. Alternan is commercially exploited as a low viscosity and extender volume agent in food and cosmetics. They are used as low glycemic index sweeteners in confectionery and as prebiotics.[4] Research on other bacterial Hetero and Homo-EPSs, as well as the discovery of new molecules in the group, especially those with bioactive properties, continue in parallel to the demand for technical advances in more efficient production processes.

Conclusion

In this context, either to increase the efficiency of the productive processes of EPS applied to the mentioned industrial segments or to discover new molecules of this biopolymer, especially those that have bioactive properties, it is necessary more exploratory studies on new natural sources and improvement of its productive processes.

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Conflict of interest

The author declares that there is no conflict of interest.

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