

Review Article

Microbial polysaccharides in food industry: Production, Applications, Legislative and toxicological affairs

Abstract

Microbial polysaccharides, including exopolysaccharides (EPS) play significant roles in various industries due to their functional properties. This review discusses the extraction and purification techniques of microbial polysaccharides from bacteria, emphasizing centrifugation, ultracentrifugation, heat treatment and specific chemical treatments based on the polysaccharide's association with cells. Techniques vary depending on whether the polysaccharides are capsular or slime forming. Notable microbial polysaccharides such as dextran, curdlan, xanthan, alginic acid, bacterial cellulose, levan and algal polysaccharides highlighted, detailing their sources, applications, and benefits in food production, stabilization, and thickening. The diverse application of these polysaccharides underlines their importance in food technology and other industries, their potential for innovation in product formulation and quality enhancement.

Keywords: polysaccharides, medical applications, cellulose, glycosaminoglycans

Introduction

Numerous creatures, including plants, animals, and microbes, receive polysaccharides from the vast array of polysaccharides found in nature. Numerous biological processes rely on polysaccharides, including cellulose for cell walls, starch for energy storage, and glycosaminoglycans for cellular communication (Ates, 2015; Held et al., 2015). Polysaccharides are widely present in nature because they are essential components of cell structure. Polysaccharides are long chains of carbohydrate molecules, composed of several smaller monosaccharides. Glycosidic linkages bind the linear or branching polymers of monosaccharides together. These polymers can either be heteropolymers, where all the monosaccharide units vary, or homopolymers, where all the monosaccharide units are the same. The wide range of properties shown by polysaccharides explained by the variations in their molecular weight, type of monosaccharide units and their linkages, and the presence of substituents like amino or carboxyl groups on the monosaccharide units (Phillips & Williams, 2020). Many of them used in food industries and human diet, as natural ingredient or as purified food additive obtained from the fermentation of microbial polysaccharides (use of yeast), as well as pharmaceuticals (as bioactive compound or media for encapsulation), cosmetics and other industries applications (Sutherland, 1998). Microbial polysaccharides are used for food, pharmaceutical, and medical applications this wide range of usefulness derives from the great diversity in structural and functional properties. Microbial polysaccharides have several advantages as compared to synthetic and plant polysaccharides and some of them are:

Production of microbial polysaccharides are independent on regional and climatic conditions, and polysaccharide obtaining from microorganisms is effortless process. Microorganisms exhibits high growth rate, and condition optimization for growth. Microorganisms genetically modified to produce polysaccharide with desired properties. The ingredients are simple and safe to use, required for microbial growth. Efforts have been made to use certain industrial food wastes as growth medium for microorganisms, which is not only a cheap way but also works as a basis for environmental cleaning (Ladtrat et al., 2014).

While seaweed and plants account for most of the polysaccharides used in the industry today, microbial polysaccharides with new and distinct physical characteristics have lately become significant biopolymers. Polysaccharides of microbial origin have proved themselves as new polymeric materials that are competing economically with other natural gums, such as Arabic gum and carrageenan produced by plants and marine algae, respectively (Milani and Maleki, 2012; Shit and Shah, 2014).

The microorganisms may produce a quite good quantity of polysaccharides when a plentiful carbon source is available. Glycogen is one of these polysaccharides that functions as a storing substance. Cells release polysaccharides called exopolysaccharides, which are important to the world economy. The medium may include exopolysaccharides by itself or in combination with the cells. The microbial polysaccharides might be neutral (dextran, scleroglucan) or acidic (xanthan, gellan). Acidic polysaccharides that include ionized groups, such as carboxyl, and may function as polyelectrolytes are becoming increasingly important in the market. Microbial polysaccharides have properties such as viscosity thickeners, gelling and film forming agents, stabilizer, texturizers and emulsifiers, thus have broad spectrum of its application.

In recent research it is found out that some microbial polysaccharides show significant immunomodulating properties (antitumor, anti-inflammatory, antimicrobial) thus making it essential ingredient in human diet (Giavasis and Biliaderis, 2006).

For particular groups of consumers, the applications of animal-based-gelatin in the food and pharmaceutical products are restricted because the animal source does not meet their religions' requirement. Muslims and Jews are prohibited to consume any pork related products, Hindus are not allowed to consume beef, while vegetarians and vegans do not include any meat in their diet. The outbreak of mad cow disease in the 1980s is another concern for consumers due to the risk of consuming gelatin from infected cattle. Food processors are already seeking substitutes for animal-sourced ingredients. Although these alternatives meet some gelatin characteristics, none is yet available that matches all the desired functional properties of gelatin. Until now macromolecules of plant origin include starch, locust bean gum, guar gum, carrageenan, and alginate, have captured the commercial market for their ease of availability and cost-effective purification process. However, renewability, stable cost, constant, and reproducible physio chemical properties of the microbial polysaccharides have provided them an edge over the macromolecules of plant origin, although so far only few of them have been commercialized. One limitation is polysaccharide based gels generally lack of molecular backbones flexibility which tend to have higher viscosities compared to animal-based gelatin.

Extraction and Purification of Microbial Polysaccharides

Various techniques are utilized to extract distinct types of exopolysaccharides from bacteria. Centrifugation is often used to remove EPS that has slime forms. The kind and viscosity of the polysaccharides determine centrifugation duration and spread. Ultracentrifugation can be used to extract microbial biomass or their waste products from the growth medium for improved outcomes in the lab (Singha, 2012). Heat treatment can be utilized to enhance the separation of microorganisms from the broth when the EPS is thermally stable. The heat treatment inactivates the enzymes in the culture broth and kills the cell by pasteurization, but it has no effect on the viscosity of these polysaccharides (Bajaj et al., 2007; Singha, 2012). Because the capsular EPS must first be separated from the cells, the extraction of capsular polysaccharide is somewhat different (Sanchez et al., 2006; Sreekanth et al., 2013; Vijayendra and Shamala, 2014). The kind of interaction between the polysaccharide to be extracted and the cell determines the procedures for separation and purification used for capsular polysaccharides. Centrifugation is used to accomplish separation when the capsular extracellular polymeric substances (EPS) have a poor association with bacteria (Sutherland, 1990). When the capsular EPS is tightly associated with the cells, more severe treatments (such as alkaline treatment or treatment with sodium chloride, EDTA, and alcohol precipitation) are performed prior to centrifugation. Besides chemical treatments, the other methods include boiling the microbial cell suspensions for 15 min, heating at 60°C in saline solution, heating in a mixture of phenol and water at 65°C, or sonicating the cell suspension (Khoddami et al., 2013).

Table 1 : *Some microbial polysaccharides, their source and application*

Microbial polysaccharide	Source	Uses or application
Dextran	<i>Leuconostoc mesenteroides</i> , <i>Lactobacillus</i> spp.	Thickener agent (ice cream), improving bread quality (softness texture)
Curdlan	<i>Agrobacterium</i> spp.	Stabilizer, thickener, texturizer (tofu, noodles, meat, jellies)
Xanthan	<i>Xanthomonas compestris</i>	Gluten free baking, thickening and stabilizing (sauces, soups, etc.)
Alginate acid	<i>Pseudomonas aeruginosa</i> and <i>Azotobacter vinelandii</i>	Thickener (sauces, syrups, ice cream), gelling, stabilizing, etc.
Bacterial cellulose	<i>Acetobacter</i> , <i>Achromobacter</i> , <i>Rhizobium</i>	Food additive, food packaging, low calorie food, etc.

Levan polysaccharides	Corynebacterium levaniformans, Aspergillus sydowii,	Sweetener and fiber (dairy products), thickener, emulsifier, stabilizer, etc.
Exopolysaccharides	Lactic acid bacteria, endophytic fungi	Thickener and stabilizer (dairy products), texture (meat products), etc.
Algal polysaccharides	Microalgae and cyanobacteria	Thickening, gelling, emulsifying, packaging, etc.

Dextran:

The first microbial polysaccharide to be approved for use in food. Dextran is produced by fermentation of sucrose by means of enzymes of leuconostoc or Streptococcus at pH 6.5 to 7. When organic solvents like methanol, ethanol, etc. are added, dextrans are precipitated. By precipitation, all unwanted contaminants are removed and resulting with white powder (Wustenberg, 2014). Dextran is produced in an extracellular process by fermentation of sucrose by enzymes of Leuconostoc or Streptococcus at pH 6.5–7. After fermentation, the culture medium is cleared of microorganisms. The solubilized dextrans are precipitated by the addition of organic solvents, such as methanol, ethanol, 2-propanol, or acetone. By reprecipitation, entrained contaminants are removed. The resulting product is a white powder (Wustenberg, 2014). Dextran is highly branched, neutral homopolysaccharide made up of glucose unit. At room temperature, dextran is readily soluble in water. Dextran forms highly viscous and slimy liquids (Kapoor et al., 2013; Werning et al., 2012).

Applications

Dextran is added in baking industry to bake goods to enhance softness, crumb texture, and loaf volume. It is used as stabilizer in confections to help with moisture retention, taste preservation, viscosity, and prevention of crystallization. It is also utilized in soft drinks, milk **beverages, and icing composition**. **Dextran is thought to offer benefits over other stabilizers since it is tasteless, odorless, and nontoxic (Bhavani and Nisha, 2010) (Kothari et al., 2014).**

Legislative and toxicology affairs

Because dextran is easily broken down in the digestive system of humans, it may be used as an energy source. Since it expands blood plasma, the kidneys can readily get rid of it. Thus, it has approval in many countries for use in food products. There is no acceptable daily intake value mentioned for dextran (Scherz, 1996).

Curdlan:

A fermentation produced polysaccharide that is a member of the glucans class of molecules. Curdlan is produced in an extracellular process by the nonpathogenic bacterium Agrobacterium biovar and mutants of Alcaligenes faecalis. The powder is colorless and odorless (Zang and Edgar, 2014). Glucan is mainly insoluble in water below 54°C. Above this point, heating the solution causes it to swell and

solidify into a strong, elastic gel. Gel strength is in the range of gelatin and agar. Aqueous alkaline solution can dissolve curdlan (Saha and Bhattacharya, 2010; Tomasik, 2004).

Physical properties

Heating causes curdlan to form an irreversible gel. The gel becomes stronger as the temperature rises. The curdlan produces insoluble films in water. These gels are edible, biodegradable, and oxygen impermeable (Ghanbarzadeh and Almasi, 2013; Khan et al., 2007).

Applications

Curdlan finds application in the food and pharmaceutical sectors as a gelling agent. Gelatin and agar is replaced by curdlan for making jellies, sweets, and confectionary. Additionally, dietetic meals (such as salad dressings, desserts, and pasta) employ it as a thickening and binder. To extend the shelf life of food goods, it is also used as an edible coating (Nishinari et al., 2009; Scherz, 1996).

Legislative and toxicology affairs

The US FDA has authorized curdlan as a food ingredient. Curdlan has also been licensed for use in food in Japan (Shetty et al., 2006; Venugopal, 2011).

Xanthan gum:

A microbial polysaccharide produced by the bacterium *Xanthomonas campestris* that is often used in foods for people with dysphagia. The 1960s saw the discovery of xanthan gum, which became commercially available in the 1970s (Huang et al., 2010; Wustenberg, 2014). It is made by using pure cultures of *Xanthomonas campestris* to ferment glucose or sucrose solution aerobically. Following the inoculation of a chosen bacterial strain, fermentation is continually carried out for around three days at 30°C. Sterilization is done on the broth after fermentation is finished in order to get rid of any living microorganisms. Centrifugation is the method used to remove the extracellular components. Isopropyl alcohol precipitation is used to recover xanthan. Xanthan fibers are separated using centrifugation or filtering, and then they are dried and ground for packing. About 50000 MT of xanthan gum are produced annually worldwide, with 50% of that amount going toward culinary applications. A marketed white xanthan powder has an approximate moisture content of 11% and an ash content of 6% to 9% (Imeson, 2010).

Chemical composition and structure

Xanthan is long chain polysaccharide having large number of trisaccharide side chain and a molecular ratio 3:3:2, d-glucose, d-mannose, and d-glucuronic acid make up the building blocks of xanthan. The average molecular weight of the xanthan is 2000kDa.

Physical properties

Xanthan gum is a fast-hydrating water-soluble hydrocolloid that can easily be dissolved at room temperature. Continuous mixing and stirring reduce the hydration time of xanthan. Long-chain xanthan polymers are easier to disperse but slow to hydrate. Generally, hydration depends on the ionic strength. If there is high ionic strength, the hydration tends to be slow. With increase in temperature, the otherwise rigid ordered state at low temperature turns to a more flexible, disordered state, and

conformational transition of the solution occurs. Xanthan solutions have the viscoelastic properties forming a weak gel, giving suspending characteristics in liquid foods, such as dressings, sauces, or cakes before baking. The behavior of xanthan solutions does not change over a wide range of pH (3–10). It is reported that it is more stable than other thickeners and even resistant to enzymes, such as amylases, proteases, and cellulases (Wustenberg, 2014).

Applications

The main use of xanthan is in dressings and sauces. Addition of xanthan in dairy desserts supports gel formation and reduces syneresis (Saha and Bhattacharya, 2010; Vuyst and Degeest, 1999).

Alginic acid:

An edible polysaccharide found in brown algae that is also known as algin. Alginic acid is commonly used natural polysaccharide that is obtained from brown seaweeds such as macroalgae, kelp, gulfweed, and Ascophyllum. It can also be produced by microbial fermentation using specialized bacteria (Aderhold, D., Williams, C. J., & Edyvean, R. G. J. (1996). The cytoplasm contains alginic acid by nature, which is important for strengthening the cell wall (A° gren, M. S. (1996). According to Myklestad research, alginic acid is mostly contained in cell as calcium alginate, but it is also partially present as magnesium alginate, potassium alginate, and sodium alginate. The extract obtained from seaweeds is usually sodium alginate.

Physical properties

Thick colloid is formed when sodium alginate is dissolved in water and its volume can increase with respect to the water absorption. Sodium alginate solution has anti charge because it maintains negative ion group and condenses hydrophobic suspension (Guo, X., Wang, Y., Qin, Y., Shen, P., & Peng, Q. (2020). Alginic acid has antianaphylaxis effect, immunomodulatory activity, antioxidant activity, and anti-inflammatory effect.

Legislative and toxicology affairs

Sodium alginate is an indispensable nutrient for the human body-edible fiber (Guo,X et al., 2020). It has an auxiliary curative effect on the prevention of colon cancer, cardiovascular disease, obesity, and the accumulation of lead and cadmium in the body. Sodium alginate is known as "health and longevity food" in Japan, and is a wonderful food additive in the United States (Aderhold et al., 1996). As a species of seaweed gum, it can improve the properties and structure of food with its inherent physical and chemical improvement.

Bacterial cellulose:

A microbial polysaccharide that can form various shapes and textures, and can change the flavor and color of food. Bacterial cellulose is produced by various bacterial species. Well known examples are Salmonella, Acetobacter, Rhizobium, Agrobacterium, Aerobacter, Azotobacter, Sarcina ventriculi, and Glucanacetobacter (Ullah MW, UI Islam M, Khan S, Shah N, Park JK (2017). Inside the bacterial cell, β -1,4glucan chains are formed, which are the precursors of bacterial cellulose. The terminal complexes,

which are located at the cell's outer membrane, allow the polymers to be expelled into the culture medium. These polymers crystallize in the medium to form highly organized structures including protofibrils, ribbons, and bundles, which are ultimately transformed into hydrogels at the medium air contact (Kim Y, Ullah MW, UI-Islam M, Khan S, Jang JH, Park JK(2019). Bacterial cellulose performs several functions that are important for the life cycle of the organisms. These include flocculation and plant attachment facilitation. When compared to cellulose derived from plants, bacterial cellulose has several unique characteristics that set it apart, including high purity, hydrophilic nature, and a finer three dimensional fiber structure (UI-Islam M, Khan S, Ullah MW, Park JK (2019).

Levan polysaccharide:

It is naturally occurring polysaccharide found in many plants and microorganisms. Many plants and microbes naturally contain levan, a fructan (Meyer, Diederick 2015). This polymer is composed up from fructose, a monosaccharide sugar, joined by 2,6 beta glycosidic links. Levan may have comparatively low molecular weight structures that are both branching and linear (Gehatia, M.; Feingold, D. S. 1957). With basal chains that are nine units long, branched levan produces a very tiny, sphere like structure (Arvidson et al., 2006). The spherical shape of methyl ethers is produced by the 2,1 branching. Levan also typically has a glucosyl residue at the ends (Srikanth et al., 2015). Compare to linear polysaccharides, branching levan is often more stable (Öner et al., 2016). Nonetheless, there is often variation in the duration of polymerization and degree of branching between species (Srikanth et al., 2015). 6-kestose, a chain consisting of two fructose molecules and a terminal glucose molecule, is the shortest levan.

Production

A few plants species, bacteria, fungus, and archaea all manufacture levan. Sucrose, a disaccharide including glucose and fructose, is the starting point for the synthesis of fructans like levan (Öner et al., 2016). Levan is also formed by bacteris using fructosyltransferase called levansucrase (Öner et al., 2016). In order for branching sites to occur, these bacterial enzymes create the 2,1 connections in the linear basal chains of levan. Levan is produced externally by several microorganisms (Öner et al., 2016). A number of variables, including pH, oxygen content, and temperature, may have an impact on its formation (Öner et al., 2016). Levan production in bacteria is usually indicative of population expansion. Soybean mucilage may also be produced by breaking it apart. Microbes create levans when they colonize a food substrate. Levan and amylovoran are released by *Erwinia amylovora* as a component of its biofilm. When combined, they add to its pathogenic potential (Mansfield et al., 2012). A sourdough technique with significant levan production (among other **exopolysaccharides**) was developed in 2016 (Ua-Arak et al., 2019)

Levan's beta 2,6 connections make it soluble in both water and oil, albeit the amount of solubility varies depending on the water's temperature (Ouweland, Arthur 2012). Levan is also insoluble in a wide range of organic solvents, including isopropanol, methanol, and ethanol. Levan's strong cohesive and tensile strength is a result of its branching, and its hydroxyl groups help it stick to other molecules (Srikanth et al., 2015). Levan typically has a relatively low intrinsic viscosity η , which is a measure of the substance's impact on a solution's viscosity. Levan can now be used in a pharmaceutical context because to this (Ouweland, Arthur 2012).

Legislative and toxicology affairs

Levan is a substance used in many sectors, including food, drinks, cosmetics, and even medical. Levan complies with all safety regulations, which is one of the reasons it can be used so extensively. Levan has no allergic effects, does not irritate skins or eyes, and does not provide a cytotoxic risk.

Exopolysaccharides (EPS):

Polysaccharides that are synthesized by lactic acid bacteria during fermentation and have prebiotic characteristics. EPS has the ability to replace commercially available food additives since it is a natural substitute for artificial additives and has physiochemical properties. The films and coatings on fruits and vegetables that are edible are eatable and have a thickness of around 0.3 mm. To improve the preservation of processed fruits and vegetables, these coatings are frequently used (Moradi et al., 2021). These films might be based on lipids, proteins, or polysaccharides. Sometimes it becomes quite difficult in the case of artificial film and coatings. Since humans directly ingest these, it is unacceptable. Thus, these coatings and films have to be entirely organic in origin. Because of this, bacterial extracellular polymerization (EPS) offers the most favorable conditions for the development of these coatings and films (Daba et al., 2021).

Applications

EPS is employed in the food business for a variety of purposes as biothickeners. Additionally, they have a few key techno-functional qualities that set them apart for application in the food sector (Ryan et al., 2015). They are also used in food packaging and guarantee creaminess, mouthfeel, and improved food texture. In addition to rheological qualities including decreased syneresis, increased viscosity, and enhanced texture, they also impart stabilizing, emulsifying, and thickening capabilities to food material (Abarquero et al., 2022).

Properties

There are multiple properties of EPS when it is used as edible films or coatings. First, they act as a barrier to gases and moisture loss. In this way, the process of oxidation and spoilage is retarded. This adds up to the shelf life of the food products. For example, gellan and xanthan gums are excellent and commonly used to attain gas and moisture barrier properties (Lynch et al., 2018). In the same way, these are widely used in food packaging as they are biodegradable. The packaging made by xanthan gums can decompose more rapidly as compared to any other artificial packaging (Kim et al., 2024).

Algal polysaccharides:

Polysaccharides that have antibacterial and anti-inflammatory properties, and are sometimes considered dietary fiber. Algal polysaccharides are renewable, abundant, biodegradable, biocompatible sources for biopolymer production and are highly used in the food industry as a thickening, gelling agents, emulsifiers, and stabilizers, and also used as packaging material (edible coating or film for active and intelligent packaging) (Ścieszka & Klewicka, 2019).

Application

The increasing awareness among consumers about foods that promote health has driven the food industry to develop products with the incorporation of bioactive compounds (Karelakis et al., 2020). In this context, nanotechnology can contribute to obtaining functional foods and nutraceuticals. Nanoencapsulation of bioactive compounds for food enrichment can prevent the degradation of these compounds during processing and storage and increase stability and bioavailability, preserving their bioactivity (He et al., 2016). Furthermore, using polysaccharides as encapsulants in the food industry can prevent the loss of volatile compounds and improve the dispersion of low-solubility compounds in the food matrix (Pateiro et al., 2021). Polysaccharides from algae have characteristics of interest as an encapsulating material, such as bioavailability, biocompatibility, bioactivity, and non-toxicity (Zavareze et al., 2019).

Legislative and toxicology affairs

Polysaccharides have been associated with many other significant biological activities such as anticoagulant, antithrombotic, and antiviral activities including anti-HIV infection, herpes, and hepatitis viruses, and their health benefit properties have been deeply studied. Generally, biological activities of sulfated polysaccharides are related to their different compositions and extent of sulfation (Mišurcová, L. 2011).

Conclusion

Polysaccharides play a crucial role in various biological processes and applications, serving as fundamental components in both plant and microbial systems. Their structural diversity—resulting from variations in monosaccharide composition, linkage types, and functional groups—enables a wide range of functionalities. Microbial polysaccharides, in particular, have emerged as significant alternatives to traditional plant-based polysaccharides, offering benefits such as consistent quality, rapid production, and potential for genetic modification to enhance specific properties. The applications of microbial polysaccharides span across multiple industries, including food, pharmaceuticals, and cosmetics. Their ability to act as thickeners, emulsifiers, and stabilizers, combined with unique health benefits such as immunomodulation and prebiotic effects, positions them favorably in the market, especially amid growing consumer demand for vegan and allergen-free ingredients.

Despite their advantages, challenges remain in the commercial utilization of microbial polysaccharides, primarily concerning extraction methods and the functional properties of polysaccharide gels compared to animal-derived alternatives. However, ongoing research and technological advancements hold promise for overcoming these limitations and expanding the scope of microbial polysaccharides in various applications. Overall, the future of polysaccharides, particularly those derived from microbial sources, appears promising, as they continue to adapt to the needs of modern industries and consumer preferences for safe, effective, and sustainable ingredients.

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