The application of microbial extracellular polymeric substances in food industry

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Abstract. Extracellular polymeric substances (EPS) are the biopolymers that naturally produced and secreted by wide species of microorganism. EPS are composed of protein, nucleic acid, lipid, and other bioactive molecules, but the main composition is polysaccharides. The EPS chemical composition and physical properties are different between species that influence their functional properties. The microbial EPS are utilized in various application such as wastewater treatment, cosmetic, pharmaceutical, and food industry mostly as flocculant, thickener, and emulsifier. This paper outlined the microbial EPS, especially their unique properties and application in the food industry. Moreover, we discussed the potential application of microbial EPS as edible coating.

Keyword: microorganism, polysaccharides, food, edible film

1. Introduction
Extracellular polymeric substances (EPS) are the natural polymers of height molecular weight compounds secreted by the microorganisms into their environment. Microbial EPS are produced from various species of prokaryotes (e.g., Archea, Bacteria, and Fungi) and eukaryotes (e.g., unicellular algae). The EPS are secreted by the cells then remains associated on the cell surface that leads to sheath formation or loosely bound from the cell surface that lead the slime formation [1-2]. It suggested that the EPS matrix served as a protective layer for the cells against the environmental stress condition. Besides, EPS also works as the primary material for bacterial biofilm that has a multipurpose functional element of microbial communities, including adhesion, structure, protection, recognition, and physiology [1-3].

The composition of EPS belongs to organic and inorganic macromolecules such as carbohydrates, protein, nucleic acids, glycoproteins, and phospholipids. Besides, the EPS are mainly composed of polysaccharides, with glucose, galactose, and mannose being the most commons monomers, therefore namely exopolysaccharides. The composition and structure of EPS are homopolymer as well as heteropolymer [4].

The microbial EPS are widely utilized in food, pharmaceutical, biomedical, and bioremediation due to their chemical structure diversity and unique properties. EPS has a high viscosity in aqueous solution, making them extensively used as stabilizing, thickening, and gelling agents [4]. For example, xanthan gum is widely used in the food industry as stabilizer and emulsifier due to its high viscosity and solubility. Moreover, some types of EPS (i.e., pullulan and levan) are recently developed as food
coatings. Those materials are suggested could increase the food shelf life due to their antimicrobial properties [4-5].

This paper is an overview of the chemical composition, structure, function, and application of microbial EPS. Moreover, we will discuss the use of EPS in food industry, especially as the edible coatings.

2. Microorganism producing EPS
EPS have been isolated from a diverse group of Archea, Bacteria, Fungi, and Alga that are found in the various ecosystem. Most of the EPS-producing microorganisms are found in a medium with high carbon/nitrogen ratio and high organic substances such as paper and food industries wastewater plants. Moreover, EPS-producing microorganisms belong to the mesophilic, thermophilic, and halophilic groups [2-4,6]. For example, Lactobacillus rhamnosus that belongs to mesophilic lactic acid bacteria are known as the producer of heteropolysaccharides EPS with Rhamnose, Glucose, and Galactose as the significant sugar composition [7-8]. Halophilic bacteria belong to the genus Halomonas are the potential producer of high-sulfate and high-uronic content EPS that is the suitable material for gelling agents [2].

EPS are suggested to have an essential role in the microorganism physiological system such as protection, nutrient exchange, stress response, and cell recognition. The formation of EPS is mainly stimulated by environmental stress such as unfavorable environment and toxins material. The EPS works as the protective layer to prevent the invasion and the interaction of toxins or foreign substance to the cell membrane. The EPS matrix also helps the absorption of nutrient as part of survival. Moreover, the EPS provides intercellular recognition during the colonization process [2, 4, 6].

EPS production is influenced by cell-growth phase, nutrient composition of medium, and environmental condition (e.g., pH and temperature). EPS accumulation is associated with cell growth, suggesting by large accumulation during stationary-phase [6]. Moreover, EPS production is high during active sugar consumption because that process needs active nucleotide as the energy source for polymerization and translocation [2,9]. The medium composition of carbon, nitrogen, phosphate, and oxygen, which carbon/nitrogen ratio strongly affects the conversion of carbon into polysaccharides of EPS [4,6]. The environmental condition influences cell growth and stress condition that indirectly affects EPS production.

3. Microbial EPS: structure, function, and application
The microbial EPS are categorized as homopolysaccharide and heteropolysaccharide based on their sugar composition. Homopolysaccharide is composed of only one monosaccharide, while heteropolysaccharide is composed of three to seven various monosaccharides [4]. Typical monosaccharides are pentoses (e.g., D-ribose and D-xylose), hexoses (e.g., D-glucose, D-mannose, D-galactose, L-fucose, and L-rhamnose), amino sugars (e.g., N-acetyl hexosamines, N-acetyl-D-glucosamine, and N-acetyl-D-galactosamine), and uronic acid (e.g., D-glucuronic and D-galacturonic)[1,4-6,10]. The EPS composition and structure are diverse among microbial species. For example, most of cyanobacteria EPS is a complex heteropolysaccharides that composed of six to ten monosaccharides [3]. While, the EPS of lactic acid bacteria, dextran, is only composed of glucose [11]. Moreover, most of bacterial EPS is composed of less than four monosaccharides [1,6].

The wide range of EPS structures and shapes are attributed to the sugar composition, monomer arrangement, glycosidic linkage, and complex entanglement. Moreover, those factors affect the physical properties of exopolysaccharides. The homopolymers composed of heaxose are neutral [1]. The high content of uronic acid gives an anionic charge. The deoxy-sugar and ester-linked acetyl groups attribute the hydrophobic properties of EPS [1,3-4,6]. The physical properties affect the interaction between EPS to the cell surface, and organic macromolecules in the medium furthermore determine the unique functional properties.

The exopolysaccharide chemical and physical properties provide their high-value application in food, pharmaceutical, and cosmetic industry as flocculant, gelling agent, and emulsifier. The
The flocculation property is attributed to the electrostatic force and neutralization charge between the organic substances and EPS that affected by the anionic charge of EPS and environmental condition such as pH and salinity [4,12-13]. The functional properties of EPS as gelling agent and stabilizer are resulted by the viscosity and non-Newtonian behavior, attributed by the structure and composition. Different type of EPS can be applied in different food product because of their ability to maintain their functional properties during the incorporation into the formulation and the addition of other food components [1]. Xanthan gum is widely used as a stabilizer in sauces due to their high solubility in the water alongside the acidic condition and wide temperature range [14, 15]. Gellan gum is a suitable thickening agent for jams and confectionery products. The functional properties of gellan gum are attributed to the ionic strength of the solution and the concentration of biopolymer [14]. Conversely, dextran is widely applied as stabilizer and emulsifier of bread to increase the airiness and softness [14, 16]. EPS is also used as the source of low-calorie sugar such as fucose and xylose [10]. Besides, EPS is recently developed as encapsulation and edible coatings material because of their capability to build the polymer matrix and non-toxic properties. Encapsulation can protect the structure and control the release of the bioactive compound such as pigment [5]. Table 1 shows the list of microorganism-producing EPS and the broad area of EPS application in the food industry.

### Table 1. Overview of microbial extracellular polymeric substance (EPS) and the main areas of application in food industry

| EPS types           | Microorganism species          | Components of EPS                        | Application in food technology                          | References     |
|---------------------|--------------------------------|------------------------------------------|--------------------------------------------------------|----------------|
| Xanthan             | *Xanthomonas campestris*       | Glucose, Mannose, Glucoronic acid, Acetate, Pyruvate | Cryoprotectan, stabilizing agent, thickening agent | 1,4,14-15      |
| Gellan              | *Pseudomonas elodea*           | Glucose, Rhamnose, Glucoronic acid, Acetate, Glycerate | Gelling agent, thickening agent, emulsifier | 1,4,14,17      |
| Dextran             | *Lactic acid bacteria* (e.g., *Leuconostoc mesenteroides*, *Streptococcus mutans*, *Acetobacter capsulatus*) | Glucose | Cryoprotectan, gelling agent, emulsifier, thickening agent | 1,4,11,16,18 |
| Pullulan            | *Aureobasidium pullulans*     | Glucose                                  | Edible coatings, thickener                             | 1,4-5,19-20 |
| Curdlan             | *Alcaligenes faecalis*         | Glucose                                  | Gelling agent, Stabilizer, emulsifier                 | 1,4            |
| Scelero glucan      | *Sclerotinum rolfsii*          | Glucose                                  |                                                        | 1,4            |
| Levan               | *Zymomonas mobilis*            | Fructose, Rhamnose, Fructose, Fucose, Galactose, Glucose | Prebiotic, Thickener, Stabilizer, emulsifier, edible coatings | 1,4,21, 3,22,23 |
| Microalgae-based EPS| *Phaeodactylum tricornutum*    | Glucose, Mannose |                                                        | 22,24         |
|                     | *Porphyridium sp.*             | Xylose, Galactose                       |                                                        | 22,25,26      |
|                     | *Rhodella reticulata*          | Xylose, Galactose                       |                                                        | 22,25         |

4. Microbial EPS as the source of edible coatings

Pullulan is a homopolymer EPS derived from *Aureobasidium pullulans* that composed of maltotriose,
the three glucose units linked through the α-1,4-glycosidic bond. Pullulan is commercially produced and widely applied as food coatings to prevent the growth of bacteria in order to extend the shelf life. In Japan, pullulan has been extensively used as edible packaging film especially for confectionery product as the suitable substitute of gelatin. It is also used in other food products such as instant beverages, creams, sauces, and meat-based product (e.g., sausages and ham) [5, 19, 27]. Recently, pullulan is developed as promising material for the encapsulation of bioactive compound to enhance stability and bioactivity [22]. Besides, pullulan is utilized as food additives, for example, as texture stabilizer, food preservation, and flavor enhancer [5, 27].

Pullulan is commercially supplied in white-colored dry powder, tasteless, and odorless. It has high solubility in water furthermore it forms a stable viscous solution in the wide range of pH and solution temperature (hot and cold). Pullulan has specific properties as edible film material such as non-toxic, mechanically strength and flexible, heat resistant, oil-resistant, and low oxygen permeable [27]. Pullulan resulting films are colorless, tasteless, and odorless, making it a suitable candidate for food coating because it will not affect the taste, color, and aroma from food [28]. Moreover, the addition of cholesterol can transform the hydrophobicity of pullulan, making it suitable to apply as protein carriers [29].

Alongside with pullulan, other microbial EPS is also a potential material to use as an edible coating. A study reported the utilization of dextran as an edible coating of guava that resulted in the better quality of fruit aroma and color. Dextran showed better functional property as a gas barrier compared to other biopolymers such as xanthan gum and sodium alginate [30]. Moreover, the addition of sorbitol as a plasticizer to dextran could enhance the elongation, tensile strength, and elasticity, making it possible to develop as food packaging material [31]. Study on xanthan gum-based edible coating reported that pear fruit coated with xanthan gum showed a delay of browning and had a longer shelf-life [32]. GalactoPol and Levan are also promising to apply as food coating or packaging. Both EPS form a transparent film that is a good oxygen barrier, flexible, and mechanically strength [1, 33]. The EPS from microalgae species is not only applicable as food emulsifier and thickener but also can utilize for edible coating. The coating material from exopolysaccharide of Porphyridium cruentum successfully prevent the browning of fresh cut banana slice during storage at 4 °C. There was no report of undesirable taste of banana when coated with P. cruentum EPS [26].

EPS has some beneficial properties, making it potential for the edible coating material, such as the carrier of antimicrobial and antioxidant substances. A study [34] reported that essential oil (rosemary and oregano) and nanoparticles (ZnO- and Ag- nanoparticles) successfully incorporated into pullulan polymer resulted in edible antimicrobial film. The resulting film was effective against foodborne pathogens such as S. aureus, S. typhimurium, L. monocytogenes, and E. coli O157:H7. In pullulan films, the nanoparticles were remained intact in the matrix polymer while essential oil was distributed uniformly in the pullulan matrix. In that study, the antibacterial properties were suggested by nanoparticles and essential oil that acted to interfere the cellular process, disturb DNA replication, inhibit the ATP production, induce the ROS generation, and disturb the membrane integrity [34-36]. Moreover, the EPS itself is suggested to have the antimicrobial effect because of the ionic interaction between EPS and bacteria cell wall that could inhibit the bacterial growth [37].

The antioxidant compounds of EPS actively scavenge the free radical to prevent the oxidation so that delaying the browning in fresh food [33, 38]. EPS such as xanthan gum has been incorporated into several antioxidant substances to produce the edible film which performed to maintain the quality of fresh-cut pear [32, 39] and apples [40]. Cinnamic acid, an aromatic polyphenol, successfully embedded to xanthan gum which reported as an active polyphenol oxidase (PPO) inhibitors. The resulting edible coating film can retard browning of fresh-cut pear. The synergic effect between xanthan gum and the cinnamic acid act as a protective barrier against oxygen. Moreover, supplementation with cinnamic acid has no negative impact on fruit-coated odor and taste [32].

5. Future study
The major drawback of microbial EPS utilization in the food industry is the low production of EPS.
The optimal condition for cell growth and EPS production is affected by the nutrient composition and environmental condition. It is worth to note that optimal condition for cell growth and EPS production are different. For example, the EPS production is higher in the early stationary phase or when the cell starts to stop the proliferation. While the optimal condition of cell growth usually does not give enough stress to trigger the EPS synthesis [1, 3-6, 38]. So, the future study shall be focused on culture system to increase the EPS mass production without causing high cell mortality.

Moreover, biotechnology method can be approached to manipulate the intracellular condition for enhancing growth and EPS production. The physical and chemical characteristic also can be modified to accustom to the variations in climatic, cultivation, production, and environmental stress condition. For example, the addition of ubiquitous promotor in EPS protein gene can promote the EPS synthesis continuously, irrespective to various environmental condition [1, 41-42]. Besides, the modification of functional groups on the surface of EPS can be modified by genetic engineering method. The specific modification to get specific chemical, physical, and functional properties will make the EPS more appealing for use as flocculating, gelling, emulsifying, and coating agents in a varied application in pharmaceutical, nutraceutical, and food industries.

6. Conclusion

The microbial extra polymeric substances (EPS) are varied in composition, structure, and function regarding the diversity of microorganism species. Microbial EPS are widely used as an emulsifier, thickening agent, and coagulant in the food industry. Moreover, EPS have potential as the material for edible coatings because of its characteristics such as gas barrier, high elasticity, and carrier of antimicrobial and antioxidant substances. Therefore, the microbial-EPS edible coating can be applied to elongate the food shelf life. Further study is essential to conducting, especially about enhancing the EPS production and EPS functional groups modification to make it more appealing to utilize in the food industry.

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