Bacterial exopolysaccharides production and their roles for human life

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Bacterial exopolysaccharides production and their roles for human life

R Malaka

Faculty of Animal Science, Universitas Hasanuddin, Jl Perintis Kemerdekaan KM 10 Makassar 90245, Indonesia

Email: malaka_ag39@yahoo.co.id

Abstract. Exopolysaccharides (EPS) is a general term for all these forms of bacterial polysaccharides found outside the cell wall. EPS are separated to two major groups are homopolysaccharides and heteropolysaccharides.The production of exocelluar polysaccharides is found in many species of both Gram-positive and Gram-negative bacteria. The function of exopolysaccharides for bacteria itself are energy reserves, role in virulence, protection against physichally adverse condition, and bacteriophage adsorption. However, Isolation or extraction of EPS from bacterial cells does not ethically violate, because based on the results of research by scientists that EPS is not an essential substance for bacterial growth and survival. Therefore, EPS can be produced industrially for commercial purposes for the benefit of the welfare of humanity and for the purpose of future new polysaccharides. EPS has been proven to be applicable to improving the quality of food products as well as new foods, and can function on a variety of benefits in health pharmaceuticals. Other uses in the polymer industry have also made it possible as a new source of environmentally friendly polymers.

1. Introduction

The history of bacterial exopolysaccharide began during the mid of 19^{th} century with the discovery of an exopolysaccharide in wine, which would later be known as dextran and the prokaryote responsible for the production was identified as *Leuconoctoc mesenteriodes* [1]. The name exopolysaccharides (EPS) as proposed by Sutherland [2] provides a general term for all these forms of bacterial polysaccharides found outside the cell wall [3,4]. Over the course of time, other polysaccharides discovered includes cellulose, alginate, xanthan, altenam, reuteran, levan, inulin, kefiran and others [5].

Based on the composition of EPS, the polymer separated to two major groups that homopolysaccharides (for example dextran and mutan) and heteropolysaccharides (EPS of lactic acid bacteria) based on their composition and mechanism of synthesis. Homopolysaccharides are polymers which consist of one monosaccharide such as glucose or fructose [2,5,6]. Heteropolysacharides usually contain 2-4 monosaccharides. Both type of polymers may contain acyl or other substituents and can be either liniear or branched. But according Cerning (1995) that the range of the extracellular biopolymers are vast and may be grouped into four major classes; polysaccharides, inorganic polyanhydrides (such as polyphosphates), polyester, and polyamides, and have been collectively termed extracellular polymeric substances, slime and microcapsular polysaccharides among others [7]. There have been reported that bacterial EPS was generally used as food addictive to increase the food quality [8, 9, 4], antihipertention, antivirus, cosmetic, or anti AID'S. One of the bacterial EPS that have produced by commercial scale are "Curdlan" is EPS from *Alcaligenes faecalis* var *myxogenes* [6]. EPS – producing LAB have received growing attention in recent years because the EPS wich they produce are food grade and have applications as food stabilizes, gelling agents, or immunostimulants [8-10]. A review about the material properties of EPS [11] has revolutionized the industrial and medical sectors due to their retinue of functional applications and prospects. These applications have been extensive in areas such as pharmacological, nutraceutical, functional food, cosmecceutical, herbicides and insecticides among others, while prospects include uses as anticoagulant, antithrombotic, immunomodulation, anticancer and as biofocculans [3].

Soil scientist have an interest in microbial exopolymer production doe to the importance of polysaccharides to soil properties and the possible role of exopolysaccharides in the symbiotic relationship between different species of *Rhizobium* and legumes. Slime production by bacteria is also an inportant phenomena in waste water management as it has been implicated in the phenomena of 'bulking' of activated sludges. This has been a noted problem in dairy, food processing, brewing and and pulp industries where slime is frequntly encountered due to large source of bohydrate available in waste effluent [12].

The industrial microbiologits has become interested in exploiting microbial exopolymer production due to increasing intrest and need for novel polysaccharides. Utilizing microorganisms to produce polysaccharides may offer both an economic and availability advantage over traditional plant and marine sources. Iincreased research effort in the production of unique polymers not presently available.

2. Extracellular polysaccharides production

Bacteria systhesize polysaccharides which are: a) located in the sytosol for a carbon or energy source (i.e.,glycogen); b) structural component of the cell wall or cytoplasmic membrane (i.e., peptidoglican, teichoic acid; c) located out-side the cell wall (figure 1). Depending on their structural relationship to the bacterial cell, they have been variously name slime, capsular or microcapsular polysaccharides.

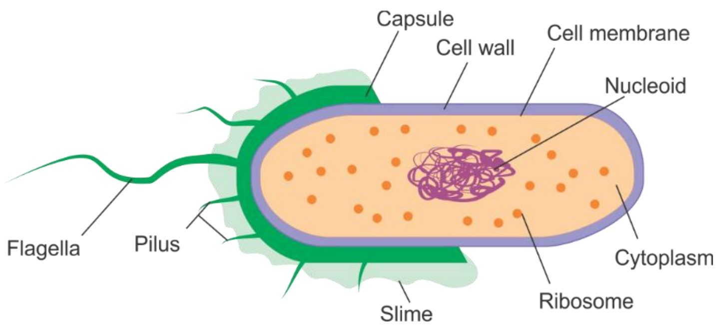

Figure 1. Bacterial cell diagram with capsules and slime.

The production of exocelluar polysaccharides is found in many species of both Gram-positive and Gram-negative bacteria [12] and has been the subject of numerous investigations in varied filds of interest. Many of the earlier studies were those of the immunologist studying happens and antigens present at the bacterial surface and their role in pathogenicity. These immunological phenomena and their elaboration were described in an excellent review article. The human pathogenic organism which owe their virulece to the presence of capsules include: *Streptococcus pneumoniae, Streptococcus pyogenes, Pasteurella pestis, Haemophilus influenzae, Neisseria gonorrhoeae,* and *Klebsiella pneumoniae*.The capsules of these organism appear to be composed of polysaccharides. One of the few exceptions to the composition of the capsule of the pathogenic bacteria is the polyglutamic acid capsule of *Bacillus antracis* [13].

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The industrial microbiologits has become interested in exploiting microbial exopolymer production due to increasing intrest and need for novel polysaccharides. Utilizing microorganisms to produce polysaccharides may offer both an economic and availability advantage over traditional plant and marine sources [14]. Increased research effort in the production of unique polymers not presently available. Numerous investigations have shown that capsules are not necessary for cell viability. Capsules or slime were removed physically for cell viability. Capsules or slime were removed physically or enzymatically without adverse effect on bacterial growth. Futher incubation of these cultures let to synthesis of new polysaccharides. Also, mutans unable to form exopolysaccarides are readily isolated. They occur spontaneusly or after mutagenesis. Althought loss of ablility to produce expolysaccharides may not couse any significant change in viability in-vitro it could be important to survival under highly competitive conditions in the natural habitat [13]. Even strain of *Escherichia coli* that are incapable of forming polysaccharide are known to do so after mutation. These polysaccharides are sometimes formed when a nitrogen compound or a certain essential inorganic ion is deficient. Microorganisms produce them to form a barrier resistant to drying and attack by amoebae, bacteriophages and leucocytes. Another function is in attachment to plants and animals. The specifies of attachment depend on the properties of the polysaccharides [6]. The fungction of exopolysaccharides are the following: energy reserves, role in virulence, protection against physichally adverse condition, bacteriophage adsorption [13].

Isolation or extraction of EPS from bacterial cells does not ethically violate since researches proved that EPS is not an essential substance for bacterial growth and survival. EPS is only a secondary metabolite product that is released when the growth environment is unfavorable. In strains of bacteria that are non-mucoid ie those unable to spontaneously form EPS can become mucoid in certain conditions. EPS capsules or mucus can be removed physically or enzymatically without affecting bacterial growth, so it does not cause microbial cells to die. Furthermore, with the incubation process the bacterial cells can synthesize new polysaccharides. Industrial microbiology is interested in EPS production research because EPS is a new polysaccharide, which can be become as future polysaccharide [15]. The use of bacteria to produce polysaccharides in economic terms will benefit more than plants polysaccharides or from seaweed [6,16]. This is due to producing these polysaccharides do not require large tracts of land and can be produced continuously with bioreactors.

Exopolysaccharide production which was first isolated by Harada in 1966 was patented in 1970 [17] and commercial in the 90s with the generic name 'Curdlan which has been utilized in various fields both as food additives, a new source of polysaccharides. In principle EPS bacteria are physically the same as plant polysaccharides. For example, EPS Curdlan is a glucan with a -D- (1-3) glucoside bond in the form of a white powder, does not dissolve in water but expands swell in warm water. This EPS dissolves in an alkaline solution and forms a gel when heated to high temperatures and forms a gel that is mushy when heated to 55^{o}C and harden when cooled. EPS can improve the texture of various food products such as tofu (tofu), jelly sweet bean paste (yokan), fish paste (kamaboko), Japanese noodles (udon), sausages, jelly, selei and so on [6].

3. Exopolysaccaride structure

Bacterial exopolysaccarides include a wide variety of classes of polysaccharides. Some are comprable to well know plant products such as amylose, amylopectin, cellulose and algin. Others such as glycogen and hyaluronic acid are similar to animal products. However, the great majority of bacterial polysaccharide are unique [6]. The structural of Exopolysaccharide from various bacteria can be seen in figure 2.

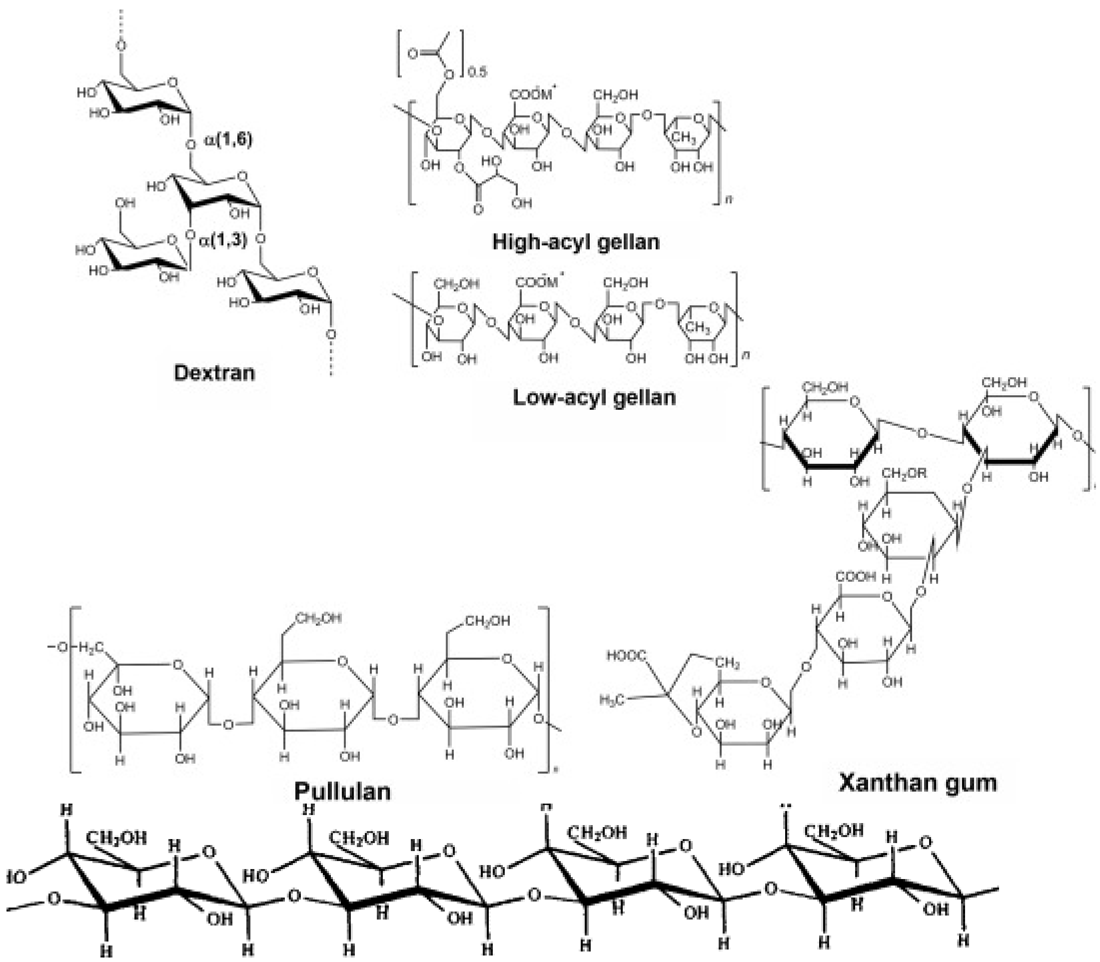

Figure 2. Structural of various polysaccharides [18, 19].

Shutherland [2] separated exopolysaccharides in two major groups based on their composition and mechanism of synthesis: homopolysaccharides and heteropolysaccarides. Homopolysaccharides are polymers which consist of one monosaccharide such as glucose or fructose. Heteropolysacharides usually contain 2-4 monosaccharides. Both type of polymers may contain acyl or other substituents and can be either liniear or branched.

One of the dairy products that are gaining in popularity and much in demand by consumers is yogurt and yakult. In making fermented milk, both yogurts, yakult and cheese, lactic acid bacteria (BAL) are used which are beneficial for health because they can be probiotic that is inhibiting the growth of pathogenic bacteria in the intestine. From various studies it is known that there are still many factors that are not yet understood from these starter bacteria, including metabolic products in the form of mucus (slime). Mucus is a component that is outside the cell wall with the main composition is the polysaccharide so it is called exopolysaccharide (EPS) or extracellular polysaccharides [2, 13, 20].

Bacteria synthesize polysaccharides, namely: (1) outside the cytosol which is used as a carbon source or energy source (eg glycogen), (2) is a component of cell wall structure or cytoplasmic membrane (eg peptidoglycan and theatric acid, (3) located outside the cell wall called EPS, exopolysaccharide in the form of mucus or capsules or microcapsule polysaccharides, the name exopolysaccharide, produced by bacterial species in both Gram positive and Gram negative bacteria and have attracted the attention of many researchers from various fields of science such as medicine, pharmacy, and food technology [21, 22]. Illustration of EPS production by cell bacteria can be see in Figure 3.

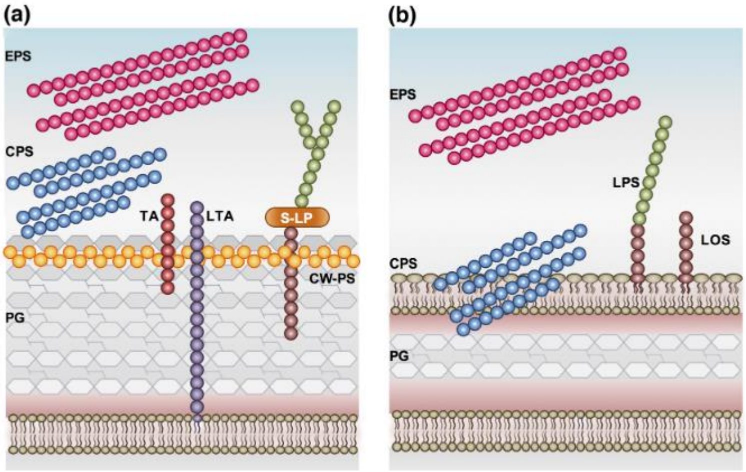

Figure 3. EPS in Gram positive (a) dan negative (b) bacteria. LPS: lipopolisaccharide, PG=peptidoglycan, LOS = lipooligosaccharide, CW – PS = Cell Wall Polysaccharide, TA = Theichoid acid, LTA = lipitheichoid acid [12].

Previous researchers have indicated that the production of mucus or capsules in certain species of bacteria is related to the pathogenicity of the bacteria. This proves that some pathogenic bacteria such as *Streptococcus pneumoniae, Streptococcus pyogenes, Pasteurella pestis, Haemophilus influenzae, Neisseria gonorrhoeae, Klebsiella pneumoniae* produce mucus or slime and *Bacillus antracis* produce capsules. This opinion was further denied by several other researchers especially those engaged in soil microbiology and food microbiology that the presence of mucus from bacteria has nothing to do with bacterial pathogenicity. This can be proven in several species of non-pathogenic soil bacteria such as *Rhizobium, Alcaligenes faecalis, Acromobacterium* and *Xanthomonas* also produce mucus. These bacteria are bacteria that can fertilize the soil [2]. Lactic acid bacteria, especially those used as culture starters for the production of fermented milk, also produce EPS [6].

Homopolysaccharides are polymers consisting of one type of monosaccharide such as glucose or fructose only. This type of EPS polymer can have straight or branched chains. Examples of homopolysaccharide EPS are dextran, levans and curdlan [23]. Heteropolysaccharides are polysaccharides usually containing 2-4 kinds of monosaccharides such as glucose, galactose, mannose, fucosa and rhamnosa. Examples of heteropolysaccharide EPS are pullulan and EPS Lactic Acid Bacteria (LAB). EPS culture starter bacteria are still being studied in terms of their composition, production and so on (Figure 4).

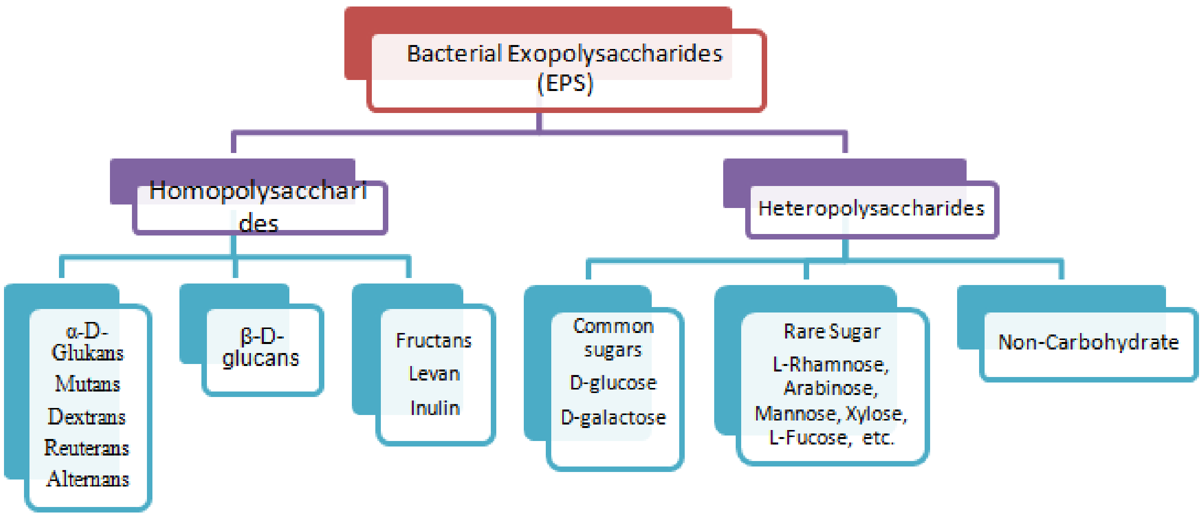

Figure 4. Two major group of bacterial exopolysaccharides.

4. Exopolysaccharide by Lactic Adid Bacteria and its role for human life

Exopolysaccharide bacteria include several classes of polysaccharides. Some of them resemble the shape of plant polysaccharides such as amylose, amylopectin, cellulose and algin. Other things like polysaccharides from animal origin are glycogen and hyaluronic acid. Most bacterial polysaccharides are unique [6]. Practically EPS has many advantages including: EPS can be produced commercially; EPS is not toxic and with very low concentrations can improve food quality; do not pollute the environment because it can easily be degraded; can be used to treat various diseases and for pharmaceutical purposes.

Indeed, the rapid development of humans and the increasingly limited land for farming forcing scientists in the field of food microbiology try to explore the various possible benefits of microbes to produce EPS. Exopolysaccharides produced by fermented milk starters have advantages compared to EPS from other microbes. This is caused by 1) The resulting EPS is safer than other bacteria because of the possibility of being contaminated by microbial toxins will be guaranteed because the starter culture of fermented milk is not pathogenic; 2) Maintenance is easier because the growth media can use milk as a natural medium of lactate culture; 3) Can be produced on a commercial scale by utilizing milk waste such as whey [16] not ethically violate since researches proved that EPS is not an essential.

An explanation of the bacteria that produce EPS is also needed to explain how bacteria can produce EPS, and under conditions of growth and media how EPS can be synthesized. The production and isolation mechanism of EPS is also then described the mechanism and biosynthesis of EPS. Finally, it is explained how EPS is applied to food and its mechanism. Also, how EPS is applied to health and made into pharmaceutical.

Exopolysaccharides isolated from fermented milk culture have been suspected to have antitumor properties. Besides that, some bacterial polysaccharides are indicated to have an effect on inhibiting the proliferation of the AIDS virus, increasing immunity, reducing cholesterol and having an anti-inflammatory effect. Malaka research [6] give results that EPS was isolated from *Alcaligenes faecalis* var. *myxogenes* can be used as a stabilizer and improve the quality of fermented milk products by increasing viscosity, elastic modulus, breaking energy. Microstructure analysis using scanning electron microscopy (SEM) indicated that EPS can function as a binding agent between one casein molecule and another casein so that it can avoid the occurrence of syneresis (whey removal from curd) in yogurt [24].

5. Conclusion

Exopolysaccharides produced by bacteria have a very important role for human life, namely acting as a binding agent in food products, as pharmaceutical ingredients for health and medicine, also in the future can be a source of new polysaccharides for new food products.

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