Influencing Factors of Exopolysaccharide Production by *Lactobacillus* and Application

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Abstract. Lactic acid bacteria are probiotics that have beneficial effects on the human body. Exopolysaccharides are one of the products of lactic acid bacteria metabolism. At present, it has been proven to play an important role in food processing, which can replace some additives and make food healthier and safer. There are many factors that affect the yield. At present, the best fermentation technology of exopolysaccharides produced by various lactic acid bacteria has been studied. In this paper, the effects of 5 different environmental factors on the production of exopolysaccharides were analyzed. It was found that carbon source was the most important factor affecting the production of exopolysaccharides. And it shows how it can be used in the food industry. The results provided a reference for the optimization of fermentation conditions of other lactic acid bacteria. However, the question that still needs to be solved is whether these effects will have an impact on the subsequent industrial applications, and researchers can pay attention to this research in the future.

Keywords: Exopolysaccharide; *Lactobacillus;* food industry.

1. Introduction

Lactic acid bacteria (LAB) are a group of Gram-positive, acid-tolerant bacteria. It is considered relatively safe and used in the food industry, because of its characteristics in degrading polymolecules, metabolizing amino acids and transferring other toxic mycotoxins [1]. Exopolysaccharides (EPS) is a mucous polysaccharides or capsular polysaccharides produced in Lactic acid bacteria and secreted out of cells. The structure of EPS determines it has high viscosity and outstanding rheological properties. The mechanism and yield of EPS produced by different Lactobacillus species are different. EPS synthesis includes both intracellular and extracellular pathways [2]. EPS can be divided into two groups: homopolysaccharides (HoPS) and heteropolysaccharides (HePS). HoPS is combined with more than 10 same monosaccharides; HePS is a repeating unit composed of multiple monosaccharides [3]. However, HoPS and HePS also have different applications.

At present, there are 28 known sources of lactic acid bacteria producing EPS [4]. Each type of EPS has a unique branching profile, monosaccharide numerical content, and physicochemical properties. There are parts of the more common *Lactobacilli* that produce EPS: *Lacticaseibacillus paracasei, Lactobacillus helveticus, Lactobacillus gasseri, Lactobacillus rhamnosus, Lactiplantibacillus splantarum* etc [5]. At the same time, environment is also a vital factor, when cultured in vitro. Some studies have demonstrated that LAB is able to adapt to medium and produce different amounts of EPS based on the conditions [6]. Monosaccharides are the main raw materials to form EPS, so carbon sources are the most influential factor. Other environmental factors also matter such as carbon sources, nitrogen sources, pH value, temperature and fermented time.

Because of its biological structure, EPS can be seen as emulsification, sweetening, gelling, or water-binding additives. It has been proven to work in fermented milk products, fruit wine and bakery [7]. Compared to macromolecular polymers, EPS is considered less harmful and biodegradable.

Therefore, research on the biosynthesis and yield optimization of EPS has a strong application potential. In order to carry out further research in EPS of LAB, this paper summarized and described the number of EPS produced in different *Lactobacillus* species and various culture conditions. In light of this, do a more complete review of its application in food processing industry and medical field. The aim is to provide more comprehensive information for the research and application of EPS.

2. Biosynthesis Mechanism

According to the research so far, there are four microbial biosynthesis mechanisms of EPS [2]: 1. The Wzx/Wzy dependent pathway 2. ABC transporter dependent pathway 3. Synthase dependent pathway 4. Extracellular synthesis pathway such as sucrase protein. Fig.1 gives the basic four biosynthesis mechanisms [2].

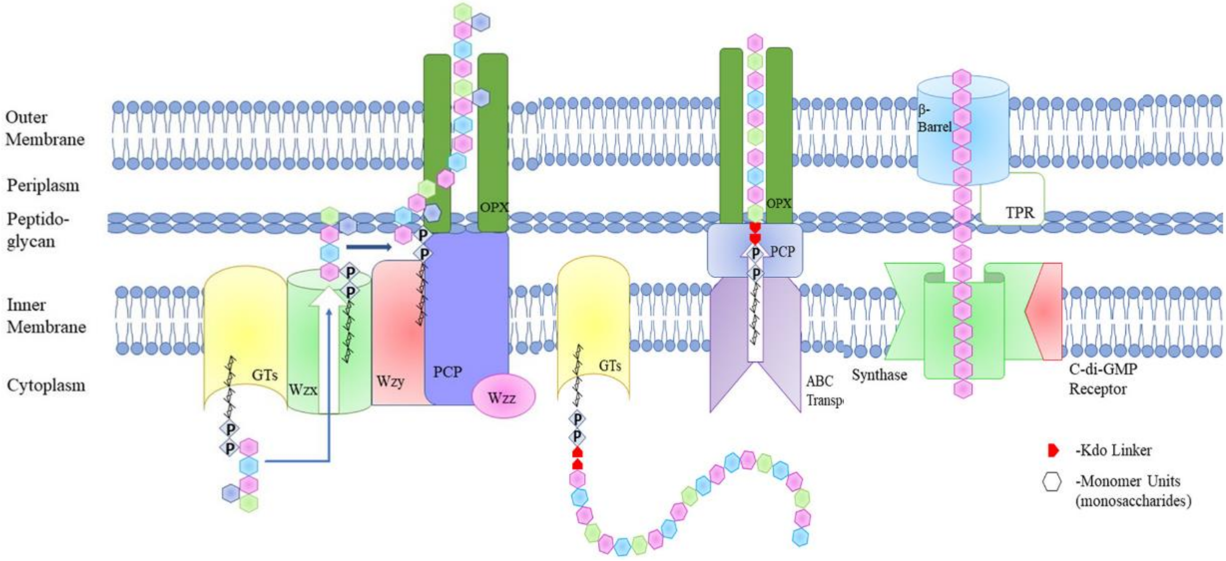

Fig. 1 The biosynthesis mechanism of EPS [2].

Wzx/Wzy pathway is the synthesis of nucleotide sugars, assembly of the repeat unit, polymerization and export are included in the Wzx/Wzy dependent pathway. Sugar nucleotide monomers are transferred to the undecaprenyl diphosphate (Und-P) linker by highly specific glycosyltransferases (GTs) combined with each other. After the flippase protein encoded by the wzx gene transfers the repeating units to the periplasmic space, the polysaccharides are excreted to the outside of the cell through the polymerase Wzy [8].

ABC transporter pathway makes polysaccharide binding to GTs, reacts with the polymerase, then is transported to an Und-P linker [2]. The polymers are transported out of the cell by ABC transporter.

Synthase dependent pathway always utilizes specific monosaccharide to produce homopolysaccharides. Membrane-embedded synthase combines UDP-glucose units together, then promotes the formation and translocation of polysaccharides on the intima [2].

The fourth path is extracellular biosynthesis by sucrase enzymes. After extracellular sucrase breaks down sucrose into units, it is polymerized into dextrans and levans with different branch levels by GTs, and finally HoPs are generated [8].

3. Factors Influencing Exopolysaccharide Production

3.1. Fermentation Time

Fermentation time plays an important role in the production of EPS. As the fermentation time increase at the beginning, the number of bacteria generated EPS rise. While once a certain peak is reached, the number of EPS begins to decline. The reason for the decrease may be because the nutrients in the medium are consumed and the concentration begins to decrease after the bacteria grow to a certain extent, requiring the consumption of additional EPS [6].

Research has shown that the optimum fermentation time varies from 16 to 48 hours. A study on optimizing the synthesis conditions of Lactobacillus plantarum PA01 compared 4 different culture time, 24 hours, 36 hours, 48 hours, and 60 hours [9]. The yield of EPS peaks at 36 hours. The yield was only about 125mg/L at 24h and reached 185mg/L after 36h of culture. After that, the production began to show a downward trend, which only produced 180mg/L at 48h. Table 1 presents the yield of EPS with other Lactobacillus and their best fermentation time.

Table 1. EPS yield of lactic acid bacteria and its optimal fermentation time

Lactobacillus species	Best fermentation time	Yield
Lactobacillus plantarum PA01 [9]	36h	185mg/L
Lactobacillus fermenti CECT 5716 [10]	16h	250mg/L
Lactobacillus pentosus YY112 [11]	24h	322mg/L
Limosilactobacillus fermentum LAN4 [12]	16h	220mg/L

3.2. Temperature

Suitable temperature is conducive to the growth of microorganisms. The rate of EPS synthesis is closely related to temperature. Temperature can change the colony morphology. At relatively low temperatures, the rate of cell wall synthesis decreases, leading to more isoprenoid lipid carrier precursor molecules being used for the synthesis of EPS [13]. If the temperature is too low, it affects synthetase activity and reduces the rate of synthesis.

Under three different temperatures, the yields of Limosilactobacillus fermentum LAN4 were significantly different, 174 mg/L at 33 degrees, 215 mg/L at 37 degrees, and 225 mg/L at 41 degrees [12]. Another study showed that the number of EPS produced by L. pentosus YY112 was 40mg/L higher at 30 degrees than at 35 degrees [11].

3.3. pH Value

There are two stages of pH influence in the fermentation of EPS [14]. The optimal pH for the growth of Lactobacillus is generally acidic. So in the first stage, in order to promote the growth and reproduction of Lactobacillus, low pH is recommended. The second stage is the fermentation metabolism of Lactobacillus to produce EPS. The optimal pH at this time may be different from the first stage, sometimes it is higher, such as pH=6 [14]. However, this has a reverse effect on bacterial growth, but promotes the production of EPS. Due to the culture in the first stage, the number of *Lactobacillus* has reached a certain extent. In the second stage, the growth rate of *Lactobacillus* slows down and has no great impact on the production of EPS. At this time, it is necessary to adjust the pH value to the optimal metabolic value to increase EPS production.

3.4. Carbon Sources

Carbon source is the decisive factor for the production of EPS by Lactobacillus. Carbon is an important factor in cell composition, growth and development, but too high or too low concentration is not beneficial to the metabolism of Lactobacillus EPS. Carbon sources commonly used in research are glucose, fructose, sucrose, galactose, lactose, and maltose. For homopolysaccharides, extracellular synthesis pathway is mainly involved. Heteropolysaccharides are generally composed of more than three simple sugars and usually contain D-glucose, D-galactose, and L-rhamnose [15].

Studies have shown that some Lactobacillus were not sensitive to fructose, and its production is the lowest among multiple carbon sources, including Lactobacillus pentosus, Limosilactobacillus fermentum, and Lactobacillus ferment [10-12].

3.5. Nitrogen Sources

Nitrogen sources are related to amino acids needed for Lactobacillus growth and therefore play an important role in metabolic processes. In the medium, the materials that provide nitrogen sources are peptone, beef powder, yeast powder, etc. Both adding different percentages of nitrogen sources and different types of nitrogen sources affect EPS yield. In a general optimization experiment, peptone, tryptone, soybean peptone, casein peptone and yeast peptone are used as a nitrogen source reference comparison. Research shown that the mixed addition of multiple nitrogen sources can effectively increase EPS yield [16].

3.6. Ratio of Carbon and Nitrogen

In the process of microbial culture, carbon source and nitrogen source are the two most important and influential factors. When the content of carbon and nitrogen sources in the medium is too low, the growth rate of bacteria will be reduced due to lack of nutrients. While, when the concentration of carbon and nitrogen is too high, the osmotic pressure in the culture medium gradually increases, inhibiting the growth of microorganisms [17]. As a result, a good carbon nitrogen ratio is necessary in culturing Lactobacillus. Before the maximum ratio, nitrogen proportion is less, making it impossible for microbes to synthesize proteins, which in turn promotes EPS production [6]. However, with the increasing number in carbon nitrogen ratio, it will reach a maximum value. Then the yield begins to decrease.

In summary, the yield of EPS is affected by various environmental factors, such as fermentation time, fermentation temperature, pH value of culture solution, carbon source and nitrogen source. Among them, carbon source and nitrogen sources are the direct sources of *Lactobacillus* growth materials and EPS production and are the main influencing factors in yield. Fermentation time, temperature and pH value were secondary factors. The structure and yield of EPS produced by different *Lactobacillus* are also different. These different influencing factors will change the structure of EPS, but the specific mechanism has not been fully explored, and future studies can focus on the specific mechanism of different environmental factors affecting the production of Lactobacillus EPS.

4. Application in Food Industry

Exopolysaccharide derived from probiotic fermentation are considered a healthy source with little harm to the human body. They composed of only three elements, hydrogen and oxygen, are natural macromolecular polymers. Due to its unique polysaccharide structure and physicochemical properties, it can be used in many aspects of life. Today EPS has been proven to play an important role in many fields.

In the food sector, there is a growing need for health and a growing focus on food safety. At present, people are slowly paying attention to the impact of some food additives on the human body, and the requirements for whether food additives are safe and beneficial are constantly increasing. In this case, Lactobacillus is a probiotic that is thought to be beneficial to the human gut. The exopolysaccharides derived from probiotics had good rheological properties. It has been proved that it can be used in the food industry in emulsification, thickening, stabilization, packaging, anti-corrosion and other aspects.

4.1. Food Packaging

On food packaging materials, some EPS with complete structure and good smoothness can be used to make films when combined with plasticizers such as glycerin. The use of EPS composite products can extend the shelf life and quality of food. However, the hydrophilicity of EPS leads the packaging made of them to absorb a certain amount of water. So, in order to increase the barrier between the packaging and water, plasticizers such as glycerin are compounded in it [18]. In the application of biofilms, probiotics can act as biofilms in an intestinal system. EPS is produced by self-fermentation of probiotics and can also provide transport and storage carrier for probiotics. In addition, in certain corrosive food processing environments, EPS can act as an isolation coating. However, the fusion of exopolysaccharides with food packaging has not been widely used, and the specific mechanism is still not fully found. Further research is needed to promote the exopolysaccharide food packaging complex in the market.

4.2. Fermented Products

In the production of fermented milk, EPS can be used as a thickening agent and has the potential to replace xanthan gum in the market. EPS can increase the viscosity of dairy products, reduce the precipitation of whey, and improve the degree of emulsification. Make the state of fermented yogurt more stable, taste more viscous, longer shelf life. EPS is a hydrophilic colloid with good emulsification [7]. In bread production, the water absorption capacity of the dough can be increased, thereby increasing the viscosity of the bread and reducing the hardness [19]. Dextran can improve the stability of dough and affect the formation of stomata during dough fermentation. Compared to regular bread, bread with EPS added is softer and more palatable. The gel properties of EPS can also precipitate proteins, making the solution of drinks such as fruit wine and juice clearer.

4.3. Antibacterial Action

EPS can inhibit the activity of bacteria, fungi, viruses and biofilms [20]. EPS has antibacterial activity against certain foodborne bacteria, such as *Salmonella typhimurium* and *E. coli*. Part of the antioxidant capacity of EPS is reflected in different functional groups, such as carbonyl groups. The sulfate in EPS causes it to take on a negative charge, which promotes the interaction between EPS and pathogens and reduces the activity of some Gram-positive bacteria and fungi. In addition, EPS prevents bacteria from attaching to surfaces by isolating the contact between pathogens and surfaces, disrupting the formation of their biofilms. Therefore, the addition of EPS to food can effectively inhibit the infection of foodborne bacteria and play the role of biological preservatives. Not only does this not change the flavor of the food, but it is also safer and healthier because it comes from the metabolism of the organisms.

5. Conclusion

Exopolysaccharides are macromolecular polymers derived from natural metabolism and are considered to be relatively safe and healthy food additives. At present, there are four ways for bacteria to produce exopolysaccharides, and the EPS structure and yield produced by each way are affected by different factors. In general, there are many factors that affect its yield, among which the most influential are carbon source and nitrogen source and their ratio. In the process of optimizing the culture medium of Lactobacillus EPS, we should also consider the influence of environment on the growth rate of Lactobacillus and the metabolism of Lactobacillus EPS.

Due to its unique structure and properties, EPS can now be used in many aspects of the food industry, such as packaging, fermented food and anti-corrosion and antibacterial. The optimization of the medium can increase the unit yield of EPS, thus reducing the cost expenditure to the maximum extent possible in the business. However, changes to the culture environment may lead to changes in the structure of EPS, which will have an impact on its application. So in the future, how different factors change its structure and the consequences in the food industry after being affected still needs to be studied.

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