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RESEARCH ARTICLE

The production-influencing factors of extracellular polysaccharide (EPS) from a strain of lactic acid bacteria and EPS extraction

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Abstract The influencing factors of extracellular polysaccharide (EPS) produced from a strain of lactic acid bacteria (LAB L15) were studied by using the phenol-H_2SO_4 method. It was demonstrated that the strain produced EPS at the most amount when it was incubated for 40–48 h and when the pH value was 4 under 30°C. Glucose was the most suitable carbon source for LAB-producing EPS. The rough EPS was obtained from L15 culture after centrifugation, dialysis, deprotein, decoloration, and ethanol-precipitation. The sample was at least composed of two polysaccharides that were completely different in molecular weight and the amount. The purified EPS was passed through the SephadexG-200 column and it showed that it was a sample purified by thin layer chromatography.

Keywords lactic acid bacteria, extracellular polysaccharide (EPS), column chromatography, thin layer chromatography

1 Introduction

Extracellular polysaccharide (EPS) of microbes is a kind of mucopolysaccharide or capsule polysaccharide that is secreted in the process of microbial growth and metabolism. On the other hand, polysaccharide is a kind of macromolecular polymers with a long chain. It has glutinous and colloid properties and can be dissolved or dispersed in water (De Vuyst and Degeest, 1999; Zhong et al., 1999). Because of its unique physical and perfect rheological characteristics, as well as safety and non-toxicity, microbial polysaccharide has become the focus of research and development during recent years.

Lactic acid bacteria (LAB) is a group of bacteria that can ferment carbohydrates to produce large quantities of lactic acid. Since many lactic acid bacteria are natural productive strains in the food industry, the EPSs of their metabolic products are widely used in the food industry and pharmaceutical industry. The main biological effect of this kind of polysaccharide is the inhibition to tumour (Gu et al., 2003). In addition, different microbial polysaccharides also have many pharmacological roles, such as delaying aging, anti-radiation, promotion of protein and nucleic acid synthesis, anti-ulceration, liver protection, resistance to mutations, anti-thrombus, extending cruor time, lowering blood glucose and blood lipid, and anti-inflammation. In the food industry, LAB polysaccharide can be used as an adhesive reagent, gel reagent, filling reagent, and stabilizer owing to its significant viscosity and stability. It also plays an important role in improving taste addiction, product quality, and distinctive structure in dairy products. Therefore, the extensive potential application to polysaccharide is significant in developing new products and improving product quality.

At present, polysaccharide-producing LAB has been studied; however, studies for the really valued and commercialized polysaccharides are still lacking. Besides bacterial strain, polysaccharide production is affected by many factors. A strain LAB L15 isolated from the gastrointestinal tract of flounder was used to extract and purify the EPS, and we analyzed its influencing factors in this paper.

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2 Materials and methods

2.1 Materials

2.1.1 Bacterial strains

LAB L15 was isolated from the gastrointestinal tract of healthy flounder (*Paralichthys olivaceus*). *Lactobacillus casei* was

stored in the laboratory. *L. fermentum*, *L. brevis* ATCC 367, and *L. acidlophilus* ATCC 4356 were purchased from China General Microbiological Culture Collection Center (CGMCC).

2.1.2 Media

The medium cultured for LAB was LBS. The different sugars, which were equal to the volume of glucose in LBS medium, were added to the LBS media respectively in the experiment to test the influence of different sugars on producing polysaccharides.

2.2 Methods

2.2.1 The identification of EPS

The LAB culture were discarded cells by centrifugation, dialyzed at 4°C, detected by the phenol–H_2SO_4 method until no purple ring appeared in the distilled water (Zhang, 1999). After being condensed, equal volume of cold pure ethanol was added to the dialysate, and then we observed if precipitates appeared with the dialyzed LBS media as control.

2.2.2 Quantitative detection of EPS

The phenol–H_2SO_4 standards curve was made using glucose, and then the optical density of fermentation culture was measured by the phenol–H_2SO_4 method. Corresponding to the glucose content of the standard curve, the polysaccharide productions of LAB and the other four *Lactobacillus* spp. were known.

2.2.3 Factors influencing EPS production

Temperature: LAB were cultured statically at 25°C, 30°C and 37°C, and the samples were taken at different times. Then, we measured the amounts of EPS in the cultures by the phenol–H_2SO_4 method.

Different culture times and bacterial biomass: LAB was inoculated and cultured at 30°C for 72 h, and samples were taken at different times. Then, we measured the bacterial biomass by the turbidity method and the amounts of EPS in the cultures by phenol–H_2SO_4 method.

Different sugars: LAB were inoculated and cultured at 30°C for 40 h in LBS media with different sugars. The cells in the cultures were discarded by centrifugation at 8 000 r/min. Then, we measured the amounts of EPS in the cultures by the phenol–H_2SO_4 method.

2.2.4 Separation and purification of EPS-II

LAB was cultured at 30°C for 40 h. The supernatant was collected after centrifugation at 10 000 r/min, condensed to appropriate volume using rotary evaporator, and dialyzed at least 48 h against distilled water. The supernatant was dialyzed again after no monosaccharide was detected in the dialysate by the phenol–H_2SO_4 method. Then, the rough EPS could be obtained after deproteination with trichloroacetic acid, decoloration with 30% oxydol, precipitation with ethanol, dialysis, concentration, and lyophilization.

The rough EPS was dissolved in a small amount of 0.05 mol/L ammonium acetate buffer, and the polysaccharide components were collected after centrifugation, chromatography with Sephadex G-200 column and detection by the phenol–H_2SO_4 method. The EPS-II was obtained after vacuum concentration, dialysis, and lyophilization.

2.2.5 The thin layer chromatography (TLC) of EPS

According to the method provided by Wang et al. (1999), lamella panels were prepared using silica gel H for the TLC. The final extraction solution of the product, EPS-II, was spotted, spread, colorized, and the purity was qualitatively analyzed.

3 Results

3.1 Identification of EPS produced from a strain of LAB

The white precipitate was observed in LAB L15 culture after dialysis, concentration, and addition of pure ethanol. It was demonstrated that the LAB L15 produced EPS. However, no precipitate was observed in the LBS liquid medium.

3.2 The detections of EPS productions from different LAB

The production of tested LAB L15 is 826.5 mg/L (Fig. 1). The strain produced more amounts of EPS compared to the output of the other LAB strains.

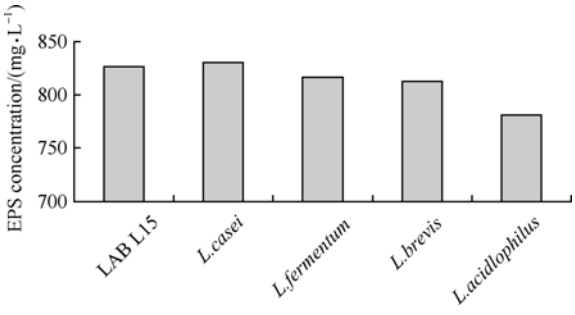


Fig. 1 The productions of EPS from different LAB

3.3 Influencing factors on the production of EPS from LAB

3.3.1 Influences of different incubation temperatures on the production of EPS

The productions of EPS in LAB L15 were completely different under different culture temperatures (Fig. 2). Throughout the experiment, the strain produced much more EPS at 30°C and 37°C than that at 25°C. The production at 30°C and 37°C reached their highest values after incubation for 48 h and 42 h, respectively, and the highest productions are both similar. The peak values of the production occurred at 37°C, which was earlier than that at 30°C.

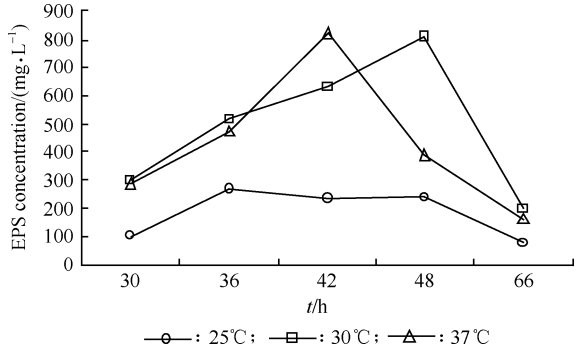


Fig. 2 The productions of EPS from LAB L15 at different incubation temperature

3.3.2 Influences of different incubation times and biomass on the production of EPS

The biomass and EPS productions were measured at certain intervals after LAB L15 was cultured at 30°C by using glucose as a carbon source. The bacterial growth reached stable growth period after incubation for 36 h and the numbers and biomass tend to be invariable. It was estimated that the bacterial biomass reached its maximum value from incubation at 40 and 48 h. Accordingly, the polysaccharide production reached its maximum value nearly during the same period (Fig. 3).

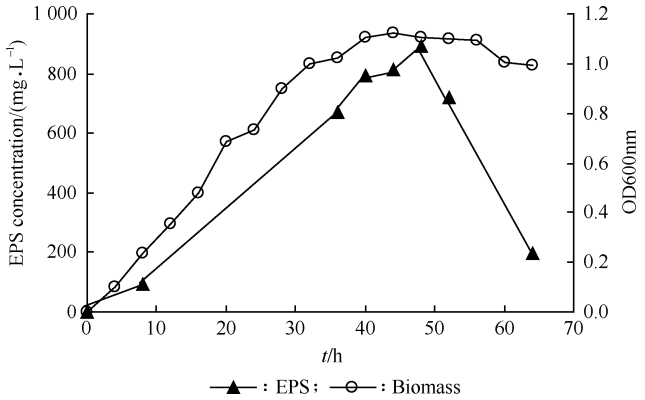


Fig. 3 The variation curve of the LAB biomas and the EPS production with time

3.3.3 Influences of different sugars on the production of EPS

EPS productions were influenced markedly by different sugars and the highest production of EPS (805 mg/L) was obtained when glucose was used as a carbon source; the next were lactose and fructose. The lowest production was 78 mg/L when mannose was used (Fig. 4).

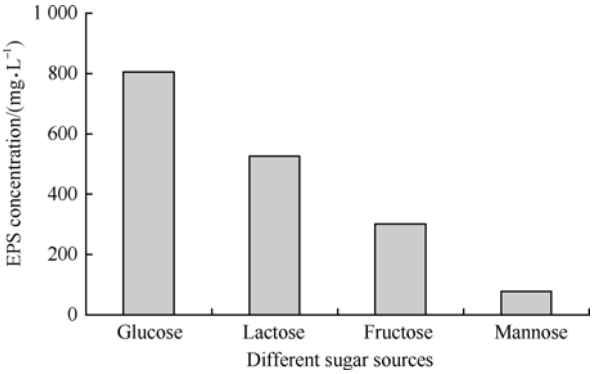


Fig. 4 The influences of different sugars on the production of EPS from LAB L15

3.4 Separation and purification of EPS-II

A high eluting peak with EPS-II appeared when elution was at 700 min (Fig. 5). The eluting peak was very high and there was no shoulder peak. It was shown that the rough EPS had a single composition. In similar experiments, the sample had another composition (EPS-I except EPS-II) and the eluting peak with EPS-I appeared when elution was at about 500 min. The content of EPS-I was so little that the eluting peak disappeared (Fig. 5) when the sensitivity of eluting column was adjusted.

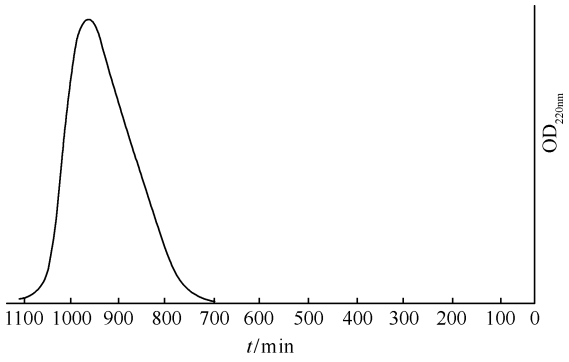
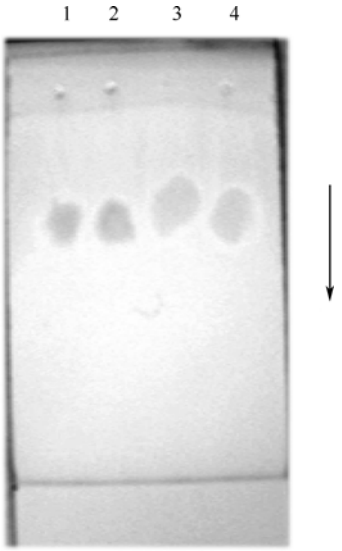


Fig. 5 SephadexG-200 column chromatographic analysis chart for EPS from LAB L15

3.5 Purity analysis on EPS-II

It was shown by the result of the thin layer chromatography

that the purified EPS in the experiment achieved the desired purity and was suitable to make further analyses on its chemical property and structure.



1–4 are all identical extracted EPS

Fig. 6 The thin layer chromatography for EPS-Ⅱ

4 Discussion

The tested LAB strain, the normal intestinal flora, is isolated from the gastrointestinal tract of flounder. The microbiological preparation made with the strain can enhance the quality of feed, promote growth, increase the animal resistance to disease, and improve the water quality in marine fish cultivation. For developing non-cell microbiological preparation by using extracellular polysaccharide (EPS), which is the metabolic product of LAB L15, its EPS is extracted and purified, and a series of influencing factors on producing EPS were analyzed. Compared to the productions of *Lactobacillus fermentum, L. brevis, L. casei* and *L. acidophilus*, the EPS production from L15 was similar to the four species. It belongs to the high productive strain for EPS and is suitable for use in producing EPS.

The EPS production in LAB growth is influenced by many factors. It was proven in the experiment that the EPS production is higher when glucose is used as a carbon source than any other sugar that was used, which is in accordance with similar domestic and foreign reports (Macedo et al., 2002). The main reason for this phenomenon is the low activity of fructose-1,6-bisphosphatase (FBPase), which can catalyse the transformation from fructose-1, 6-biphosphate to fructose-6-phosphate, and the essential stages of the biosynthesis from fructose to carbohydrate and nucleotide are restricted in LAB. Looijesteijn et al. (2001) proved that the low activity of FBPase limited the large biosynthesis of EPS using fructose as carbon source. The bacterial biomass was the highest when the maximum EPS production was achieved. The EPS production decreases quickly with the prolonging of time and the declining of pH, especially under 30°C. The possible reason is that EPS could have been gradually decomposed into monosaccharides and compensated as carbon source for continuous growth in LAB culture.

The low production of EPS and high degeneration rate of EPS in LAB culture have become a significant bottleneck in using the food-safety level microbes for producing EPS in industrial fermentation. Therefore, finding a way to improve the excessively low production of EPS will be an aspect of EPS study in the future (Cerning, 1990). The bacteria cultured under a lower temperature have a prolonged logarithm and stable period. Accordingly, the EPS production may increase. In this paper, we used a lower culture temperature compared with that in literature and obtained a higher EPS production (Zisu and Shah, 2003). The highest productions at 30°C and 37°C are similar. The reasons for earlier production peak and quicker production decline under 37°C may be that it is more suitable for the enzyme activity under 37°C, and the metabolism rate of the polysaccharide is quick. Therefore, it is better for the EPS collection to be under 30°C from the point of polysaccharide accumulation. Then, we can conclude that the best condition for LAB L15 to produce polysaccharide is by incubation at 30°C for 48 h.

Finally, the effective method for extracting EPS was explored in this experiment. It was shown by using thin layer chromatography that our experimental method is reasonable and effective. However, in the separation and purification of EPS, protein and other charged polysaccharides can mutually combine through the complicated chemical bonds, which brings about the difficulties for separation and purification of EPS (Li and Xia, 2003). It is the absorption among macromolecules that results in rather more loss in the repetitious separation and purification. It has become another setback in the low production and limited application in EPS.

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