High yield of exopolysaccharides in lactobacillus bulgaricus by optimization of culture condition

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Abstract. Exopolysaccharides derived from lactic acid bacteria play a crucial role in antitumour activity, immunomodulating bioactivity and anticarcinogenecity. With MRS as lactobacillus basic culture, the high yield of exopolysaccharide in lactobacillus bulgaricus strain AK-1 optimized fermentation condition was analyzed in this paper. The optimum pH, volume, fermentation time, carbon sources, nitrogen, and microelement were studied by single factor experiment. The orthogonal test was used for seed volume, fermentation temperature and fermentation time. The results showed that the optimum medium was include 35 g/L Glucose based on MRS culture, the optimum inoculum size was 2%, initial pH was 5.3, the optimum temperature was 40℃, and fermentation time was 8 hours. The yield of EPS is 409mg/L under the optimum condition, more than that in reported value (354mg/L).

Keywords: Exopolysaccharides, lactobacillus bulgaricus, optimization, culture condition.

1. Introduction

Immunomodulation by probiotic microorganisms has become a topic of increasing interesting food microbiology. Lactic acid bacteria (LAB) have health-promoting attributes, including antimutagenic activity, anticarcinogenic activity, hypocholesterolemic properties, and inhibition of intestinal and food-borne pathogens, antitumor effects, and promotion of T- and B-cell proliferation [1]. Exopolysaccharides (EPS) derived from lactic acid bacteria (LAB) play crucial role in antitumour activity, immunomodulating bioactivity and anticarcinogenecity [2-3]. These hetero-polysaccharides are composed of linear and branched repeating units varying in size from tetra- to heptasaccharides. The final EPS of high molecular mass ($1X10^6$ to $2X10^6$ Da) is formed by polymerization of several hundred to a few thousand of these repeating units [4]. Production of exopolysaccharides (EPS) by lactic acid bacteria in milk is not only an important factor in assuring the proper consistency and texture of fermented food, but also an important role in increasing the immunomodulation bioactivity of LAB [5]. The total yield of EPS produced by the LAB depends on the composition of the medium, LAB strain and growth conditions like temperature, pH, and oxygen tension and incubation period.

Lactobacillus delbrueckii subsp. bulgaricus (until 2014 known as *Lactobacillus bulgaricus*) is commonly used as a starter for making yogurt. It is also found in other naturally fermented products. In this work, we developed a high yield EPS culture condition in a strain of Lactobacillus delbrueckii which used in yogurt production.

2. Materials and methods

2.1. Materials.

Bacterial strains. EPS-producing L. bulgaricus strains AK-1 were obtained from the freeze-dried culture collection of the Alpha bio-technology co., LTD (Taiwan).

MRS medium. MRS typically contains (w/v):1.0 % peptone,0.8 % egg extract,0.4 % yeast extract,2.0 % glucose,0.5 % sodium acetate trihydrate,0.1 % polysorbate 80 (also known as Tween 80),0.2 % dipotassium hydrogen phosphate,0.2 % triammonium citrate,0.02 % magnesium sulfate heptahydrate,0.005 % manganese sulfate tetrahydrate. The pH adjusted to 6.2 at 25 ℃. The medium was autoclaved at 110 ℃ for 10 min.

2.2 Methods

EPS determination. After removal of cells by centrifugation (16,000 ×g for 10 min),the crude EPS was precipitated at 4 ℃ by addition of 2 volumes of ethanol (100%). The resulting precipitate was collected after centrifugation (16,000 ×g for 15 min) and redissolved in water. The crude EPS solution was dialyzed at 4 ℃. The total sugar concentration was determined by the anthrone sulfuric acid method using glucose as a standard. The results were expressed as milligrams of glucose per liter.

Glucose standard curve and regression equation. Under the anthrone sulfuric acid procedure,glucose serious concentration range is 10–500 mg/L, absorbances were measured at 620 nm with a spectrophotometer (Perkin Elmer model 512). A standard curve of corrected absorbance vs. glucose concentration was drawn, The regression equation of this standard curve was y=0.0063x-0.0194(R2 =0.9988).

Experimental design. Batch fermentations were carried out in N2-flushed 100-ml screw-cap flasks with 150 ml of MRS. The orthogonal test used for seed volume(1%,2%,3%), fermentation temperature(35 ℃ ,40 ℃ , 45 ℃) and fermentation time(4h,6h,8h). The optimum pH(4.3,4.8,5.3,5.8,6.3,6.8), fermentation time(4h,8h,12h,16h,20h,24h), carbon sources content(0.5%,1%,1.5%,2%,2.5%,3%,3.5% and 4% glucose),carbon source ration(Glucose:Lactose , 0.5:3,1:2.5,1.5:2,2:1.5,2.5:1,3:0.5,3.5:0.5,3.5:0) were studied by single factor experiment.

3. Result

3.1 Effect of fermentation time on the yield of EPS

The result showed that EPS is decreased after 12hours fermentation. The optimal fermentation time can from 4-12 hours (Fig 1).

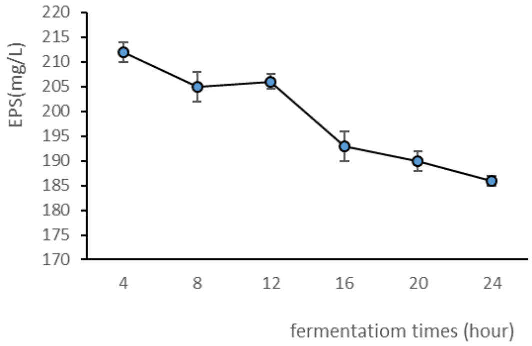

Fig.1 Effect of fermentation time on the yield of EPS in L. bulgaricus strains AK-1 grown in 40℃, the culture initial pH=6, inoculum size is 1%.

3.2 Effect of initial culture pH on the yield of EPS

The results suggested that the optimal initial culture pH is 5.3 for high yield EPS (Fig 2).

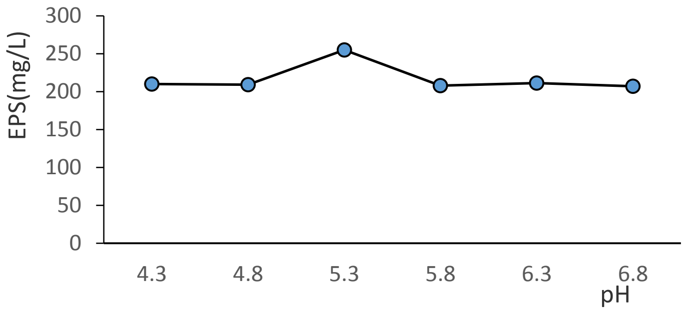

Fig.2 Effect of initial culture pH on the yield of EPS in L. bulgaricus strains AK-1 grown in 40℃, inoculum size is 1%, fermentation time is 8 hours.

3.3 Effects of inoculum size, fermentation temperature and time on the yield of EPS.

The range result showed that the EPS yield increased with the extension of fermentation time. Taken together 3.2 and 3.1 results strongly suggested that the optimal fermentation time is 8 hour. With the culture condition under 2% inoculum size and 40℃ for 8 hours can get highest EPS production, the yiels is 409.02mg/ml (Table 1).

Table1 Effects of inculum size, fermentation temperature and time on EPS production examined by orthogonal array

samp	factor			EPS yield
	inoculum size	Fermentation temperature	fermentation time	
1	1(1%)	1(35)	1(4h)	260.44 mg/L
2	1	2(40)	2(6h)	388.38 mg/L
3	1	3(45)	3(8h)	221.08 mg/L
4	2(2%)	1	2	262.03 mg/L
5	2	2	3	409.02 mg/L
6	2	3	1	324.25 mg/L
7	3(3%)	1	3	389.02 mg/L
8	3	2	1	245.84 mg/L
9	3	3	2	264.57 mg/L
K1	289.97	303.83	276.84	
K2	331.86	347.75	304.99	
K3	299.81	269.97	339.7	
range	41.89	77.78	62.87	

3.4 Effect of Lactose/glucose ratio on the yield of EPS.

The result showed that glucose as carbon source favors the production of EPS than lactose (Fig 3). Results suggested that the lactose in milk does not prefer for EPS production in LAB.

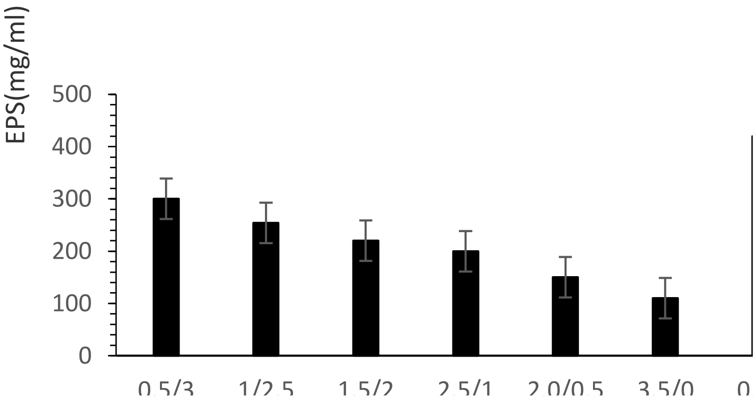

Fig.3 Effect of lactose and glucose ratio on the yield of EPS in L. bulgaricus strains AK-1 grown in 40℃, inoculum size is 2%, initial culture pH is 5.3, and fermentation time is 8 hours.

3.5 Effect of glucose concentration on the yield of EPS.

The 3.4 suggested the higher glucose concentration in culture produce the higher EPS by LAB. So the further test for glucose concentration on the yield of EPS was designed. The results showed the same EPS yield with the initial at 3.5% and 4%. Considering the culture cost-saving, 3.5% is as the optimal glucose concentration.

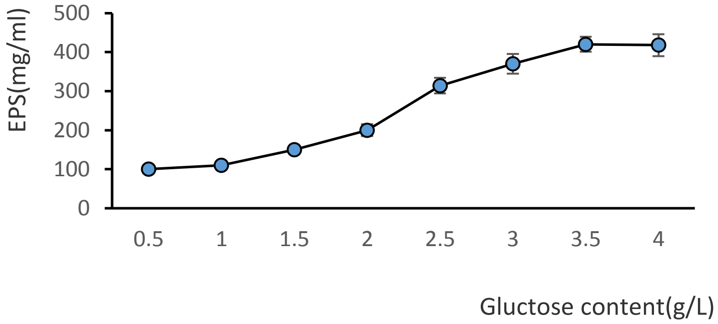

Fig 4 Effect of glucose content on the yield of EPS in L. bulgaricus strains AK-1 grown in 40℃, inoculum size is 2%, initial culture pH is 5.3, and fermentation time is 8 hours.

4. Conclusion

High yield of exopolysaccharides in *l. bulgaricus* strains AK-1 by optimization of culture condition was analyzed in this paper. As MRS as the basic culture, the optimum glucose concentration is 35 g/L. The optimum seed volume was 2%, initial pH was 5.3, the optimum temperature was 40℃, and fermentation time was 8h. The yield of EPS is 409mg/L under this optimum condition, more than that in reported value (354mg/L).

References

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