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Production of high molecular weight of pullulan with agro-industrial byproducts

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Abstract

Production of pullulan by *Aureobasidium pullulans* HP-2001 with agro-industrial byproducts was investigated. Agro-industrial byproducts from the rice processing industry for the traditional Korean food (AIB-A), apple juice production (AIB-B), and soybean sauce production (AIB-C) were used for carbon and nitrogen source for production of pullulan. Major components of AIB-A were glucose, maltose, maltotriose, and dextran. AIB-A and B were found to be good substitute to glucose as carbon source. Productivity of pullulan with AIB-A and B as carbon source was similar to that glucose. Molecular weight of pullulan produced with AIB-A and B was higher than that with glucose. Major components of AIB-B and C were carbohydrate, protein, fat and ash. AIB-C was also a good substitute to yeast extract as nitrogen source. Some of physiological conditions were examined for the large scale production of pullulan.

Introduction

Pullulan, an α-1,6-linked homopolymer of maltotriose, is produced by *Aureobasidium pullulans*, a member of the *Fungi imperfecti*. *A. pullulans* is yeast-like fungus that has been used for industrial production of pullulan from starch. Pullulan possesses distinctive flim and fiber-forming characteristcs which is not found in amylose. Pullulan may be used as a coating and packaging material, a sizing agent for paper and a starch replacer in low-calorie food formulations, cosmetic emulsions, and other industrial and medicinal applications. Production of pullulan with AIB-A, B, and C was investigated in this study.

Material and methods

Bacterial strain *Aureobasidium pullulans* HP-2001, UV induced mutant of *A. pullulans* ATCC 42023, was transferred monthly to the nutrient agar medium. The medium used for cell growth and production of exopolymer contained the following components(g/l): K_2HPO_4, 5.0; NaCl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; $(NH_4)_2SO_4$, 0.6; yeast extract (Difco Lab., Detroit. MI), 2.5. The pH of medium was adjusted 6.8 to 7.0 before sterilization. Carbon source was autoclaved separately for 15 min at 121℃ and added to the medium under aseptic conditions.

Production of pullulan Starter cultures were prepared by transferring cells from agar slants to 50㎖ medium containing 2%(w/v) glucose in 250㎖ Erlenmeyer flasks. The resulting cultures were incubated for 2 days at 30℃ and 200 rpm. Each starter culture was used as an inoculum for 100㎖ of medium in a 500㎖ Erlenmeyer flasks. The culture were incubated for 5 days under the same condition used to prepare the starter cultures. Samples were periodically withdrawn from the cultures to examine cell growth and production of pullulan.

Purification of pullulan Cultured broth after 120 hr was centrifuged at 8000×g for 15 min to remove fungal cells. Supernatant was mixed with 2 volume of isopropyl alcohol and incubated at 4℃ for 24 hr to precipitate the crude product which were separated by centrifugation at 8000×g for 20 min. The precipitated material was repeatedly washed with acetone and ether, dissolved in deionized water, and dialyzed against deionized water by using dialysis tubing with a molecular weight cut off 12,000 to 14,000. After dialysis for 2 to 3 days with four or five changes of deionized water, the solution was lyophilized and the exopolymer yield was determined by weighing. To determine biomass, the cells were washed with distilled water and dried at 100 to 105℃ until the weight was constant.

Determination of molecular weight by gel permeation chromatography The average molecular weight of pullualn samples were determined by the gel permeation chromatography equipped with HP 1100 series 20RBAX PSM HPSEC columns and RI detector. Pullulan standards with narrow polydispersity and molecular weights ranging from 5.80×10^3 to 1.6×10^6 were used to construct a calibration curve. Distilled water was used as the mobile phase at a flow rate of 1.0 ml/min. The sample concentration and injection volume were 5.0 mg/ml and 100㎕. All of the sample solutions were filtered through 0.45 ㎛-pore-size filters(Adbentec MFS, Inc., Japan) before injection.

Results and discussion

The effect of AIB-A and B on molecular weight and production of pullulan was shown in Table 1. Molecular weight of pullulan produced with AIB-A,B was higher than that glucose.

Table 1. Effect of AIB-A and B as carbon source on production and molecular weight of pullulan

Carbon sources (w/v)	Pullulan (g/l)	Conversion rate (%)	Molecular weight
2% Glucose	5.96	29.8	0.90×10^6
10% AIB-A	15.8	15.8	2.11×10^6
20% AIB-A	10.7	5.4	4.79×10^6
1% AIB-B	2.99	29.9	6.27×10^6
2% AIB-B	5.61	28.1	5.62×10^6

The effect of AIB-C on molecular weight and production of pullulan was shown Table 2. Production yield of pullulan with 2% glucose as carbon source and 0.05% AIB-C as nitrogen source was 8.67g/l and its conversion rate was 43.4% whereas that with 2% glucose and 0.25% yeast extract was 5.96g/l and its conversion rate was 29.8%.

Table 2. Effect of AIB-C as nitrogen source on production and molecular weight of pullulan

AIB-C (%, w/v)	Pullulan	Converion rate(%)	Molecular weight
control[1)]	5.96	29.8	0.90×10^6
0.05	8.67	43.4	1.75×10^6
0.10	8.60	43.0	2.99×10^6
0.15	8.03	40.2	2.25×10^6
0.25	7.66	38.3	2.66×10^6
0.35	7.41	37.1	5.66×10^6
0.50	7.28	36.4	3.18×10^6

1) 0.25% yeast extract

Effect of AIB-C on molecular weight of pullulan was shown Fig. 1. Molecular weight of pullulan with various concentration of yeast extract ranged from 1.68 $\times$ 10^5 to 9.26 $\times$ 10^5 and that with various concentration of AIB-C ranged from 1.75 $\times$ 10^6 to 5.66 $\times$ 10^6. Molecular weight of pullulan with AIB-C was higher than that of yeast extract.

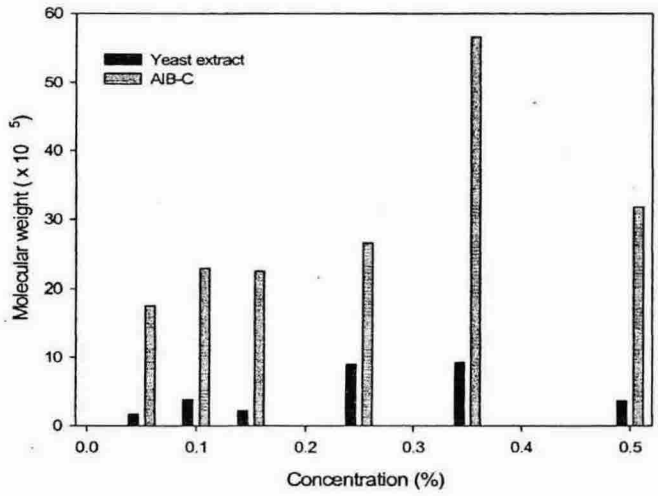

Fig 1. Effect of AIB-C as nitrogen source on molecular weight of pullulan

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