A Study on the production of pullulan by *Aureobasidium pullulans* and its properties

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Abstract

Pullulan as useful biopolymer has been focused on its industrial production. The objectives of this thesis were to enhance the production of pullulan for the industrial production and to characterize pullulan physical properties. In order to find the economical optimum of the fermentation conditions and promote pullulan productivity, effect such as the carbon source, pH have been estimated and measured molecular weight of produced pullulan.

Introduction

Mass production of polysaccharides from microorganism is also available through a proper strain selection and a method of cultivation. The investigation of pullulan production by Aureobasidium pullulans has studied concerning the morphology and the physiology of the cultivation. A. pullulans well grows on various carbon sources like glucose, sucrose, starch, and so on, but particularly high growth yield was shown on sucrose, starch soluble and starch hydrolyzed.[1),2)] Using waste resource and cheap material is considered because the carbon source influences mostly on the production cost, so potato starch, whey and peat hydrolyzed are used to research pullulan production.[3),4)] Further more, crop waste and sugar of byproduct from food process are prospected as carbon sources for pullulan. Pullulan is possibly used for a low calory food additive because of non-toxic and hardly degradable in a digestive organ. Pullulan can be decomposed well by pullulanase, a secretion of Aerobacter aerogenes, and isopullulanase from Aspergillus niger, so not caused any environmental problems.[5),6)] Pullulan can be used

as a film, food coating and packaging agents, food ingredients, adhesives, fabrics, and so on with these various properties. In the near future, the market will be extended as the uses develop and improve.

Materials and methods

Micro-organism and cultivation medium : *Aureobasidium pullulans* ATCC 9348 was used as fermentation organism in this study. The strain was stored at 4℃ on PDA slant (Difco, USA). The standard cultivation medium contained (g/L) : Starch soluble, 50 (Glucose, 50 or Sucrose, 50 for comparative study); K_2HPO_4, 0.5; NaCl, 0.1; $MgSO_4 \cdot 7H_2O$, 0.02; $(NH_4)2SO_4$, 0.06; yeast extract(Difco), 0.1. The pH of medium was adjusted 7.5 before sterilization. The medium was autoclaved for 15min at 121℃.

Inoculum preparation and fermentation : The inoculum was grown for 48hr at 25℃ and 200rpm in a shaking incubator (New Bruswick Scientific Co., USA). Each culture was used as an inoculum for 200mL of medium in a 1000mL erlenmeyer flasks. Fermentations were carried out in a 4.5L jar fermenter (KFC, Korea). After every 12hr, fermentation broth from the fermenter was sampled and analysed.

Measurement procedure : Cultured broth was centrifuged at 8000rpm for 20min. to separate fungal cells. Pullulan was obtained from fermentation solution by isopropyl alcohol precipitation (1.5 volumes). The precipitate obtained was filtered through a pre-weighed Whatman No.1 filter and then dried at vaccum oven(20hr, 80℃). The dry weight of pullulan was expressed as g/L and pullulan yield was expressed as gram polysaccharide per 100g of carbon source consumed.

Measurement of pullulan molecular weight : To determine the molecular weight of pullulan, gel permeation chromatography (GPC) was used.

Thermogravimetric analysis : Thermogravimetric analysis was examined using TGA for checking a thermal stability. The increasing rate of temperature was 10 ℃/min in nitrogen mood. A sample of 20 mg was applied to, and the range of 20 - 500 ℃ was measured.

Results and discussion

Viscosity of pullulan : The each pullulan, produced from glucose, sucrose and starch, was compared with on a point of viscosity. As shown in Table 1, the pH of the fermented broth using sucrose was 4.12, that using glucose was 4.80, and that using starch was 4.58 when initial pH of the broth was 7.5. The pH of the pullulan 1 % solution recovered

from sucrose medium was slightly acidic at 5.28, whereas it was 5.51 for the pullulan from starch.

Molecular weight of pullulan : The peculiarity and the useage of pullulan are depending on its molecular weight. *A. pullulans* ATCC 9348 grow well around a natural pH, but produced well pullulan of high molecular weight on a acidic pH. The Table 2 is shown up that high molecular weight pullulan over 1.0×10^6 was produced under pH 6.0 controlled. The fermentation without pH control produced pullulan over 1.0×10^6 because the pH decreased spontaneous to less 4.5 with an increased duration of cultivation.

Figure 1(Left) is gel permeation chromatograph curves of pullulan and pullulan was produced at different pHs. Pullulan with different curves were shown at different time to estimate the own molecular weight.

Therefore, economically optimum condition for the production of the high molecular weight pullulan around 1.0×10^6 was initial pH 7.5 uncontrolled.

Thermal analysis : TGA was used to analyze a thermal stability. Pullulan was stable and As shown in Figure 1(Right), two steps were in the reduction of weight, it reflected on the linkages of structure. In a comparison of pullulan between from pH 7.5 and from pH 3.0, pullulan produced at pH 3.0 was more thermally stable. The result was considered that higher molecular weight might be, more stable in a thermal decomposition.

Reference

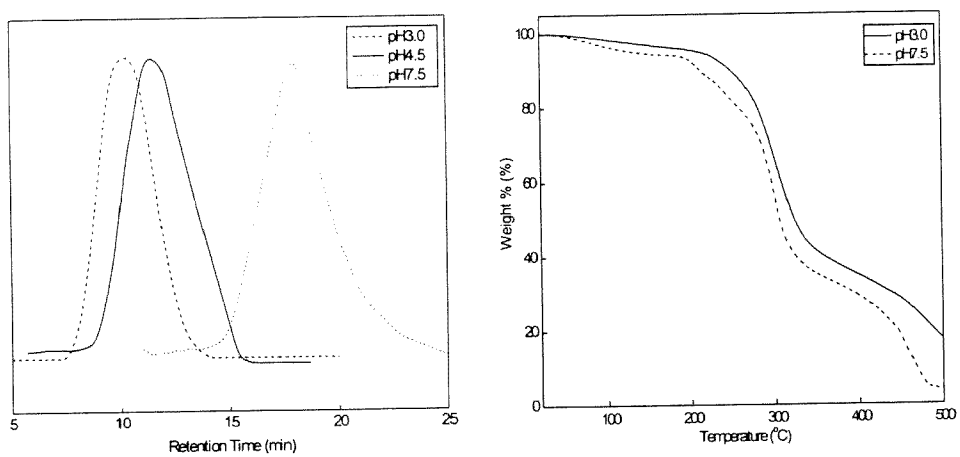
1) Shin, Y. C., Kim, Y. H., Lee, H. S., Cho, S. J., and Byun, S. M. Biotechnology Bioengineering, **3,** 129-133 (1989).
2) 양승남, "Aureobasidium pullulans GM21에 의한 Pullulan 생산", 성균관대학교 화학공학과 석사학위 논문 (1998)
3) Leduy, A., Yarmoff, J. J., and Chagradui, A., Biotechnology Letters, **5,** 49-54 (1983).
4) Leduy, A., and J. M. Boa. Canadian Journal of Microbiology, **29,** 143-146 (1983).
5) Bull, A. T, Arch. Biochemical and Biophysical research communications, **137,** 345 (1970).
6) Brown, R. G., Hanic, L. A., and Hsiao. M, Canadian Journal of Microbiology, **19,** 163 (1973).

Table 1. Physical properties of pullulan with different carbon sources

Property		Source of pullulan		
		Glucose	Sucrose	Starch
pH of fermented medium		4.80	4.12	4.58
pH of 1 % solution		5.63	5.28	5.51
pH change		17 %	28 %	11.7 %
Viscosity of solution (cP)	1 %	37 ±0.68	33 ±0.61	60 ±0.7
	5 %	111 ±1.32	99 ±1.21	165 ±1.93
	10 %	350 ±3.50	308 ±3.02	480 ±4.83

Table 2. Change in molecular weight of pullulan at various culture pHs

Culture pH	Pullulan yield (%)	Mw	Mn	Polydispersion (Mw / Mn)
pH 7.5, uncontrolled	41.2	103×10^4	54×10^4	1.90
pH 6.0, uncontrolled	33.0	125×10^4	71×10^4	1.76
pH 7.5, controlled	12.0	9×10^4	6×10^4	1.50
pH 6.0, controlled	35.0	68×10^4	42×10^4	1.62
pH 4.5, controlled	30.2	137×10^4	99×10^4	1.38
pH 3.0, controlled	23.2	160×10^4	123×10^4	1.30



pH 3.0 (--) pH 4.5 (-) pH 7.5 (···) pH 3.0 (-) pH 7.5 (···)

Figure 1. GPC curves of pullulan(Left) and TGA thermograms of pullan(Right)