Production of pullulan by *Aureobasidium pullulans* HP-2001 with continuous culture

서형필, 이유정, 김성구[1], 신명교[2], 이진우*

Division of Biotechnology, Faculty of Natural Resources & Life Science,
Dong-A University, Pusan 604-714, Korea, Tel (051)200-6995 Fax (051)200-7593
Division of Food & Biotechnology, Pukyung National University, Pusan 608-737, Korea[1]
KBP Co., Ltd, Chilgoi-dong, Pyongtaek-si Gyeonggi-do, Korea[2]

Abstract

In batch culture of *A. pullulans* HP-2001, maximal production of pullulan was 57.90 g/l at 96 hr. The maximal production of pullulan with 76.55 g/l occurred when substituted solution was 10% (w/v) sucrose, 0.25% (w/v) yeast extract and mineral salts. The maximal production of pullulan was 75.00 g/l with a dilution rate of 0.015 h^{-1}. Productivity of pullulan in the continuous culture fed with the fresh medium was higher than that fed with the fresh medium without nitrogen source.

Introduction

Pullulan, an α-1,6-linked homopolymer of maltotriose, is produced by *Aureobasidium pullulans*[1]. Pullulan possesses distinctive film and fiber-forming characteristic which is not founded in amylose[2]. Pullulan may be used as a coating and packaging material, as a sizing agent for paper and as a starch replacer in low-calorie food formulation, in cosmetic emulsions and in other industrial and medicinal applications[3,4]. In this study, effect of composition of feed solution on production of pullulan and optimal dilution rate for continuous culture was investigated.

Material and methods

Bacterial strain & culture medium

Aureobasidium pullulans HP-2001, UV induced mutant of *A. pullulans* ATCC 42023[5], was transferred monthly to the nutrient agar medium. The medium used for cell growth

and exopolymer production 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 and yeast extract (Difco Lab., Detroit. MI), 2.5.

Continuous culture for the production of pullulan

Experiments employed a 7 l bioreactor with two six-bladed impellers and 3 baffles. The dilution rate ranged from 0.003 to 0.043 h^{-1}. Working volume was 4 l. Aeration rate and agitation speed were 1.0 vvm and 500 rpm.

Production of pullulan

Starter cultures were prepared by transferring cells from agar slants to 50 ml medium containing 2% (w/v) glucose in 250 ml Erlenmeyer flasks. Each starter culture was used as an inoculum 5% (v/v) for 100 ml of medium in a 500 ml Erlenmeyer flasks. The culture were incubated for 4 days under the same condition used to prepare the starter cultures. Samples were periodically withdrawn from the cultures to examine cell growth and pullulan production.

Purification of pullulan

Cultured broth was centrifuged at 8,000 × g for 15 min to remove fungal cells. Supernatant was mixed with 2 vol of isopropyl alcohol and incubated at 4℃ for 24 hr to precipitate the crude product, which were separated by centrifugation at 8,000 × 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 change of deionized water, the solution was lyophilized.

Analytical methods

To determine biomass, the cells were washed with distilled water and dry cells weight (DCW) measured by directly weighing the biomass after drying to constant weight at 10 0℃ to 105℃. The concentration of pullulan was determined colorimetrically by the phenol-sulfuric acid method. A standard curve for pullulan was prepared from pullulan (Sigma, St. Louis, USA).

Results and discussion

The effect of medium substitution after 72 hr on cell growth and production of pullulan by *A. pullulans* HP-2001 was examined (Fig. 1). In batch culture of *A. pullulans* HP-2001, maximal production of pullulan was 57.90 g/l at 96 hr. Substituted solutions used in this study were 1) 10% (w/v) sucrose, 2) 10% (w/v) sucrose and 0.25% (w/v) yeast extract and 3) 10% (w/v) sucrose, 0.25% (w/v) yeast extract and mineral salts, which is the medium for the production of pullulan. The composition of mineral salts were 5.0 g/l K_2HPO_4, 1.0 g/l NaCl, 0.2 g/l $MgSO_4 \cdot 7H_2O$ and 0.6 g/l $(NH_4)_2SO_4$. The pHs of substituted solution was adjusted to 6.0 before sterilization. The maximal production of pullulan with 76.55 g/l occurred when substituted solution was 10% (w/v) sucrose, 0.25% (w/v) yeast extract and mineral salts.

Continuos culture of *A. pullulans* HP-2001 for the production of pullulan was performed in a 7 l bioreactor and the effect of dilution rate on cell growth and the production of pullulan was examined (Fig. 2). Feeding material was the fresh medium for the production of pullulan. Dilution rates ranged from 0.015 to 0.043 h^{-1}. The production of pullulan increased with increased dilution rate up to 0.015 h^{-1}. The maximal production of pullulan was 75.0 g/l with a dilution rate of 0.015 h^{-1}.

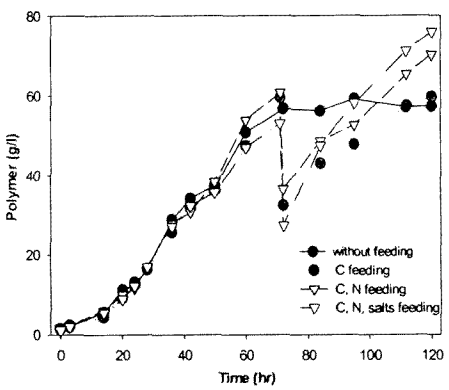

Fig. 1. Effect of feed solution on production of pullulan by fed batch production of pullul an by continuous culture.

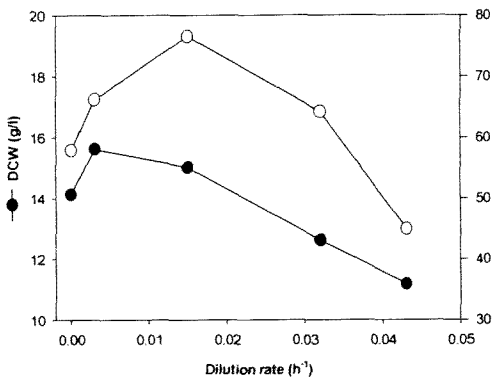

Fig. 2. Effect of dilution rate on production of p ullulan by continuous culture.

Continuos culture of *A. pullulans* HP-2001 with a dilution rate of 0.015 h^{-1} was performed

for 12 days in a 7 l bioreactor (Fig. 3). Feeding materials were the fresh medium and the fresh mediun without nitrogen source. Productivity of pullulan in the continuous culture fed with the fresh medium was higher than that fed with the fresh medium without nitrogen source. Cell growth in the continuous culture fed with the fresh medium maintained for 12 days, which was longer than that fed with the fresh medium without the nitrogen source.

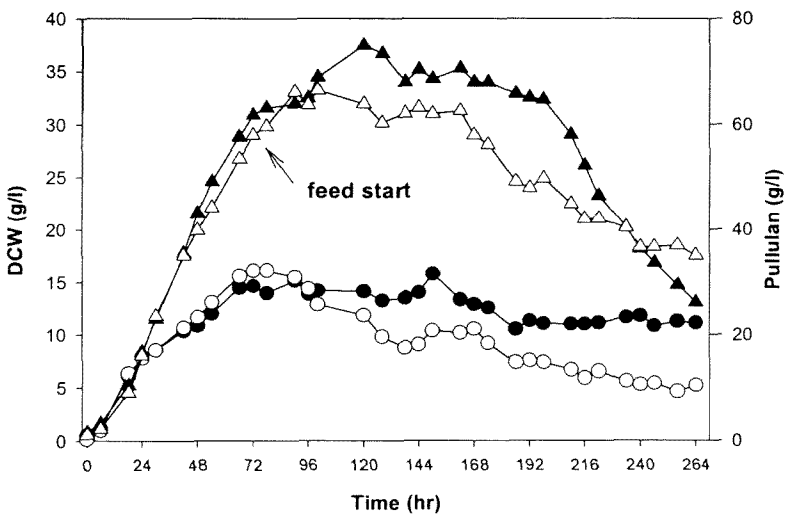

Fig. 3. Continuous culture of *A. pullulans* with a dilution rate of 0.015 h^{-1}. ●, DCW (feed solution contained carbon, nitrogen source and minerals); ○, DCW (N-limitation feed solution); ▲, pullulan (feed solution contained carbon, nitrogen source and minerals); △, pullulan (N-limitation feed solution)

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