Molecular Weight Distribution of Pullulan and Degrading Enzyme Activity of *Aureobasidium pullulans*

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Abstract

The effects of DO and pH on the mass production of pullulan with high-molecular weight from *A. pullulans* ATCC 42023 were evaluated. The maximum pullulan production yield (51%) was obtained at pH non control (initial pH 6.5) and DO control (above 50%) condition. The pullulan degrading enzyme was activated when the pH of broth reached lower than 5.0 and portion of low molecular weight pullulan was increased. The formation of a black pigment was observed at the initial stationary phase, 40hr of fermentation. Therefore, the fermentation should be carried out in pH non control and DO control condition and harvested before reaching stationary phase around 40h for the production of high molecular weight pullulan.

Introduction

Pullulan is an extracellular water-soluble microbial polysaccharide produced by the yeast-like fungus *Aureobasidium pullulans*. It consists mainly of maltotriose units interconnected via $\alpha(1\rightarrow6)$ linkages (Deshpande *et al.*, 1992). A number of potential applications have been reported for pullulan using transparent, oil resistant and oxygen impermeable film-forming properties. In this study, the effects of dissolved oxygen and pH on pullulan fermentation and the molecular weight distribution of pullulan during the fermentation were evaluated for determining the optimum fermentation condition for pullulan production.

Materials and Methods

Microorganism and Culture Conditions : *Aureobasidium pullulans* ATCC 42023 (Ueda *et al.* 1963) was used as fermentation organism. The medium used for cell growth and pullulan production contained the following composition (g/*l*): K2HPO4 5.0, NaCl 1.0, MgSO47H2O 0.2, (NH4)2 SO4 0.6, yeast extract 2.5, and glucose 50 (Kim *et al.*, 2000).

Determination of Enzyme Activity : The effect of pH on enzyme activity of fermentation broth was determined by the standard procedure (Bollag *et al.* 1996) and using the appropriate buffer. From pH 2.0 to 3.0, 50mM citrate buffer was used and 50mM acetate buffer was used for pH 4.0 to 5.0 whereas 50mM phosphate buffer was used in pH range of 6.0 to 8.0. The released reducing sugars were determined by the dinitrosalicylic acid method (Miller, 1959).

Results and Discussion

Cell growth, pullulan production and molecular weight distributions of pullulan produced from *A. pullulans* were determined at the various culture conditions with dissolved oxygen (DO) and pH. Pullulan concentrations increased steadily at the logarithmic phase (40h) in each condition. However, those slightly decreased in the late period of stationary phase. The total amounts of pullulan at pH non-control conditions were much higher than that of pH control conditions. Besides the total amounts of pullulan, the amounts of high molecular weight pullulan was maximum of 6 g/*l*, at 30hr of fermentation under pH non-control and DO control condition. After reaching to the maximum level, the amount of high molecular weight of pullulan decreased and consequently the low molecular weight portion increased.

However, the amount of high molecular weight pullulan produced at pH control conditions (pH 6.5) were maintained the level throughout the fermentation period. The molecular weight shift at pH non-control condition was

due to the activation of pullulan degrading enzymes.

The activities of standard pullulanase and crude enzyme solutions from culture broth on the pullulan obtained from fermentation were determined at various pH. Standard pullulanase showed the optimum activity at pH 4-8, crude enzyme solutions of fermentation broth showed optimum activity at pH 3-4. Crude enzyme from the fermentation broth showed 70% of pure pullulanase activity.

Generally, pullulanase is one of the starch-debranching enzymes and the optimum pH for activity was known as pH 5.0-7.0. However, crude pullulanase from this study showed an optimum activity at pH 3.0. Therefore, the pH decrease at the culture with pH non control and DO control condition promoted the degradation of pullulan with high molecular weight, whereas the molecular weight of pullulan produced at the pH control condition at pH 6.5 could maintain the high molecular weight pullulan portion throughout the fermentation process.

As a conclusion, the maximum production yield of pullulan was obtained from the culture of *A. pullulans* at pH non control and DO control condition. In addition to the pullulan yield, fermentation should be less than 40hr to avoid the production of melanin. Therefore, optimum condition for the production of the high molecular weight pullulan without pigment was the pH non control (initial pH 6.5) and DO control (above 50%) condition with fermentation time of 40h.

References

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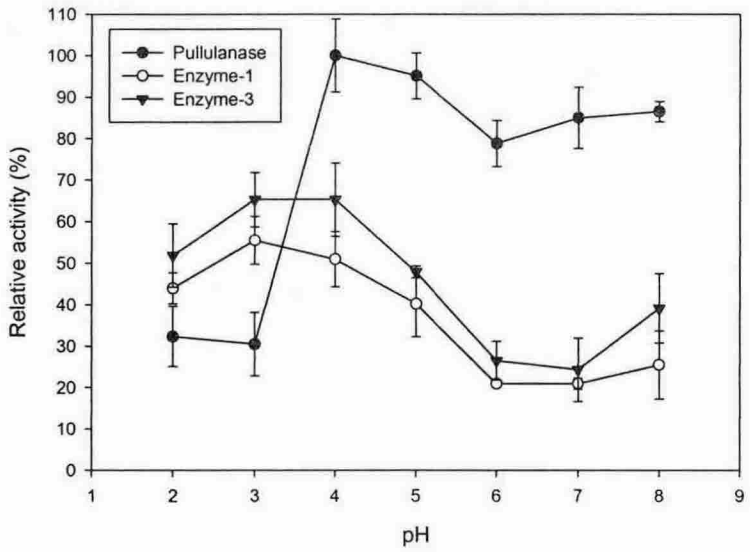

Figure. Effect of pH on the activity of enzyme from culture broth by *A. pullulans* and pullulanase activity.
Enzyme 1 : Crude enzyme solution with fermentation time of 1day.
Enzyme 3 : Crude enzyme solution with fermentation time of 3day.