Study on the Cultivation Conditions of Aeromonas hydrophila Too12 to Produce N-acetylchitooligosaccharides

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ABSTRACT

The aim of this study is to explore the effects of various factors such as carbon source, nitrogen source, and concentrations of α-chitin and β-chitin on the production of N-acetylchitooligosaccharides by Aeromonas hydrophila Too12. The microbe was cultivated separately in chitin broth (CB) media of α-chitin and colloidal chitin. The main hydrolysate product in these media was N-acetyl-chito- triose. The concentrations of N-acetylchitotriose in the α-chitin medium and in the colloidal chitin medium were 0.48 and 0.47 g/L, respectively, at 72 h. When the microbe was cultivated in a β-chitin broth medium, N-acetylchitotriose reached 0.3 g/L at 24 h, and N-acetylglucosamine 0.45 g/L at 96 h. When the microbe was cultivated in a 5% α-chitin CB medium, the main products included N-acetyl-glucose- amine and N-acetylchitotriose. The concentrations of N-acetylglucosamine and N-acetylchitotriose were 2.85 and 2.42 g/L, respectively, at 144 h. The microbe was also cultivated separately in a 5-L batch fermenter in the α-chitin broth (CB) medium with various levels of dissolved oxygen (designated as the high level, the low level, and no aeration) to examine the effect of oxygen level on the varieties and contents of N-acetylchitooligosaccharides. At 96 h, under the high oxygen level, the highest production of N-acetylchitotriose was 0.64 g/L. At 72 h, under the low oxygen level, the highest N-acetylglucosamine and N-acetylchitotriose were 1.26 and 1.1 g/L, respectively. At 96 h, with no aeration, the highest production of N-acetylglucosamine was 2.55 g/L. Further purification was carried out for the supernatant culture of Aeromonas hydrophila Too12 cultivated in a 5-L batch fermenter in the α-chitin broth (CB) medium with the low level of dissolved oxygen. Crude chitinases were obtained through a sequence of steps including protein precipitation using ammonium sulfate, dialysis, anion exchange of DEAE-Sepharose CL-6B, and gel filtration of Sephacryl S-100 HR. A peak in anion exchange chromatographic diagram showed chitinase activity, and fractions of this peak were collected for further analysis. The specific activity of the chitinase was 9.62 U per mg of protein, the chitinase recovery was 5.34%, and the chitinase was purified by 3.14 fold. The optimum reacting temperature for the chitinases was 40℃, and the optimum pH was 6.0. Metal ions such as Hg2+, Zn2+ and Mn2+ showed an inhibitive effect on the chitinase activity and EDTA could enhanced the activity.

Keywords : N-acetyl-chitooligosaccharides ; N-acetylchitotriose ; chitinase

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