Production of N-Acetylchitooligosaccharides by Aeromonas sp. DYU-Too13 and Characterization of Its Chitinases

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ABSTRACT

In this study, the target strain, Aeromonas sp. DYU-Too13, was cultured in various nitrogen sources and ammonium chloride (NH4Cl) concentrations to examine the effect of different culture conditions on the chitinase activity, and yields of reducing sugars and N-acetyl-chitooligosaccharides. The results showed that, simpler types of N-acetyl-chitooligosaccharides were obtained, and high yields of N-acetyl-chitotetraose reached 0.32 g/L at 72 h and 0.31 g/L at 96 h using NH4Cl as the sole nitrogen source. Maximum yields of reducing sugars and N-acetyl-chitotetraose were obtained in the medium containing 0.1 g/L NH4Cl as the nitrogen source, and they were 7.2 g/L and 0.70 g/L, respectively, at 72 h. After Aeromonas sp. DYU-Too13 was cultured in a medium containing 5% β-chitin and 0.1 g/L NH4Cl for 96 h, the culture was further purified through precipitation, ultra-filtration, and chromatographic separation. The final product has a specific chitinase activity of 5.06 U/mg and a purity fold of 3.77, and the chitinase recovery ratio was 2.80%. Three chitin isozymes with molecular weights of 58, 66, and 70 kDa were identified through analysis by SDS-PAGE. The purified chitinases were also characterized. The optimum reaction pH for the chitinases is 7.0, and the optimum reaction temperature is 40°C. The chitinase activity is substantially inhibited by metal ions such as Cu2+, Hg2+ and Zn2+.

Keywords : Aeromonas sp. DYU-Too13、N-acetyl-chitotetraose、characterization、chitinase、purification、β-chitin

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