Production of N-acetylchitooligosaccharides by Chitin Hydrolysis Using a Chitinase from Aeromonas caviae DYU-BT4

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ABSTRACT

The purpose of this study is to isolate an indigenous microorga- nism to degrade chitin and to produce hydrolysates of chitin. Hydrolysates of chitin were analyzed by the method of HPLC. The effect of carbon concentration on biomass, reducing sugar, chitinase activity and N-acetylglucosamine in a batch fermenter was studied. Purification of chitinases produced at different culture times was carried out by an ion exchange chromatography column with DEAE-sepharose CL-6B gel and by a gel filtration chromatography column with Sephadex G- 100 gel. The molecular weight and the characteristics of each of chitinase proteins was identified with an SDS-PAGE. A microbe, DYU-BT4, isolated from the soil of Zwou-Shau-Shi in the Yunlin county was used to degrade chitin. The microbe had been identified to be Aeromonas caviae by the Food Industry Research Development Institute at Hsin-Chu of Taiwan and was named as Aeromonas caviae DYU-BT4. The microbe was cultivated in media with different carbon-sources, such as shrimp shell powder, squid pens, chitin powder and colloidal chitin. The major chitin hydrolysate from the cultivation of strain DYU-BT4 was N-acetylglusamine. The highest yield of N-acetylglucosamine (about 39% (w/w)) was obtained by using the colloidal chitin as a carbon source. Experimental results have shown that the time required to produce N-acetylglucosamine can be shortened and the yield of N-acetylglucosamine can be raised as the carbon concentration in the medium was elevated during a cultivation of strain DTU-BT4 in a batch fermemter. After the supernatant of the culture broth of Aeromonas caviae DYU-BT4 was purified, the portion of the protein with chitinase activity was examined by the SDS-PAGE containing 1% glycol chitin, and its molecular weight was identified to be 75 kDa. The optimum reacting temperature of the 75-kDa enzyme was 40℃, and the optimal pH was 7.0. The enzymatic reaction value, Km, for the 75-kDa enzyme was measured to be 1.87 g/L and the value of Vmax was 213 U/L. Metal ion Co2+ can promote the chitinase activity, and other metal ions such as Ag+, Ba2+, Hg2+, K+ and Zn2+ will inhibit the chitinase activity.

Keywords : chitinase ; Aeromonas caviae

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