Purification and Characterization of Chitinases by Aeromonas sp. DYU-Too 7 and an

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ABSTRACT

Abstract A chitin-degrading bacterium, DYU-Too 7, was isolated from the soil in Hsin-Chu. The bacterium was identified as Aeromonas sp. The supernatant of 120 h-old culture of Aeromonas sp. DYU-Too 7 cultivated in medium CB (chitin broth) at 30°C was collected by centrifugation. Two chitinases were purified from the culture super- natant by ammonium sulfate precipitation, DEAE Sepharose CL-6B and Sephadex G-100. The two chitinases have molecular masses of 35 and 60 kDa, respectively, determined by SDS-PAGE. The 35 and 60 kDa chitinases were purified 3.66-fold with 5.07% yield and 2.25-fold with 6.76% yield, respectively. The optimum pH and temperature were 5 and 70°C for the 35 kDa chitinase and 4 and 60°C for the 60 kDa chitinase. Effects of pH and temperature on stability of chitinase have been explored. Experimental results showed that the 35 kDa chitinase was stable for pH 5-8 and 10-60°C, and the 60 kDa chitinase was stable for pH 4-8 and 10-50°C. Effects of metal ions on chitinase activity were also explored. Results showed that the activities of both chitinases of 35 and 60 KDa remained over 50% in a solution containing 10 mM of Ba2+, Hg2+, Mg2+ or Ag+. The main hydrolysate of colloidal chitin was (GlcNAc)2 that had 2.21 g/L for the 35 kDa and 2.3 g/L for the 60 kDa chitinase, respectively. The Km and Vmax were 8 g/L and 2,000 U/L, respectively, for the 35 kDa chitinase, and they were 4.3 g/L and 1,428.6 U/L for the 60 kDa chitinase. A chitin-degrading bacterium, JR1, was isolated from the soil in Taipei with a medium containing 0.5% colloidal chitin. The strain reached its log phase and stationary phase at 2 h and 12 h, respectively, in medium LB. The activity of chitinases produced by strain JR1 was 366 U/L when it was cultured in medium CB containing 2% chitin powder at 30°C for 120 h. The 75 kDa chitinase was obtained from the purification of the culture supernatant by ammonium sulfate precipitation, DEAE Sepharose CL-6B, Sephadex G-100 and SDS-PAGE. The optimum substrate concentration of crude enzyme was 15 g/L. The optimum pH and temperature were 6 and 40°C for crude enzyme and 7 and 40°C for the 75 kDa chitinase. The main hydrolysate of chitin powder was N-acetylglucosamine (GlcNAc), 4.79 g/L, when strain JR1 was cultured in medium CB after 24 h.

Keywords : chitin ; Aeromonas sp. DYU-Too 7 ; chitinase ; strain JR1 ; N-acetylglucosamine

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