

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 was collected by centrifugation. Two chitinases were purified from the culture supernatant 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 for the 35 kDa chitinase and 4 and 60 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, and the 60 kDa chitinase was stable for pH 4-8 and 10-50. 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 K_m and V_{max} 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 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 for crude enzyme and 7 and 40 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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