Thesis Title Chitinolytic Enzymes of *Bacillus licheniformis*; Partial Purification, Characterization and Mosquito Larvicidal Property

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ABSTRACT

The *Bacillus licheniformis*, isolated by our laboratory, produced chitinolytic enzymes when cells grown in medium supplemented with crude chitin entered the stationary phase. This *Bacillus licheniformis* strain produced at least 5 types of chitinolytic enzymes and their molecular weights were 70.8, 68.1, 63.7, 53.0, and 49.5 kDa. The chitinolytic enzyme from culture supernatant of cells grown in medium supplemented with chitin was purified by fractionation with ammonium sulfate ranges from 35-65 percent and passed through column chromatography with CM- and 2 columns of DEAE-sephadex matrix respectively. The pure fraction of chitinase with molecular weight of 70.8 kDa was obtained. Results of physicochemical properties of purified enzyme showed optimal temperature of 60°C, relatively stable when kept at 40 and 50°C with

optimal pH of 5.0. Its activity was rapidly lost when kept in strong acid, strong alkali or high temperature (≥ 70°C), but very stable in deionized distilled water. Enzyme kinetics study showed that Michaelis's constant (K_m) and maximum velocity (V_m) value were 3.33 % (w/v) and 781.90 mU/ml/mg of enzyme, respectively. Crude fraction and purified 70.8 kDa chitinolytic enzyme were assayed for toxicity against mosquito larvae. The LC_{50} of crude chitinolytic enzymes against 2nd instar of *Aedes aegypti* larvae was 60.67 mU/ml. These enzymes enhanced toxicity effect when assayed against mosquito larvae in combination with *Bacillus thuringiensis* subsp. *israelensis* strain 4Q2-72. The crude enzyme fraction alone was completely lost its larvicidal property upon heating at 100°C for 10 min. The LC_{50} of purified 70.8 kDa chitinolytic enzyme against 2nd instar of *Aedes aegypti* larvae was equal to 66.67 mU/ml.