Thesis Title	Purification and Characterization of Chitinase from *Bacillus* No.4.1
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ABSTRACT

Bacillus No. 4.1, a chitinase producer was identified to be *B. circulans*. The bacterium produced high level of chitinase when cells were grown in tryptic soy broth supplemented with 0.3% colloidal chitin at 35°C for 5 days. Its supernatant was separated by centrifugation and subjected to be purified by column chromatography. Purification was carried out by protein precipitation with 80% saturation ammonium sulfate, dialyzed with distilled water, and concentrated by lyophilization. The crude enzyme was purified by anion-exchange chromatography with DEAE-Sephacel and gel filtration with Sephadex G-100, sequentially. The

purified enzyme could be demonstrated as a single band on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight was estimated by SDS-PAGE to be 45 kDa. This enzyme could hydrolyze colloidal chitin, purified chitin, glycol chitin, CM-chitin and 4-methylumbelliferyl-β-D,N,N′-diacetylchitobioside [4-MU-$(GlcNAc)_2$]. In addition, the chitinase activity could be detected after separating by native gel electrophoresis when overlaid with agarose containing [4-MU-$(GlcNAc)_2$]. A bright purplish fluorescent band was detected under ultraviolet light. The optimum pH and the optimum temperature of this chitinase were found to be pH 8.0 and 40°C, respectively. The isoelectric point of chitinase was determined to be 5.1. The amino acid composition and the initial 20 amino acid residues of the N-terminal of chitinase from *B. circulans* No.4.1 was determined to be alanine (A), proline (P), tryptophan (W), asparagine (N), serine (S), lysine (K), glycine (G), asparagine (N), tyrosine (Y), alanine (A), leucine (L), proline (P), tyrosine (Y), tyrosine (Y), arginine (R), glycine (G), alanine (A), tryptophan (W), alanine (A) and valine (V), respectively.