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# 学 位 論 文 要 旨
# SUMMARY OF DOCTORAL THESIS

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題目 Title: **Purification and characterization of a novel chitinase from silkworm, *Bombyx mori* and observation its effects on Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) as biopesticide**

Chitinases are glycosylhydrolases that catalyze the random hydrolysis of the β-(1, 4)-glycosidic bonds in chitin, yielding *N*-acetylchitoologosaccharides. In insects chitinases are present in molting fluids, venom glands and midguts. Chitinases participate in the periodic shedding of old exoskeletons and the turnover of peritrophic membrane.

In this study a novel isozyme of chitinase (*Bm*-CHI) was purified from the integument extract of the fifth instar larvae of *Bombyx mori* L. (Lepidoptera: Bombycidae) and proved to be the fourth chitinase, by liquid chromatography on DEAE-Toyoperal 650 (M), hydroxlapatite, and Fractogel EMD DEAE 650 (M) columns. The purity of the isolated enzyme was confirmed as a single band by staining for both proteins and chitinase activity in SDS-PAGE gel, and the apparent molecular mass was estimated to be 75-kDa. The purified chitinase had an optimum pH of 6.0 toward the short substrate, *N*-acetylchitopentaose ($GlcNAc_5$), while in its reaction with long substrate, glycolchitin, it showed a pH of 10.0. The chitinase hydrolyzed *N*-acetylchitooligosaccharides except $GlcNAc_2$ as follows: $GlcNAc_6 > GlcNAc_5 > GlcNAc_4 > GlcNAc_3$ suggested that the 75-kDa chitinase is an endo or random type of chitinase and preferred the longer-chain substrates. The anomeric forms of the products of *N*-acetylchitooligosaccharides in the reaction with 75-kDa chitinase cleave the substrate to produce the β anomeric product at the reducing end site, suggesting that *B. mori* 75-kDa chitinase belongs to family 18 of glycosyl hydrolases.

Furthermore, we evaluated the insecticidic activity against the Japanese pine sawyer, *Monochamus alternatus* beetle (Coleoptera: Cerambycidae) (JSP). The bioassay results showed that the purified chitinase at concentration of 3 μM and 0.3 μM produced entomotoxic responses to adult JPS, and caused high mortalities, a significant decrease in the bark consumption and, in high concentration only, slight reduction of body weight. Fluorescence assay with chitin binding dye using fluorescence microscope indicated that chitin, which is an important structural component of the PM of JPS, was degraded due to the action of *Bm*-CHI of 3 μM concentration, when orally ingested. Observation with scanning electron microscope distinctly showed that the beetles which were ingested with *Bm*-CHI of the same concentration had their PM perforated and disrupted. However, brief ultrastructural studies showed that ingested enzyme did not affect the midgut epithelia. These findings open up the possibility to use insect chitinase as a biopesticidal protein and should have agronomic potential for insect control.