

Cloning, Expression, and Characterization of the Cellulase Gene of the Mulberry Longicorn Beetle, *Apriona germari*

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Cellulase cDNA was cloned from the mulberry longicorn beetle, *Apriona germari*. The cDNA encoding the cellulase of *A. germari* is 711 base pairs long with an open reading frame of 237 amino acid residues. The deduced protein sequence of the cellulase of *A. germari* showed 83.8% and 80.7% identity to *Phaedon cochleariae* and *Reticulitermes speratus* hindgut symbiont, respectively. The putative catalytic sites (-TTTRYWDCCKPSC-) are conserved in *A. germari* cellulase. Southern blot analysis of genomic DNA suggested the presence of the *A. germari* cellulase gene as a single copy and Northern blot analysis confirmed midgut-specific expression at the transcriptional level. The cDNA encoding the cellulase of *A. germari* was expressed as a 29-kDa band in the baculovirus-infected insect cells and the culture supernatants of the recombinant baculovirus-infected cells showed activity in the cellulase enzyme assay using carboxymethyl cellulose as a substrate. Furthermore, the cellulase enzyme assay exhibited high activity in only midgut tissue, evidencing the midgut is a site where large quantities of cellulase are synthesized for degrading the absorbed cellulose from the diet. The enzyme assay of the cellulase expressed in baculovirus-infected insect cells showed that the cellulase activity was most effective at 50°C and decreased at over 65°C when treated for 12 hrs.