A Novel Cellulase of the Mulberry Longicorn Beetle, *Apriona germari*: Molecular Cloning, Expression and Enzymatic Characterization

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We have previously cloned a cellulase [b-1,4-endoglucanase (EGase), EC 3.2.1.4] cDNA (Ag-EGase I) belonging to glycoside hydrolase family (GHF) 45 from the mulberry longicorn beetle, *Apriona germari*. We report here the cloning, expression and characterization of a novel cellulase from *A. germari*. The cDNA encoding a novel EGase of *A. germari* (Ag-EGase II) is 717 base pairs long with an open reading frame of 239 amino acid residues. The Ag-EGase II was closely related to another beetle, *Phaedon cochleariae*, cellulase and one symbiotic protist cellulase in the hindgut of the termite *Reticulitermes speratus*, those belonging to GHF 45. The putative catalytic sites of GHF 45 are conserved in Ag-EGase II. The Ag-EGase II has 14 conserved cysteine residues and three putative N-glycosylation sites. To identify the genomic structure of the Ag-EGase I and Ag-EGase II, furthermore, we designed a primer set based on the sequences of the Ag-EGase I and Ag-EGase II cDNAs. The genomic structure of the Ag-EGase I and Ag-EGase II spans 2,713 bp with three exons and 1,033 bp with three exons, respectively. Northern blot analysis confirmed midgut-specific expression at transcriptional level. Similarly, the Ag-EGase II enzyme assay exhibited high activity only in midgut tissue, suggesting that the midgut is the prime site where large quantities of EGase are synthesized for degrading the absorbed cellulose from the diet. The cDNA encoding Ag-EGase was expressed as a 36-kDa polypeptide in baculovirus-infected insect Sf9 cells and the enzyme activity of the purified recombinant Ag-EGase II expressed in baculovirus-infected insect cells was approximately 812 U per mg of recombinant Ag-EGase II. The enzymatic properties of the purified recombinant Ag-EGase II showed the highest activity at 50℃ and pH 6.0, and were stable at 60℃ at least for 10 min.