Distribution, Purification, Partial Characterization, Sequencing, Cloning and Expression of *Musca domestica* Chymotrypsin

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M. domestica chymotrypsin (MdChy) is mainly present in the posterior two thirds of posterior ventriculus in *M. domestica* larvae. MdChy was purified to homogeneity through one affinity cromatography onto PBA-Agarose and characterized, showing an pH optimum in the range of 7.5-8.5 and a K_m value of 1.65 uM for Suc-Ala-Ala-Pro-Phe-MCA. One partial sequence of chymotrypsin was obtained from *M. domestica* transcriptome. This sequence was completed, and the alignment of its amino acid sequence with bovine chymotrypsin shows that the MdChy has the conserved catalytic triad His-Asp-Ser of serine proteinases. Specific primers for MdChy were designed and it was cloned from a *M. domestica* cDNA library onto pAE shuttle vector, which adds a N-terminal His-tag to the expressed protein. Successful expression tests were performed in *E. coli* strains BL21 Star (DE3) induced by 1mM IPTG at 37ºC. Western blot analysis using anti-His-tag antibody confirmed that MdChy is best expressed within three hours, being degraded afterwards. Anti-MdChy antibody production and MdChy biochemical characterization are in progress.

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