MOLECULAR CLONING AND PARTIAL CHARACTERIZATION OF THREE ASPARTIC PROTEINASE FROM MUSCA DOMESTICA LARVAE

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Aspartic proteinases are key digestive enzymes in larval flies and some peetles. Knowledage on these enzymes is scarce and for this a study, was undertaken with our fly model, Musca domestica. cDNAs coding for three procathepsin-D (AspMDs - Aspartic proteinase from Musca domestica - AspMD01, AspMD02 and AspMD03) were cloned and sequenced from a cDNA library prepared from Musca domestica larval midguts. The strategy consisted in using an oligonucleotide primer designed with hybridize to the region encoding the consensus catalytic site D₃₂TGSSNLW (Harrop et al., 1996) of aspartic proteinase. The deduced proteins have a signal peptide, two conserved aspartic acid residues that are characteristic of aspartic protease active site and their sequences are homologous to cathepsin-D enzymes. The cDNA fragment coding for AspMD01 was cloned into a pAE vector (Ramos et al., 2004) and expressed in E. coli. The majority of the expressed recombinant proteins was present in inclusion bodies. Sequences trees of cathepsin D sequences from insects and a for other organisms was prepared, including the *Musca domestica* sequences. AspMD03 was most closely related to lysosomal enzymes of insects. AspMD01 and AspMD02 sequences branch alone and are more derived than the other sequences. They are obvious candidates to be digestive enzymes.

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