

Cloning, Expression, Characterization and Distribution of a Lipolytic Enzyme
from *Musca Domestica* Midgut Larvae

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The house fly, *Musca domestica* is one the best known and most widely distributed insects known to humans. A lipolytic enzyme (LipMD) was identified from expressed sequence tags (EST) constructed from midgut larvae cDNA library. The deduced amino acid sequence is homologous to lipase enzymes and contained three, well-conserved amino acid residues, Ser¹⁸³, His²⁷³, and Asp²⁰⁸, which composed the catalytic triad of the enzyme. The cDNA fragment coding for LipMD was cloned into a pAE vector (Ramos et al., 2004) and expressed in *E. coli* produced an enzyme with a molecular weight of 37.3 kDa. The recombinant Lip06MD was purified and was able to hydrolyse tributyrin and a broad range of substrates, from C₂ to C₁₈ p-nitrophenyl esters and displayed an optimal pH of approximately 7.5. RT-PCR analysis in tissue homogenates (anterior, middle and posterior midguts, hemolymph, fat body and Malpighian tubules) showed that LipMD mRNA transcripts were expressed only in anterior midgut. Western-blotting after SDS-PAGE of proteins from different tissues and stained with anti-LipMD serum revealed that translation occurs mainly in the anterior midgut lumen. This is candidate to be digestive lipolytic enzyme in that midgut region.

Key words: insect, digestive, lipolytic enzyme

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