IN SILICO ANALYSIS, MOLECULAR CLONING AND DIFFERENTIAL TISSUE EXPRESSION OF CATHEPSIN D FROM DYSDERCUS PERUVIANUS (HETEROPTERA)

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Trypsin and chymotrypsin are enzymes widely distributed among insect taxa and act, in most cases, as the major digestive endopeptidases. However, there are remarkable exceptions that deserve our attention. As a rule, cathepsin activity is restricted to the insect midgut lysosomes. But, the presence of acid enzymes, such as aspartic peptidases, actually cathepsin D, acting in luminal digestion is now widely accepted. Digestive cathepsin D lacks a consensus sequence, which is characteristic of lysosomal cathepsins D and may be used to separate lysosomal from non-lysosomal cathepsin Ds.

Given the possibility of distinguish typically lysosomal from extracellular cathepsin D of Dysdercus peruvianus, the aim of this work is to describe cathepsin D identities, features, and sites of expression.

A 454 transcriptome from three D. peruvianus tissues generated the data for cathepsin D screening. Ten sequences called DpCatD1 to 10 were found. Two selected sequences (DpCatD1 and 10) were cloned in PGEMT and PAE plasmid.

DpCatD2, 3, and 8 do not present the conserved catalytic residues and are considered as non active. The others are probably active enzymes and present at least one N-glycosylation site. DpCatD1 is the most expressed, considering the reads counting. Comparing all sequences, only DpCatD10 presents the consensus sequence of lysosomal cathepsin D and also is the only one expressed in non-digestive tissues. DpCatD10 branches in a phylogenetic analysis within lysosomal lineages of cathepsin D. So, DpCatD10 is a good candidate to be a lysosomal protease.

This analysis indicates that D. peruvianus, and probably other Heteroptera, kept a gene responsible for standard cathepsin D expression in all tissues and evolved another set of genes with expression restricted to midgut.

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