

Cloning and Functional Studies of Intestinal Peptidases from a ESTs Database of the Sugarcane Weevil *Sphenophorus levis*.

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Among the pests that damage crops of sugar cane is the larva of the beetle *Sphenophorus levis*, popularly known as the boll weevil from sugarcane. The larvae of this insect feeds from the rhizome of sugarcane, causing large damage to the crop decreasing productivity and causing death of plants. The lack of an efficient method to control this pest and total lack of genomic information reinforces the need of studies for this species, which attacks one of the most important crops of Brazilian agribusiness. Considering the feeding and development of these larvae are directly related to the digestive proteolysis in the gut of the insect, the objectives of this study are the isolation, characterization and heterologous expression of intestinal peptidases of *S. levis*. To achieve these goals we built a cDNA library from *S. levis* larvae gut and sequenced hundreds of clones. The analysis of the sequences allowed the identification of various digestive enzymes from insect as cathepsin (L, B and D), glycosyl hydrolases and salivary proteins. Considering that the majority cathepsins found in the larvae transcriptome are cathepsin L-like, we chose a clone for expression in the methylotrophic yeast *Pichia pastoris* containing the alpha factor in the N-terminus that guides the production of the protein directly to the culture medium. The recombinant enzyme of 41 kDa containing a His-Tag peptide in the C-terminus was highly expressed (240 mg per liter of culture) and was easily purified by chromatography affinity. The recombinant enzyme was subjected to kinetics studies and tests of inhibition with recombinant sugarcane cystatins produced in our laboratory (CaneCPIs).

Keywords: *Sphenophorus levis*, sugarcane, phytopathogens, coleopterous, cysteine peptidase.

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