

Purification and Characterization of a Midgut Cysteine Peptidase from the Sugarcane Weevil *Sphenophorus levis*

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Sugarcane is today one crop of great importance for several countries, especially Brazil. However, the culture can suffer productivity losses when attacked by insects. The *Sphenophorus levis* is one of the most important pest of this culture. Therefore, it is necessary to develop efficient methods to control this insect. Inhibition of digestive peptidases of the insect can be an efficient procedure to impair the insect development. The objectives of this work are purification, characterization of peptidases present in the midgut of *Sphenophorus levis* and inhibition of activity enzyme. The purification of *Sphenophorus levis* enzymes was accomplished through ionic exchange chromatography. Experiments of Mass Spectrometry resulted in peptides that match with cysteine peptidases from ESTs obtained from a *S.levis* cDNA library. Kinetic studies demonstrated the enzyme specificity for the substrates Z-Phe-Arg-MCA and Z-Leu-Arg_MCA (K_{cat}/K_m values of 20 and 30 μM), respectively. Inhibitory activity assays revealed the behavior of enzyme as a cysteine peptidase. The enzyme was efficiently inhibited by the specific cysteine peptidase inhibitor E-64 and also by the sugarcane recombinant cystatins CaneCPI-1, CaneCPI-2, CaneCPI-3 and CaneCPI-4 ($K_i = 0.59, 0.36, 0.57$ and 0.02 nM, respectively). These studies may contribute to understating the interaction of cysteine peptidases from *Sphenophorus levis* with inhibitors from sugarcane, and also suggest the potential use of these inhibitors for the production of transgenic sugarcane resistant to this insect.

Key-words: *Sphenophorus levis*, cysteine peptidase, proteinase inhibitors

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