

Colorado potato beetle (*Leptinotarsa decemlineata*) is an economically important herbivorous pest. Cathepsin D-like aspartic peptidase (LdCD) plays an important role during protein degradation in the midgut of Colorado potato beetle. This work describes the preparation of two expression systems, namely in *Escherichia coli* and *Pichia pastoris*, for the production of recombinant LdCD. The protocol for refolding of denatured LdCD was designed and optimized. Activation of the inactive LdCD zymogen and cleavage of the propeptide (activation peptide) were investigated. This process proceeds autocatalytically at acidic pH or with the assistance of the cysteine peptidase legumain. The proteolytic activity of LdCD was characterized using fluorogenic peptidic substrate and protein substrates, and kinetic parameters and pH optimum were determined. The inhibition specificity of LdCD was analyzed using a panel of peptidase inhibitors. LdCD was significantly inhibited by PDI (potato cathepsin D inhibitor), a protein inhibitor produced in potato leaves. This suggests that PDI is a natural defense protein, which is directed against LdCD in the midgut of Colorado potato beetle in order to block the digestion. The potential application of PDI in the construction of transgenic crops resistant against insects is discussed.