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MIDGUT PROTEINASE ACTIVITIES OF CERAMBYX CERDO (COLEOPTERA, CERAMBYCIDAE) LARVAE

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Different proteolytic activities using natural and synthetic substrates were analyzed in the midgut of *Cerambyx cerdo* larvae. Significant leucyl-aminopeptidase and elastase-like activities, as well as some trypsin-like activities were detected.

KEY WORDS: Cerambycidae, *Cerambyx cerdo*, proteinase activitiy, chromogenic substrates

There are some literature data about the proteinase of *C. cerdo* (IVANOVIĆ & MILANOVIĆ, 1970) , also from the view of metabolic response to different diets (NENADOVIĆ *et al*., 1999) and temperatures (NENADOVIĆ *et al*., 1982; 1994), but there are no data about the specific proteinase class. This is of great interest in last years, especially because it has been shown that some Cerambycidae have both, serine- and cysteine proteinase what is not common for Coleoptera. Among serine proteinases, trypsin- and chymotrypsin-like enzymes have been most frequently detected in Coleoptera. Cysteine proteinases also have been found (WIEMAN & NIELSEN, 1988) and among metallo-proteinase, aminopeptidase are the most frequently identified (NOVILLO *et al*., 1997). Leucyl-aminopeptidase is the most abundant digestive enzyme in the midgut of cerambycid beetle *Morimus funereus* larvae (BOŽIĆ *et al*., 2003). Characterization of digestive proteinase could lead to some elucidation concerning larval growth, development, dispersion and evolution, having in mind law abundance of proteins in wood.

In this work, larvae from the environment, collected in December from Klenak, were used. Midguts were dissected out, weighed and homogenized with a

pre-chilled mortar and pestle in 4. vol. (g/mL) of ice-cold, 50 mM Tris buffer, pH 7,5 with the addition of quartz sand. After the centrifugation resulting supernatants were treated with an equal volume of carbon tetrachloride for lipid removal, followed by centrifugation (BOŽIĆ *et al*., 2003). In each extract protein concentration was determined (BRADFORD, 1976).

Total proteolytic activity was assayed by using 1% casein and 1% gelatin in appropriate buffers. Reaction mixtures contained 10 μL of crude midgut extracts and 1 mL casein or gelatin solution in buffer. The reaction was terminated after 2 h at 37^{0}C by adding 0,5 mL 15% TCA. The content of free amino groups in the supernatants was determined using TNBS method as was described (BOŽIĆ *et al*., 2003). Data are the means of triplicate measurment. Standard errors were within 5% of the means.

The activities of trypsin-like, chymotrypsin-like, elastase-like enzymes and leucyl-aminopeptidase activity were determined using specific chromogenic substrates (LEE &ANSTEE, 1995). Reaction mixtures contained 5 μL of crude midgut extracts in 0,5 mL of appropriate buffer and 1 mM substrates dissolved in DMF. Reaction times for the substrates were: 10 min for LpNA, 60 min for SAAPLpNA, 100 min for BApNA and 210 min for BTpNA at 30^{0}C. After that enzymatic reac-

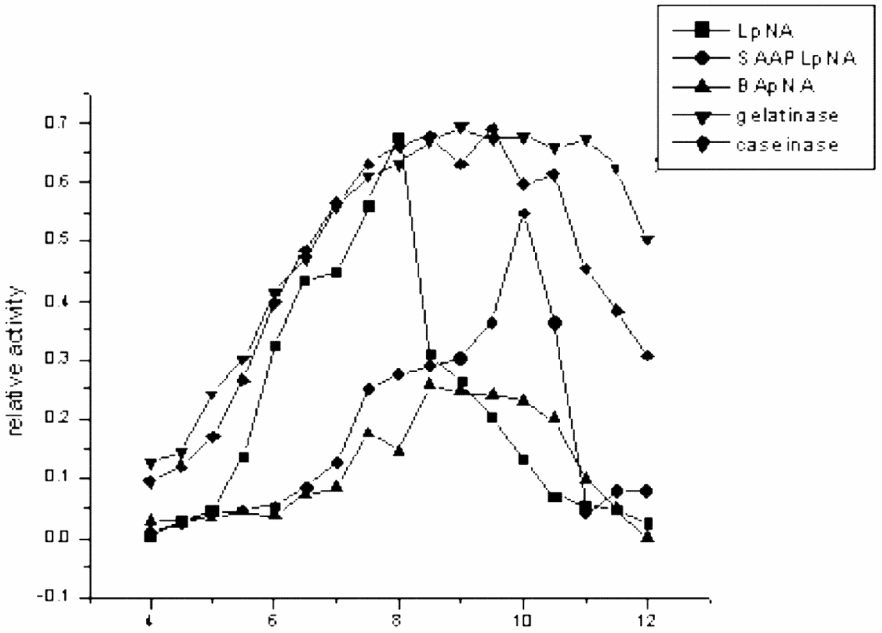

Fig. 1. Midgut proteinase activities of *Cerambyx cerdo* (Coleoptera, Cerambycidae) larvae

tions were terminated by adding 0,1 mL 30% acetic acid. The concentration of the resulting p-nitroaniline was estimated by measuring the apsorbance at 410 nm (ERLANGER *et al*., 1961).

To determine the pH optimum of different enzyme activities against specific chromogenic substrates a series of 50 mM buffers in the pH range from 4,0-12,0 were used (acetate, pH 4,0-6,5, phosphate I, pH 6,5-8,5, borate, pH 8,0-10,5 and phosphate II, pH 10,0-12,0). Incubation of substrates only, indicated that buffers did not induce autohydrolysis. Results are shown in Figure 1.

Application of specific chromogenic substrates enabled us to identify several proteinase classes in the midgut extract of *C. cerdo* larvae. Among the metalloproteinases, LAP was detected. Analysis of serine proteinase showed elastase-like activities and less trypsin-like activities, but no chymotrypsin-like activities. These data agree with the reports on some other Coleoptera (CHRISTELLER *et al*., 1989; WAGNER *et al*., 2002; BOŽIĆ *et al*., 2003). To be sure that there are no aspartic and cysteine proteinase activities in the midgut of *C.cerdo* larvae, some other studies whit inhibitors need to be done.

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ПРОТЕИНАЗНЕ АКТИВНОСТИ СРЕДЊЕГ ЦРЕВА ЛАРВЕ *CERAMBYX CERDO* (COLEOPTERA, CERAMBYCIDAE)

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Коришћењем природних и синтетичких супстрата анализиране су различите протеолитичке активности у средњем цреву ларве *Cerambyx cerdo*. Детектоване су значајне активности леуцил-аминопептидазе и еластази сличних ензима, као и слабија активност трипсину сличних ензима.

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