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Comparative Study on the Characteristics of Midgut Protease in Different Multivoltine Races of Silkworm, *Bombyx mori* L.

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ABSTRACT : Proteolytic activity from the midgut of the lepidopteran larvae *Bombyx mori* was studied in relation to compare the characterization in three different multivoltine races, Pure Mysore, Nistari and Kolar gold. The optimum pH for the midgut protease was 11.2 in Pure Mysore and Nistari, while it was 11.0 in Kolar gold. The temperature optimum for Pure Mysore was 50°C, while it was 45°C for Nistari and Kolar gold. Incubation of the gut protease for 15 minutes was found as a linear time period in Nistari while that of 20 minutes in Pure Mysore and Kolar gold. The 50% reduction in activity at 55°C, for 7.3 minutes was found in Nistari, 9.6 minutes for Pure Mysore and 10.7 minutes for Kolar gold. Km values recorded were 0.278%, 0.167% and 0.222% in Pure Mysore, Nistari and Kolar gold respectively. The specific activity of Pure Mysore was 0.611 µg tyrosine/µg protein/h; Nistari was 0.916µg tyrosine/µg protein/h, while that of Kolar gold was 0.546µg tyrosine/µg protein/h.

Keywords : Midgut protease, Pure Mysore, Kolar gold, Nistari.

INTRODUCTION

Enzymes responsible for the complete hydrolysis of proteins down to amino acids are the proteases. The proteolytic activity of the alimentary canal in relation to feeding or proteins was studied in many insects (Dadd, 1956; Engelmann, 1969; Hamano and Mukaiyama, 1970; Persaud and Davey, 1971; Briegel and Lea, 1975). Proteases of the midgut epithelia have long been recognized to contain peptidases mainly and scarcely any protease activity (Shinoda, 1930; Horie *et al.*, 1963). In studies on midgut proteases of the pharate adult of *Bombyx mori* (Eguchi *et al.*, 1972) and *Antheraea pernyi* (Eguchi and Iwamoto, 1973), several lines of evidence suggested that midgut protease of the pharate adult is utilized as a source of the cocoon digesting enzyme. The midgut epithelial cells are protected by an extracellular sheath, the peritrophic membrane. This structure is generally supposed to protect the midgut cells from mechanical damage caused by abrasive food particles, but little is known about the function of this thin membrane. The occurrence of proteolytic activity in the peritrophic membrane has been demonstrated (Yamazaki, 1955; Smith, 1968; Wigglesworth, 1972; Nishida & Hayashiya, 1974b; Peters, 1976; Richards & Richards, 1977). In the silkworm, *Bombyx mori*, the presence of intestinal proteinases has been reported by Shinoda (1930), and several investigations have been done mainly in Japan (Horie *et al.*, 1963; Eguchi and Yoshitake, 1967; Hamano and Mukaiyama, 1970; Nishida and Hayashiya, 1974). Jadhav and Kallapur (1988) were studied the influence of age, sex and feeding on the protease activity of certain tissues of fifth instar silkworm *Bombyx mori.* Kangayam *et al.*, (1999) studied effect of alkalinity and protease in the digestive juice of silkworm, *Bombyx mori* on BmNPV infection. Rawling and Barrette (1993) proposed an evolutionary scheme for protease based on amino acid sequence data for 600 of these enzymes. Regulation of digestive proteolytic activity in the larvae of Spilosoma obliqua was studied by Anwar *et al.*, (2001). Bharati *et al.*, (2006) were studied impact of prolactin on day to day changes in the protease activity in the midgut of fifth instar silkworm.

The present communication deals with the comparison of characteristics of enzyme activities of proteases from the midgut tissue in different multivoltine races i.e. Pure Mysore, Nistari and Kolar gold and discusses the physiological significance of the results.

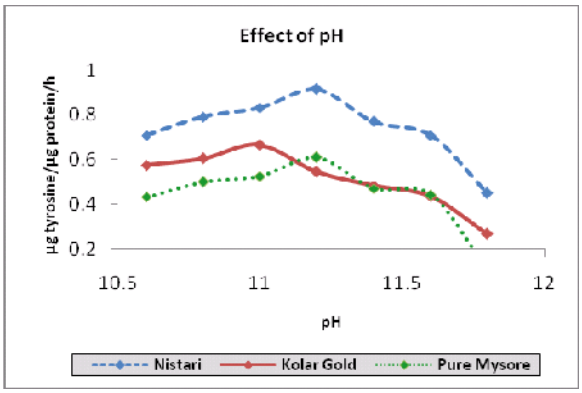
MATERIAL AND METHODS

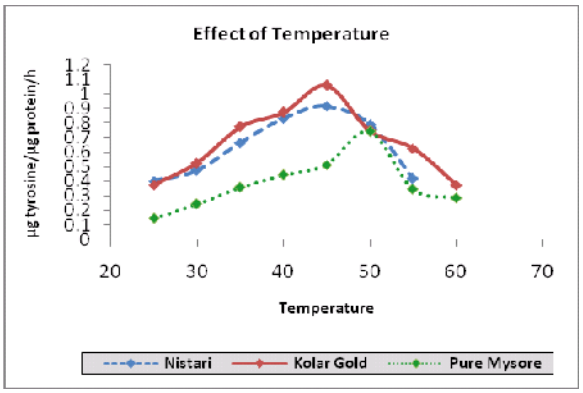
The DFLs of multivoltine pure race of *Bombyx mori* i.e. Pure Mysore were procured from Directorate, Sericulture, Govt. Grainage Centre, Ganhinglaj, Dist. Kolhapur, Maharashtra, India; Nistari were procured from Central sericulture Germplasm Resource Centre, Hosur, Krishnagiri District, Tamil Nadu, India while that of crossbreed multivoltine race i.e. Kolar gold were procured from District Sericulture Grainage Centre, Shahupuri, Dist. Kolhapur, Maharashtra, India and larvae were reared as per the recommended regimen of Krishnaswami (1978, 1979) in the rearing house of the Department of Zoology, Shivaji University, Kolhapur on the fresh leaves of Morus alba.

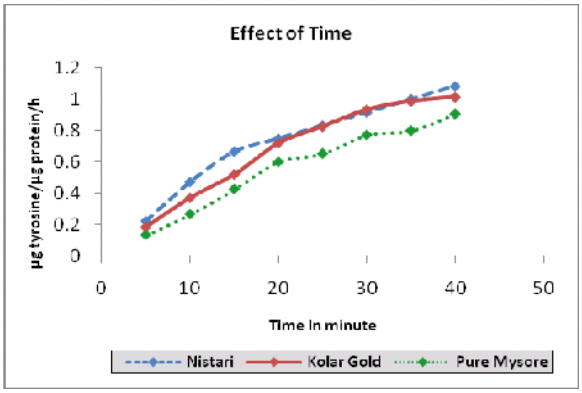
The fifth instar larvae of all the races were dissected in chilled insect ringer. The mid gut was homogenized in 0.8% saline. The homogenates were centrifuged at 3000 rpm for 15 minutes and supernatants were used for characterization

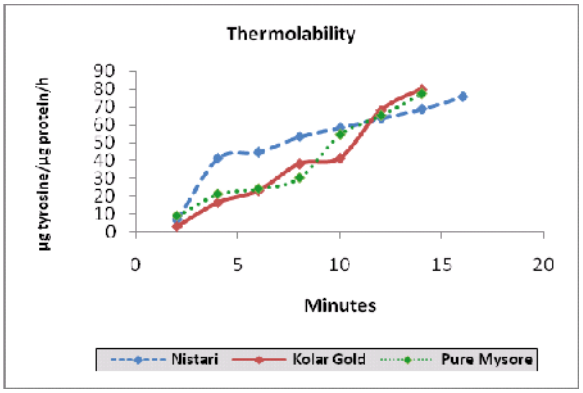
of protease. The reaction mixture contains 1% casein, 5 ml appropriate buffer, 1 ml supernatant. Incubation was done at 45°C for 15 minutes and reaction was terminated by adding 6% TCA. The precipitate formed is then centrifuged and the aliquot was used. The 2ml of solution is taken from it and diluted with 5 ml 2N NaOH then 1 ml of phenol reagent is added and colour developed was measured at 660nm.

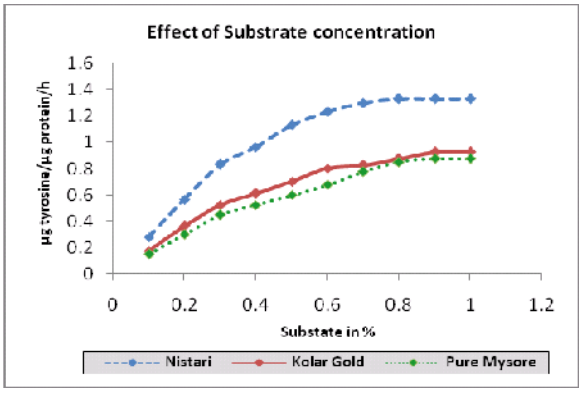
Optimum pH was determined by using appropriate buffers. To determine the optimum temperature, the reaction mixture was incubated for 15 minutes in case of Nistari, while for 20 minutes for Pure Mysore and Kolar gold at various temperatures ranging from 25–60°C. Km values were determined by using 1% casein and the concentration of the substrate varied from 1 to 10 mg/ml. To determine thermolability, for Pure Mysore enzyme extract was subjected to 60°C treatment, while for Nistari and Kolar gold it was subjected to 55°C in water bath for different periods of time. One tube of enzyme extract was stored at 5°C until needed, which served as control. After treatment, tubes were cooled in ice–cold water. The residual protease activity was described as usual. The mean activity of control was taken as 100% activity. For each experiment the values are the mean of three observations.











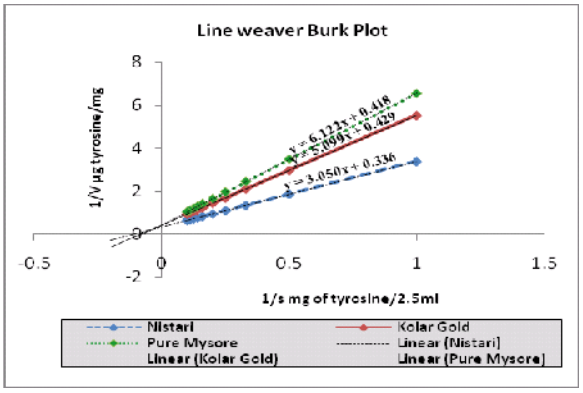


Fig. 1–6. Characteristics of midgut protease in Pure Mysore, Kolar gold and Nistari.

The protein concentration of enzyme extract was determined as per Lowry *et al.*, (1951).

RESULTS AND DISCUSSION

The effect of pH on the proteolytic activity is represented in Fig. 1 in which two races i.e. Pure Mysore and Nistari showed optimum activity at pH 11.2, which is similar to the result reported by Kangayam *et al.*, (1999) in *Bombyx mori* while Kolar gold showed the optimum activity at 11.0. In case of *Spilosoma obliqua* also there is presence of alkaline protease i.e. pH 11.0 (Anwar and Saleemuddin, 2001). The presence of alkaline proteases in the guts of lepidopteran larvae has been well documented (Ishaaya *et al.*, 1971; Ahmed and Saleemuddin, 1980; Kloke and Chan, 1982; Hamed and Attias, 1987; Johnston *et al.*, 1991; Christeller *et al.*, 1992; Saumels *et al.*, 1993).

The proteolytic activity was also assayed at different temperatures (Fig. 2) ranging between 25°C and 60°C. The results obtained from the study in the present insect suggest that the proteolytic activity peaked at 45°C followed by a decline in activity with the increase in temperature in Nistari and Kolar gold while it was found at 50°C in Pure Mysore. In case of *Spilosoma obliqua* the peak observed at 40°C (Anwar and Saleemuddin, 2001). In Fig. 3, time course of the proteolytic activity is depicted. The reaction product increases almost linearly within 15 min in Nistari while 20 minutes in Pure Mysore and Kolar gold.

The heat stability in the midgut protease is compared in Fig. 4, which shows that there is 50% loss activity when enzyme is heated at 60°C for 9.6 minutes in pure Mysore while it was at 55°C for 7.3 minutes in Nistari and 10.6 minutes in Kolar gold. In *Spilosoma obliqua* when enzyme is heated at the same temperature for 40 minute, about 65% activity still remained (Anwar and Saleemuddin, 2001). The relationship between sucrose concentrations and rate of hydrolysis is shown in Fig. 5. Lineweaver Burk plot was employed by using regression equation $Y = ax + b$ (Fig. 6) and the regression line obtained were $Y = 6.122x + 0.418$ for Pure Mysore; $Y = 5.099x + 0.429$ for Kolar Gold and $Y = 3.050x + 0.336$ for Nistari. The Km values obtained for Pure Mysore was 0.278%; for Nistari 0.167% and in case of Kolar gold it was 0.222%, which shows Nistari having efficient proteolytic activity than Kolar gold and Pure Mysore. These results indicates that protease is more efficient in Nistari than Kolar gold and Pure Mysore, because it shows lower Km value.

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