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Research Article

Proteases from dump gut excreta of wild tasar *Antheraea mylitta*

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Abstract

In the present study wild tasar silkworm *Antheraea mylitta* dump gut excreta proteases were studied. Proteases were detected by Dot blot assay on X-Ray film and by Zymographic methods. Molecular weight of major proteases was determined by SDS PAGE. The approximate molecular weight of these proteases ranged between 45-60 kDa which is similar to serratiopeptidase. This seems that the proteases found in the gut of *Antheraea mylitta* may show activity like serratiopeptidase.

INTRODUCTION

Antheraea mylitta is a natural fauna of tropical India which is distributed in different geographical locations. Possibly because of their distinct ecological conditions in that particular locality, several morphological variants called ecoraces have been identified in *Antheraea mylitta*. As high as 44 ecoraces of this species has been reported which primarily feed on *Terminalia species, Shorea robusta* and also on a number of secondary plants (Jolly *et al.*, 1974). Depending on geo-ecological conditions the ecoraces are uni, bi or trivoltine and differ from each other with respect to their qualitative and quantitative traits (Srivastava *et al.*, 2002). It is one of the commercial exploited silkworm reared outdoor (Fletcher, 1914). Daba and Sukinda along with a little of Jata are semidomisticated and commercially applied ecoraces in India. (Hansda *et al.*, 2008; Ojha *et al.*, 2009). Cocoons of tasar are reported to be the largest among all silk producing insects in the world (Akai, 2000). Digestive amylases of *Antheraea mylitta* are purified and characterized (Nagaraju and Abraham, 1995). But it seems that proteases from the drained out gut have not been yet studied from this commercially important insect. Hence present study is focused on the proteases from the drained out gut of *Antheraea mylitta.*

MATERIALS AND METHODS

Experimental insects

The Eggs of wild silkworm, *Antheraea mylitta* were collected from Sarsam (Bk), village, Dist. Nanded Maharashtra, India and were maintained under optimum environmental conditions (25 ± 3^0C until hatching. After 8^{th} days these eggs were hatched. After eggs hatching, newly emerged larvae were reared on wild almond leaves collected from the campus of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

Collection of drained out gut from *Antheraea mylitta* and study of protease activity

Before cocoon formation insect drain out the gut. This drained out gut was collected in falcon tubes. The protease activity was studied by using Dot blot method (Pichare and Kachole, 1994).

This drain out gut was stored in the deep freezer at -20^{o}C.

Zymographic analysis

Casein, Gelatin, Collagen, Gliadin and Cocoon (dissolved in Glycine-NaOH buffer pH 9.5) were used as substrates for zymographic analysis. In gel activity analysis of the gut proteases was performed by using 10 % SDS polyacrylamide gel electrophoresis. The electrophoresis was carried out according to the procedure of Laemmli. 10% SDS Acrylamide gel was co-polymerised with addition of 0.1% casein, 0.1% gelatine type A (porcine pancreas), 0.1% gliadin in 60% ethanol, 0.1 % cocoon and 0.1% collagen. After electrophoresis the renaturation was carried out by washing the gel with 2.5% triton-X 100 for 1 hr. Then the gel was extensively washed with deionised water followed by incubation in activation buffer (Glycine-NaOH 10mM, pH 9.5) at 37^{0}C for overnight. The gels were stained with Coomassie brilliant blue R-250 and the zones of proteolysis were detected.

Molecular weight determination by SDS PAGE

For molecular weight determination the dump gut excreta and molecular weight markers were resolved on 10% separating Acrylamide gel (pH 8.8) and a 5% stacking gel (pH 6.8) containing 0.1% SDS. Electrophoresis was performed using Tris–Glycine buffer (pH 8.3) polyacrylamide gel. The electrophoresis was carried out according to the procedure of Laemmli. After electrophoresis the gel was stained with silver staining. The molecular weight of the enzyme was estimated by comparing its Relative Mobility (RF) value with the molecular weight markers.

RESULTS AND DISCUSSION

Tasar (Tussah) is a crude form of silk which is usually used for furnishings and interiors. It has its own feel and appeal less lustrous than mulberry silk. Tasar silk is produced by the silkworm, *Antheraea mylitta* which mainly thrive on the food plants Asan and Arjun. Besides Maharashtra, West Bengal and Andhra Pradesh tasar silk is mainly produced in the states of Jharkhand, Chhattisgarh and Orissa, in India. (Suryanarayana et al.,2005; TNAU Agritechportal, Sericulture, Government of Tamilnadu, India).

Laboratory hatching and rearing stages are summarized in figure no 1.

Fig.1A] Early stage larvae feeding on wild

Fig.1B] Adult larvae feeding on wild Almond leaves. Almond leaves.

Fig.1C] Collection of dump gut excreta

Fig.1D] Early stage of cocoon formation

Fig.1E] Progress in cocoon formation

Fig1F] Cocoon formation

Fig.1G] Cocoons

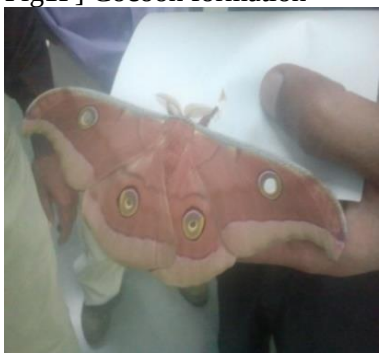
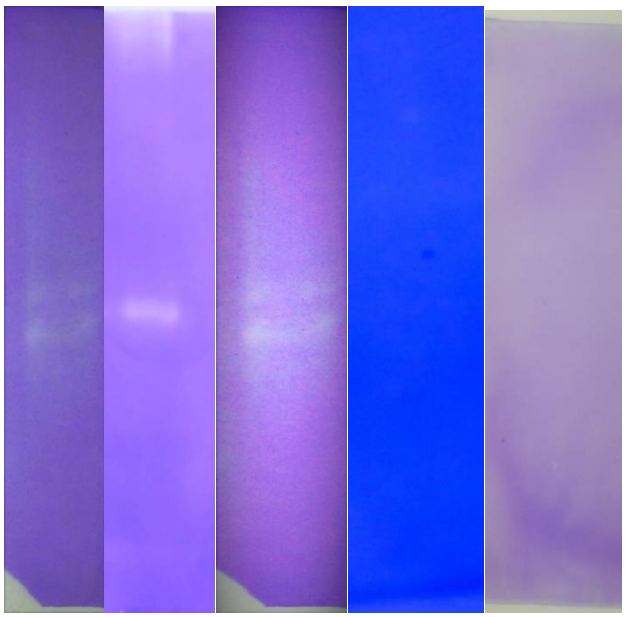


Fig.1. H] Moth

Fig1. In laboratory rearing of *Antheraea mylitta from egg to moth.*

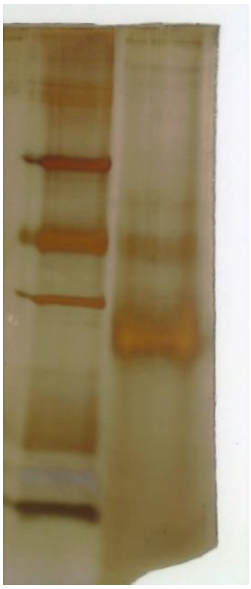
Zymographic study:

Zymographic analysis of the gut excreta proteases showed casein, cocoon and gelatin hydrolysis at positions that correspond to molecular weight range between 45 to 60kDa (Fig.2. A, B, C) respectively. However, no zymogram was detected in gels where gliadin and collagen were incorporated as substrates. Hence there is no gliadinase and collagenase activities in the dump gut excreta.



A B C D E

Fig.2. Zymographic analysis of *Antheraea mylitta proteases with different substrates.*
A: Casein, B: cocoon, C: Gelatin, D: Collagen and E: gliadin



A B

Fig.3. SDS-PAGE silver staining protein profile of *Antheraea mylitta proteases with standard molecular weight markers. A- Standard molecular weight markers. B- Antheraea mylitta dump gut proteases.*

Among the three largest groups of industrial enzymes, proteases account for about 60% of total worldwide sale. In the late 1960 serratiamarcescens E15 was isolated from intestine of Silkworm *Bombyx mori* .Silkworm use the enzyme to dissolve the cocoon. It is found that it secrete a potent proteolytic enzyme named as serratiopeptidase its origin is from serratiamarcescns. (Alan and Rebekan, 2011; Ammar *et al.*,1998). Serratiopeptidase have multiple applications in medicine showing anti-inflammatory activity which reduces swelling by decreasing the amount of fluid in the tissue, thinning the fluid and by facillating the drainage of fluid. In addition to this it also dissolves dead tissues surrounding the injured part and accelerates the healing process (Kee et al., 1989; Klein and Kullich, 2000). Serratiopeptidases also exhibit analgesic activity which helps to alleviate pain by inhibiting the release of pain inducing amine like bradykinin from inflamed tissues (Mazzone *et al.,* 1990). Furthermore fibrinolytic activity of serratiopeptidase is beneficial in atherosclerotic disease because it acts by breaking down fibrin and other damaged tissues without harming the living tissues which could help to dissolve blood clots and atherosclerotic plaques (Bhagat *et al.,* 2013; Brewer science library website, 1999). The molecular weight of this enzyme was reported to be 45-60 kDa consisting of 470 amino acids (Nakahama et al., 1986). Though this is very preliminary study, the detailed study of these proteases may prove its significance. These proteases are from dump gut, which is not used in silk production. So this waste can become a best if we could explore proteases from this dump. Another beauty of this dump is that all proteins present show protease activity; hence need not to separate it from other proteins.

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Conflict of interest

The authors declare no conflict of interests.

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