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Electrophoretic Patterns of Esterases in Eri silkworm *Samia Cynthia ricini*

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Abstract: *The present study was carried out to investigate the patterns of esterase isozymes extracted from the silk gland, haemolymph and mid gut of Eri silkworm (Samia Cynthia ricini). The qualitative analysis of esterases was carried out by 7.5% of native Polyacrylamide Gel Electrophoresis (PAGE). The inhibitor sensitivity of the enzymes towards paraxon, eserine and pCMB was used to classify the individual zones of esterases. Three zones of esterases were observed in different tissues of Eri silkworm. Silk gland esterases were classified as CHsp (Cholinesterase like enzymes) esterases. The haemolymph and mid gut esterases were classified into Esdp (Enzyme inhibited by paraxon and pCMB).*

Key words: *Eri silkworm, Electrophoresis, Esterases.*

I. Introduction

The sericulture is playing an important role in the economic life of man [1]. India is the largest producer of silk and also the largest consumer of silk in the world. It has a strong tradition and culture bound domestic market of silk and the unique distinction of producing all the four commercial varieties of natural silks i.e. Mulberry, Tasar, Eri and Muga. Eri culture is a household activity practiced mainly for protein rich pupae, a delicacy for the tribal. Resultantly, the Eri cocoons are open-mouthed and are spun. The silk is used indigenously for preparation of *chaddars* (wraps) for own use by these tribals. The Eri silkworm is raised especially in Assam, West-Bengal, Manipur, Bihar, Orissa, Nagaland, Meghalaya, Karnataka, Andhra Pradesh and Tamil Nadu states. *Samia Cynthia ricini* is belonging to the order Lepidoptera and family saturniidae is a domesticated multivolatile and polyphagous insect feeding on castor leaves. [2]. Taking this into consideration, some enzymological work was under taken with special reference to esterases.

Esterases are a group of hydrolytic enzymes occurring in multiple forms with broad substrate specificity. Esterases comprise a diverse group of enzymes catalyzing the hydrolysis of organic esters. Esterases (EST, 3.1.1.2) are ubiquitous in living organisms. Several esterases have been isolated from various tissues of microbes, plants and animals and investigated for their biochemical properties [3, 4]. They have been found widespread in insect species and function in the digestion of nutritional materials, detoxification of xenobiotics [5, 6, 7], and in reproduction [8]. Insect esterases are classified according to their reactions to different substrates. Use of α- or β-napthylacetate, a hydrolyzing substrate, for nonspecific esterases (α and β) have been reported by several workers [9, 10]. Some authors have used esterases as genetic markers for studying enzyme polymorphism in different insect species [11, 12, 13, 14, 15].There is also the possibility of esterases showing post-translational modifications and formation of hybrid polymers. The band pattern also exhibits profound variation with varying electrophoretic conditions [16]. As a consequence of these problems, use of inhibitor techniques and substrate specificity studies becomes inevitable for characterization and genetic interpretation of esterase zymograms. So far no work has been done on the esterases of the Eri silkworm and the present investigation deals with the characterization of the tissue esterases in order to understand their possible role.

II. Materials & Methods

2.1 Animal materials

The Eri silkworm **(***Samia cynthia ricini)* V^{th} instar larvae with maximum mature larval weight fed on local castor variety are chosen for the study. The larvae after 2 days of attainment of V^{th} instar are collected for the experiment.

2.2 Extraction and collection of samples

The haemolymph was collected into prechilled eppendorf tubes by cutting the prolegs of the larvae. Silk gland is obtained by dissecting the larvae and midgut is isolated and collected into prechilled eppendorf tubes and weighed to the nearest milligram. The samples were homogenized in 10% 0.01M Tris-HCL buffer (pH 7.4) containing 0.9% NaCl. The homogenate was centrifuged at 2000 rpm in a clinical centrifuge at room temperature (30±2).

2.3 Experimental procedure for preparation of native gels

The supernatant was mixed with equal volumes of 20% sucrose containing 0.01% bromophenol blue as tracking dye. An aliquot of 0.1ml of this solution was loaded directly on to the separating gel. Esterase patterns were separated on thin layer (1.5mm thickness) Native polyacrylamide gels (7.5%). The gel mixture was prepared according to [17]. Gelling was allowed for 45 minutes. After loading on to the gel, the samples were over laid with electrode buffer and gel plates were connected to the electrophoretic tank. Tris (0.05M), glycine (0.38M), buffer (pH 8.3) was used as the electrode buffer. A constant current of 50 volts for the first 15 minutes followed by 150 volts for the rest of the run was supplied during electrophoresis. The electrophoretic run was terminated when the tracking dye migrated to the distance of 6 cm from the origin.

2.4 Staining and inhibition studies

Esterases were visualized on the gels by adopting the staining procedures of [18, 19]. Physostigmine (10^{-4}M), pCMB (Para chloro Mercuric benzoate (10^{-3}M) and Paraxon (0, 0-di-ethyl-4- nitro phenyl phosphate ($2x10^{-3}$M) were used for inhibitor sensitivity studies. The gels were pre-incubated in the buffer containing the above concentrations of inhibitors for half an hour. Then they were stained for esterase activity with 1-napthyl acetate as substrate. The inhibitors in concentrations used for pre-incubation were added to the stain buffer to prevent reversal of inhibitory action of the compounds.

III. Results And Discussion

Results obtained on the electrophoretic patterns of esterases of different tissues of silk gland, haemolymph and mid gut of Eri silkworm are presented in Fig.1, 2 and 3 respectively. The details about the relative mobilities of individual esterase zones, visual end points and classification of these esterases are presented in Table.1. The relative mobility (Rm) activity of zones was determined according to **Klebe** [20] **described earlier [21**]; In short, the Rm is the distance travelled by zone relative to that of tracking dye. The serial two fold dilution of the samples followed by the visibility of the zone in electrophoresis was used to score activity intensity of the zones.

The results presented indicate that the silk gland and haemolymph extract contains single hyperactive band which leave heavy deposition of 1-naphthol on cathodal side of the bands after staining. Comparison of the relative mobilities indicates (Table-1) that the slow moving bands with Rm value 35.71 in zone 3 is present in the silk gland and haemolymph (Fig.1 & 2). The mid gut extract (Fig.3) contains three additional bands with Rm 35.71 in zone 3 in slow moving zone; Rm 57.14 in zone 2 in middle region and Rm 71.42 in zone 1 in fast moving zone. 1-naphthyl acetate was used as a substrate to score the activity of non-specific esterases on gels.

Based on the sensitivity of individual zones towards the three inhibitors paraxon, pCMB and **physostigmine (eserine)** used for inhibitory studies in the present investigation to classify the zones. These non-specific esterases were further classified. The silk gland extract zone 3 esterases which were inhibited by paraxon, eserine and pCMB were classified as CHsp (Cholinesterase like enzymes) esterases. The haemolymph zone 3 and mid gut extract Zones 1, 2, 3 are inhibited by paraxon and pCMB were classified as Esdp (Enzyme inhibited by paraxon and pCMB) esterases.

Esterases (E.C.3.1.x) represent a diverse group of hydrolases catalyzing the formation and breakdown of ester bonds .Inhibition of esterases by the organophosphates, carbomates had been used traditionally to classify them. [22] Used OP compounds to distinguish esterases into 2-distinct groups, (1) the **A- esterases** – which hydrolyse OP compounds: e.g. paraozonases, disopropyl fluorophosphatases and phosphor triesterases and (2) the **B-esterases** – which are sensitive and their activity is inhibited by OP compounds. The CHsp and Esdp esterases come under this group.

CHsp (Cholinesterase like enzymes) esterases inhibited by all the three inhibitors and Esdp esterases which are inhibited both by pCMB and paraxon organophosphates. These are implicated in biotransformation and detoxification of the pesticides [23, 24] they have important biotechnological applications as antidotes against poisoning and are useful in bioremediation of organophosphate sensors [25].

Various authors reported different number of esterase fractions in the gut spectrum of different breeds of B.*mori* [26, 27, 28, 29]. Esterase isozyme exhibited higher level of polymorphism in vertebrates and invertebrates [30, 31]. Gillespie and Kojima [32] reported a relationship between **the level** of polymorphism and metabolic enzymes such as esterases. The differences in fractions of esterase may be due to the degree of genetic heterogeneity.

Studies on esterases of fishes and other organisms **like** crustaceans, insects, molluscs and amphibians revealed similar types of inhibition patterns are observed [33, 34, 35, 36, 37, 38].

Observations on the esterase isozyme pattern have revealed that the banding pattern differs between the silk gland, haemolymph and mid gut. The zymograms indicated the variation in Rm value and intensity of bands among the three tissues of silkworm.

IV. Conclusion

The present results clearly indicates that the mid gut and haemolymph esterase activity levels indicate high intensity in **normal**, moderate activity with **physostigmine** and no activity in the presence of paraxon and pCMB. The silk gland esterase activity levels indicate high intensity in control, no activity in the presence of paraxon, **Physostigmine** and pCMB.

In view of the above results the esterases can be used as tools in establishing the genetic relatedness among the closely related species [39], and also as a marker molecule during the evolution of new breeds of Eri silkworm.

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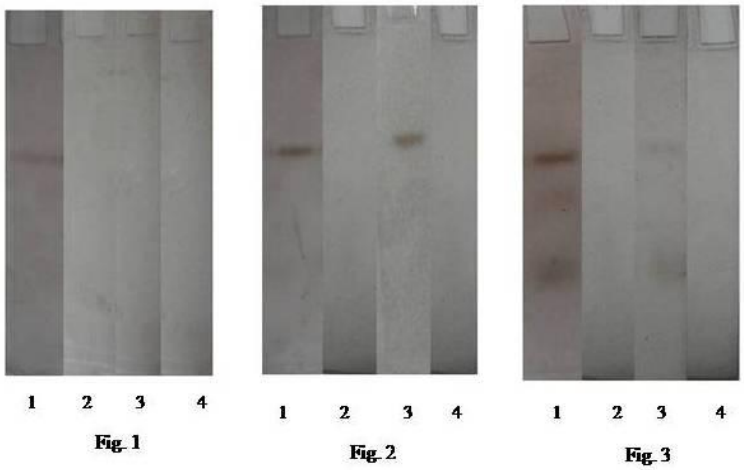

Fig.1: Electrophoretic patterns of esterases of 1. Silk gland extract (Normal); 2. Silk gland extraction in the presence of pCMB; 3. Silk gland extraction in the presence of **physostigmine**; 4. Silk gland extraction in the presence of paraxon.

Fig.2: Electrophoretic patterns of esterases of 1. Haemolymph extract (Normal); 2. Haemolymph extraction in the presence of pCMB; 3. Haemolymph extraction in the presence of **physostigmine**; 4. Haemolymph extraction in the presence of paraxon.

Fig.3: Electrophoretic patterns of esterases of 1. Mid gut extract (Normal); 2. Mid gut extraction in the presence of pCMB; 3. Mid gut extraction in the presence of **physostigmine**; 4. Mid gut extraction in the presence of paraxon.

TABLE-1

Sample		**Zone**			
		1	**2**	**3**	**Classification**
Silk gland	**Rm Values**	**71.42**	**57.14**	**35.71**	**CHsp**
	Inhibitor	-	-	+++	
	Normal				
	Paraxon	-	-	-	
	Physostigmine	-	-	-	
	pCMB	-	-	-	
Haemolymph	Normal	-	-	+++	**Esdp**
	Paraxon	-	-	-	
	Physostigmine	-	-	++	
	pCMB			-	
Mid gut	Normal	+++	+++	+++	**Esdp**
	Paraxon	-	-	-	
	Physostigmine	++	++	++	
	pCMB	-	-	-	

Table.1: Patterns of Esterases of silk gland, haemolymph and mid gut of eri silk worm. Rm (Relative mobility) =Shown as percent migration of the zone from the origin to that of tracking dye; (+++) = Indicates the high activity; (++) = Indicates the normal activity; (+) = Indicates low activity; (-) = Indicates no activity; Esdp = Enzyme inhibited by paraxon and pCMB; CHsp= **C**holine esterase-like enzymes (The residual activity is shown in the case of inhibition).

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