INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
VOLUME-2, ISSUE-4, OCT-DEC-2012 ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 10th August-2012 *Revised: 13th August -2012* *Accepted: 16th August -2012*

Research article

PATTERNS OF ESTERASES IN THE DEVELOPING STAGES OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

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ABSTRACT: Changes in qualitative patterns of esterases are observed in the developing stages of the red flour beetle, Tribolium Castaneum. pCMB , paraoxon and eserine are used to classify the esterases into different categories. 8 zones of esterases are observed in different developmental (larval to adult) stages of Tribolium Castaneum.
Key words: *Tribolium Castaneum, esterase, electrophoresis.*

INTRODUCTION

Esterases are hydrolases which split carboxylic acid esters into alcohol and acid with the addition of water molecule. They are promiscuous in their choice of substrates and have important application in food, pharmaceutical, detergent, fine chemical, waste water treatments, biodiesel production, pharmaceutical industries and in bioremediation processes (Rao et al., 1998; sharma et al, 2001; Bornscheuer et al., 2002; Jaeger and eggert, 2002; Reetz 2002; Maurer, 2004; Cammarota and Freire, 2006; Hasan et al., 2006). These enzymes play vital role in detoxification of pesticides, and the metamorphism of insects. Although the role of juvenile hormones is well characterized in insects, only few studies were made on the patterns of non specific esterases in development of organisms. In an earlier study from this lab the stage specific changes in the pattern of esterases were reported in hemimetabolous insect Chrysochoris purpureus (Shobha Rani and Lakshmipathi, 1995) and the crab Barytelphusa guerini (LAKSHMIPATHI AND SUJATHA, 1991). The present paper describes the qualitative changes in the patterns of esterases at different development stages of Tribolium Castaneum (Herbst)

MATERIALS AND METHODS

The insects, Tribolium Castaneum, used for this work were obtained from different stored product godowns and kirana retail shops in the Warangal district. They were reared in small plastic vessels containing sufficient amount of autoclave sterilized bran at 32±2°C, 70±5% relative humidity and 14:10 light dark period.

Native gel Electrophoresis: larvae, pupae and adult insects were pooled, weighed to the nearest milligram and homogenized (10%) in 0.01M Tris-Hcl, 0.9% Nacl buffer pH 7.5 in cold conditions. The homogenates were centrifuged at -4ºc at 5,000rpm for 10minutes. The supernatant were mixed with 20% sucrose solution containing bromophenol blue as tracking dye in 2:1 ratio. An aliquot of 0.1ml of this solution was used for electrophoresis. The native gel electrophoresis was performed with 7.5% separating gel in vertical slabs. A discontinuous buffer system with 0.05m Tris containing 2.9% Glycine pH 8.3 used as gel buffer and this buffer diluted to 1:9 with distilled water was used for electrophoresis as electrode buffer. A constant power of 100volts was supplied for first 15minutes following which it was raised to 200volts. The current was terminated when the tracking dye migrated to a distance of 6cm from the origin. The gels were stained for esterases and the classification of the zones was done as per the procedures described earlier by Lakshmipathi and Reddy (1989). α-naphthyl ester of acetate was used as substrate. Physostigmine (Eserine, 4mg/10ml, 1mM), pCMB (parachloromercuribenzoate, 1mM) and Paraoxon (o, o-diethyl o-(4-nitrophenyl) phosphate, 0.01% V/V in acetone) were used for inhibition studies.

RESULTS AND DISCUSSION

Patterns of esterases of different developmental stages of Tribolium Castaneum are presented in Figure 1. Electrophoretic studies indicate that there are eight detectable zones in the different stages of Tribolium (EL to adult). Since the patterns in larval stages upto 3rd stage (3L) was almost similar and 4th and 5th larval stages also exhibited similar patterns in the preliminary investigation, the patterns of 3rd (3L) and 5 th stages (5L) were studied for their inhibitory patterns. The results presented in the table and figure1 indicate that zones1, 3, 4, 5 and 6 are present in all stages. Zone 1 is present in all stages but its activity decreases in in prepupal and pupal stages. Zone 2 is observed in larval stages only but it is absent totally in prepupal and pupal stages and a very low intensity band is observed in adult stage. Zone 7 is only observed in prepupal and pupal stages. Zone 8 is observed in larval and prepupal stage.

Details about the relative mobility and the classification of the zones are presented in Table 1. The relative mobilities of the individual esterases and the relative mobility (RM) activity of zones was determined according to the procedure of Klebe(1975), described earlier by Lakshmipathi and sujatha1991; in short, the RM is the distance travelled by the 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.

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. Aldridge (1953) used OP compounds to distinguish esterases into 2-distinct groups, (1) the **A- esterases** – which hydrolyse OP compounds: eg paraozonases, disopro pyl fluorophosphatases and phosphotriesterases and (2) the **B-esterases** – which are sensitive and their activity is inhibited by OP compounds. The carboxyl esterases and choline esterases come under this group.

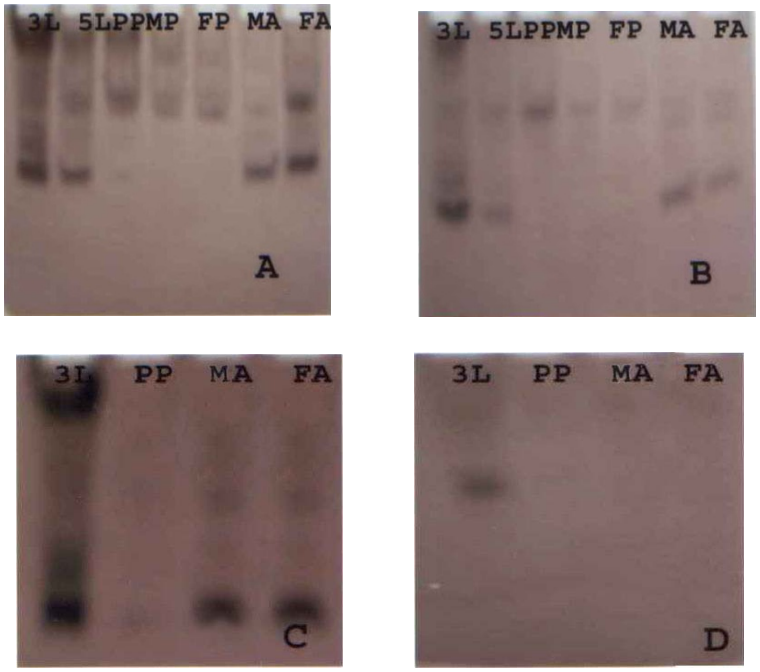

Figure 1: Patterns of esterases observed at different stages of development of Tribolium.
(A) Patterns of esterases of larval stages: Third stage (3L), Fifth stages (5L), Pre Pupae (PP), Male Pupae (MP), Female Pupae (FP), Male adult (MA), Female adult (FA). **(B)** Patterns observed in the presence of inhibitor pCMB. **(C)** Patterns observed in the presence of inhibitor paraoxon. **(D)** Patterns observed in the presence of inhibitor Eserine (Physostigmine).

Table 1: Relative mobility, activity and classification of esterase zones of different stages of development.

Zone No		1	2	3	4	5	6	7	8
Relative mobility		58	48	36	33	20	16.6	13.3	8
Stages	Inhibitors								
3L	-	+++	+++	++	++	++	+++		+++
	Pa		+++			+	++		
	E	+++			++		++		
	pCMB	+++	++	+	+	+	+		
5L	-	+++	++	++	++	++	+++		++
	Pa		+++			+	++		
	E	+++			++		++		
	pCMB	+++	++	+	+	+	+		
PP	-	+		++	++	++	++	++	++
	Pa					+	+		
	E	+	++		+		++		
	pCMB	++				+	+		
P	-	+		++	++	++	++	+	
	Pa					+	+		
	E	+	++		+		++		
	pCMB	++				+	+		
A	-	+++	±	++	++	++	++		
	Pa					+	+		
	E	+++			+		++		
	pCMB	++				+	+		
Classification		CE	ESE	CHE	CHE	ESE	ER	CHE	CHE

Note: Relative mobility is shown as percent migration of the zone from the origin to that of tracking dye. Inhibitors: Pa = Paraoxon, E= eserine, pCMB = parachloromercuribenzoate; Normal and residual activity is represented as +++ = high intensity; ++ = Moderate intensity; + = Low intensity; ± = Very low intensity;
Classification: CE = Carboxyl esterase; ESE = Eserine esterase; CHE = Choline esterase and ER = Enzyme resistant to OP compounds physostigmine and pCMB.

The phosphotriesterases and carboxyl esterases are implicated in biotransformation and detoxification of the pesticides (Jokanovic 2001; casida and quistad 2005) they have important biotechnological applications as antidotes against poisoning and are useful in bioremediation of organophosphate sensors (Sogorb and vilinova, 2002).

Three inhibitors, Paraoxon (an organophosphate), Physostigmine (the carbamate and pCMB (a thiol inhibitor were used for inhibition studies in the present investigation to classify the zones. The pattern of esterases observed indicates that zone 1 is a carboxylesterase inhibited by Paraoxon; zones 2 and 5 are Ese esterases inhibited by Eserine alone, zone 3, 4, 7 and 8 are cholinesterases inhibited by all the three inhibitors and zone 6 is ER esterase resistant to all inhibitors. Zone I which is a carboxyl esterase is present in all the stages of Tribolium castaneum. Structurally carboxyl esterases belong to a family of proteins and enzymes commonly known as α/β hydrolases fold (ollis et al., 1992), which is a large family of proteins consisting among the other proteases, lipases, esterases, dehalogenases, peroxidases and epoxide hydrolases (Nardini and Dijkstra 1999). Insect carboxylesterases may contribute to insecticide resistance.

The carboxyl esterases of Luclia Cuprina (Newcomb et at., 1997; Heidari et al., 2004), Musca domestica (Claudianos et al., 1999), and Anisopteromalus calendar (Zhu el al., 1999) have been extensively studied. Carboxylesterase cDNA(TCE) of T.castaneum was isolated and expressed in the methylotropic yeast, Pichia pastoris (Delroisse et al., 2005) where they designed and constructed a synthetic TCE gene (syn TCE) The significance of sex dependent (Male predominant) carboxylesterase expression in Mytilus galloprovincialis and Drosophila virilis was discussed in relation to the sexual differentiation and functioning of the male reproductive system. Mikhailov et at., (1997) reported the biochemical characteristics of male associated polypeptide(MAP) identified in the gonad tissue of bivalve molluscus, M.galloprovincialis in comparison to those of male specific carboxylesterase (esterase s). D.Virilis ejaculatory bulbs. They have also shown that MAP is characterized by esterase activity towards α and β naphthylacetates

Zone 8, which appeared sensitive to all the inhibitors shown to be active in only larval and prepupal stages. Juvenile harmone esterases(JHE) were shown to be active during larval development of manduca sexta (Sanburg et at., 1975). Recently for the first time, the JHE gene from the Tribolium castaneum has been cloned and shown highest similarity to TmJHE and also to P.hilaris JHE (PhJHE) (Tsubota et al., 2010) suggesting that this gene functions as a JH-degrading enzyme present in the last larval stage. A further study is needed whether this larval specific gene is a JHE!

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