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Research Article

COMPARATIVE TISSUE ESTERASE POLYMORPHISM OF *MACROBRACHIUM MALCOLMSONII* AND *PENAEUS MONODON*

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ABSTRACT

Esterase polymorphism were studied in *Macrobrachium malcolmsonii* and *Peneaus monodon* of two prawns belonging to fresh water and marine water respectively, Six tissues were studied viz; gill, hepatopancreas, intestine, muscle, brain and eye. CE esterases are noticed in gill, hepatopancreas of fresh water prawns. ER esterases are also noticed only in fresh water prawns in gill and hepatopancreas, but completely absent in marine water prawns. ChE and CHsp esterases are present in both the prawns. But Esdp, ArE, esterases are noticed in marine prawns only i.e. *P. monodon* where as in fresh water prawns this type of esterases are completely absent.

Keywords: Electrophoresis, Esterases, Tissues, Prawns.

INTRODUCTION

Esterases are the hydrolyze enzymes that splits esters into an acid and an alcohol. Two categories of such enzymes were recognized first by Loevenhart, (1906), enzymes, which hydrolyze the esters of short chain (C_2-C_4) fatty acids were recognized as esterases, while those which hydrolyzed the long chain fatty acid esters (>C8) were recognized as lipases (Seligman & Nachlas, 1950).

$$\text{Esters} \xrightarrow{\text{Esterases}} \text{Alcohol} + \text{Acids} + H_2O.$$

$$\text{Alcohol} + \text{Carboxylic acid} \leftrightarrow \text{Ester} + \text{water}.$$

$$\text{R-OH} + \text{R-COOH} \text{ --------> } \text{R-COO-R} + H_2O.$$

Esterase enzymes are involved in important physiological process such as nervous impulse control, reproduction, developmental process, detoxification and tolerance of xenobiotics besides being good biomarkers to predict environmental pollution and they have been used as gene markers in a wide variety of organisms. These enzymes also attracted the action of industry in past few decades due to their application in food, detergent, fine chemical, waste water treatments, bio-diesel production, and pharmaceutical industries and in bio-remediation (Bornscheuer, 2002; Cammarota & Freire, 2006; Jaeger & Eggert, 2002; Rao *et al*., 1998; Reetz, 2002; Sharma *et al*., 2001; Vimala & Rajaiah, 2014; Alam *et al*., 2015). The high region and spacio specificity of these enzymes has applications in the Kinetic resolution of optical isomers for synthesis of optically pure substances in pharmaceutical and chemical industries (Alam *et al*., 2015; Bornscheuer, 2002). Their ability was to catalyze a variety of esterase without the aid of cofactors is an additional advantage (Bornscheuer, 2002). Esterases play a vital role in the metamorphosis of insects (Yu *et al*., 2009). The present study was aimed to investigate the comparative tissue of esterase polymorphism of selected prawns.

MATERIAL AND METHODS

Fresh water, prawns (*Macrobrachium malcolmsonii*) were collected from ponds (tanks) located within the radius of 60 kms from Kakatiya University Campus by netting with the help of local fishermen and marine water prawns (*Peneaus monodon*) were collected from Vishakhapatnam of Andhra Pradesh. They were immediately brought to the laboratory in water in plastic buckets and acclimatized to laboratory conditions for about a week in aquaria. They were fed on

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natural plankton collected from their natural habitats. Prawns were immobilized by hitting them on the head and the tissues were dissected out of animals. Six tissues were selected for the study gill, Hepatopancreas, intestine, muscle, brain and eye. The dissected tissues from about three (big fish) to six (small fish) individuals were pooled, weighed to the nearest milligram and were homogenized in 0.01M Tris-Hcl buffer (pH 7.5) containing 0.9% of NaCl. The concentration of tissue homogenates varied from tissue to tissue. I) Gill - 10 %, ii) Hepatopancreas - 10%, iii) Intestine-10%, IV) Muscle - 20%, v) Brain-10 %, vi) Eye - 10%. The tissues after homogenization were placed in ice-jacketed centrifuge tubes. The extracts were centrifuged at 2,000 rpm for 10 minutes in a clinical centrifuge at room temperature. The supernatants were mixed with equal volumes of 20% sucrose solution containing 0.05% bromophenol blue as the tracking dye. An aliquot of 0.1ml of this mixture was used for loading the sample on to the gel for electrophoretic separation of esterase patterns.

Esterases were classified in accordance with the procedures of (Holmes & Masters, 1967;Hart & Cook, 1976; Haritos & Salamastrakis, 1982) and (Lakshmipathi & Reddy, 1989) on the basis of their sensitivity of specific inhibitors. Physostigmine (Carbomate), pCMB (the thiol active compound) and paraoxon (OP compound) were used in the study. The scheme of classification employed in the study is as hereunder: Carboxylesterases (CE): These esterases were sensitive to inhibition by the organophosphate but were not affected by physostigmine or pCMB. 2. Arylesterases (ArE): They were sensitive to inhibition by sulphydryl Agent pCMB and were not affected by paraoxon or physostigmine. 3. Cholinesterases (ChE): Enzymes, which were inhibited by paraoxon and physostigmine. 4. ER Esterases: Enzyme which was not affected by any of the three inhibitors used.5.Esdp Esterases: Enzymes, which were inhibited by pCMB and paraoxon. 5. Ese Esterases: Enzymes, which were inhibited by physostigmine alone. 6. CHsp Esterases: Enzymes, which were inhibited by paraoxon, physostigmine and pCMB.

RESULTS AND DISCUSSIONS

Electrophoretic separation of tissue esterases gel coating in *Macrobrachium malcomsonii* and *Penaeus monodon* is given in the Plate I.

Macrobrachium malcolmsonii

Gill: This tissue exhibited three zones with Rm value .66, .50 and .33. The zones with Rm value .66 and .50 were inhibited by Paraoxon alone; they were classified as CE esterases with low activity, while the other zone with Rm value .33 was an ER esterase with high activity. **Hepatopancreas:** There are three zones in this tissue. The zones with Rm value .66.50 and .33. Among these, the zone Rm .50 .33 were not inhibited by any of the inhibitors used. So they were classified as ER esterases. While the zone with Rm value .66 was a CE esterase with low activity. **Intestine:** Intestine had three active zones on the zymogram with Rm value .66, .50 and .33. The zones with Rm value .50 and .33 were inhibited by paraoxon and eserine. So they were classified as ChE esterases. The other zone with Rm value .66 is a CHsp esterase with moderate activity. **Muscle:** This tissue contains two esterase zones on the zymogram with Rm value .66 and .33. The zone with Rm value .66 is a CHsp esterase and while the other zone with Rm value .33 is a ChE esterase. The zone with Rm .66 was exhibited very low activity which was appears in inhibitors not in general stain. **Brain:** Brain also exhibited two active esterase zones with Rm .66 and .33. Both of zones were inhibited by paraoxon and eserine. So they were classified as ChE esterases. The zone with Rm value .33 is a moderate activity and other zone is very low activity. **Eye:** Eye contains two zones in the zymogram with Rm value .66 and .33. Both of three zones are inhibited by paraoxon alone, so they were classified as CE esterases.

Peneaus monodon

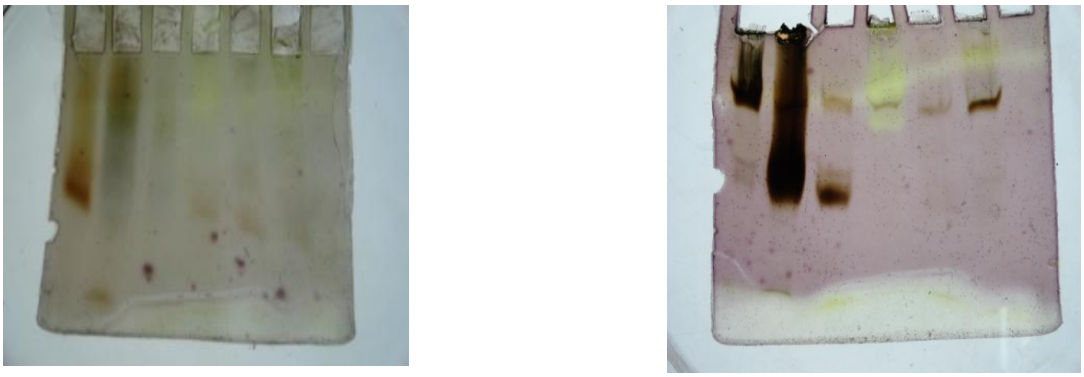
Gill: Gill contain two esterase zones on the zymogram with Rm value .50 and .33, the zones with Rm value .50 is inhibited by paraoxon and pCMB So it was classified as Esdp esterases and other zone is CHsp esterase with very low activity. **Hepatopancreas:** This tissue contains three zones with Rm value . 85, .50 and .33. Among these, the zone with Rm value .33 is an Ese esterase with high activity. The other zones with Rm value .85 and .50 exhibited ChE, ArE esterases respectively. **Intestine**: Intestine exhibited two zones with Rm value .50 and .33. Among these, the zone with Rm .50 exhibited Esdp esterases and remaining zone with Rm .33 exhibited CHsp esterases with moderate activity. **Muscle:** Muscle exhibited only one zone with Rm value .50. It is ChE esterases, with very low activity. **Brain:** This tissue also exhibited only one zone on the zymogram with Rm value .50, it is inhibited by paraoxon and Eserine. So, it was classified as ChE esterase. **Eye:** Eye contain one zone on the zymogram with Rm .50, it is a CE esterase, because it is inhibited by paraoxon alone.

Based on relative nobilities of esterase zones found in *M. malcolmsonii* (Table 1 & 2) exhibited three active zones with Rm value .66, .50 and .33 in different tissues. The zones with Rm .66 and .50 are found in gill, hepatopancreas and intestine. The zone with Rm .66 was CE esterases in gill and hepatopancreas, but in intestine and muscle, it is CHsp esterase, in brain and eye, it is a ChE and CE esterases respectively. The zone with Rm .50 was found in gill, it is CE esterase while in other tissues like hepatopancreas and intestine ER and ChE esterases are found. The zone with Rm value .33 was found in almost all the tissues. In gill and hepatopancreas it is ER esterase. In intestine, muscle and brain, it is ChE esterases. But eye exhibited CE esterases. In *Macrobrachium malcolmsonii***,** CE and ChE esterases are predominant and followed by other esterases. Based on relative nobilities of esterase

zones found in the tissue of *P. monodon* (Table 3 & 4) can be grouped into three zones with Rm values .85, .50 and .33 which are present in six tissues. The zone with Rm value .50 was found in all the tissues, in gill and intestine it is Esdp esterases and in hepatopancreas it is an ArE esterase. But muscle and brain exhibited ChE esterase, while in eye it is CE esterase. The zone with Rm .33 was found in three tissues namely gill, hepatopancreas and intestine. In gill and intestine, it is CHsp esterase, but in hepatopancreas it is Ese esterase. The fast moving zone with Rm .85 was found in hepatopancreas with ChE esterase. In *Peneaus monodon*, ArE, Esdp and Ese specific esterases are found.

Plate I. Electrophoretic separation of tissue esterases gel coating in *Macrobrachium malcomsonii* and *Penaeus monodon.*

Macrobrachium malcomsonii *Penaeus monodon*



1 2 3 4 5 6 1 2 3 4 5 6

1. Gill, 2. Hepatopancreas, 3. Intestine, 4. Muscle, 5. Brain, 6. Eye.

Table 1. Inhibitor sensitivity of individual esterase zones in *Macrobrachium malcolmsonii.*

Name of Tissue	Gill			Hepatopancreas			Intestine			Muscle		Brain		Eye	
Rm values	.66	.50	.33	.66	.50	.33	.66	.50	.33	.66	.33	.66	.33	.66	.33
Activity	+	+	+++	+++	+	+	++	+	++	+	++	+	++	+	++
pCMB	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+
Eserine	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+
Paraoxon	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-
Classification	CE	CE	ER	CE	ER	ER	CHsp	ChE	ChE	CHsp	ChE	ChE	ChE	CE	CE

Rm: Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye. CE: Carboxylesterase. ChE: Cholinesterase. CHsp: Cholinesterase like enzymes. ER: Esterases resistant to inhibitors; ArE: Arylesterases; Esdp: Esterase sensitive to organophosphates and pCMB Ese: Esterases sensitive to Eserine alone; +++: High activity; ++: Moderate activity; Low activity; +: Very low activity.

Table 2. Tissue specific distribution of esterases in *Macrobrachium malcolmsonii.*

Rm values Tissues	1 .66	2 .50	3 .33
1). Gill	+ CE	+ CE	+++ ER
2). Hepatopancreas	+++ CE	+ ER	+ ER
3). Intestine	++ CHsp	+ ChE	+ ChE
4). Muscle	+ CHsp		++ ChE
5). Brain	+ ChE		++ ChE
6). Eye	+ CE		++ CE

Rm: Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye. CE: Carboxylesterase. ChE: Cholinesterase. CHsp: Cholinesterase like enzymes. ER: Esterases resistant to inhibitors; ArE: Arylesterases; Esdp: Esterase sensitive to organophosphates and pCMB Ese: Esterases sensitive to Eserine alone; +++: High activity; ++: Moderate activity; Low activity; +: Very low activity.

Table 3. Inhibitor sensitivity of individual esterase zones in *Peneaus monodon.*

Name of Tissue	Gill		Hepatopancreas			Intestine		Muscle	Brain	Eye
Rm values	.50	.33	.85	.50	.33	.50	.33	.50	.50	.50
Activity	++	+	+++	+	+++	+	++	+	+	+
pCMB	-	-	+	-	+	-	-	+	+	+
Eserine	+	-	-	+	-	+	-	-	-	+
Paraoxon	-	-	-	+	+	-	-	-	-	-
Classification	Esdp	CHsp	ChE	ArE	Ese	Esdp	CHsp	ChE	ChE	CE

Rm: Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye. CE: Carboxylesterase. ChE: Cholinesterase. CHsp: Cholinesterase like enzymes. ER: Esterases resistant to inhibitors; ArE: Arylesterases; Esdp: Esterase sensitive to organophosphates and pCMB Ese: Esterases sensitive to Eserine alone; +++: High activity; ++: Moderate activity; Low activity; +: Very low activity.

Table 4. Tissue specific distribution of esterases in *Peneaus monodon.*

Rm values Tissues	1 .85	2 .50	3 .33
1) Gill		++ Esdp	+ CHsp
2) Hepatopancreas	+++ ChE	+ ArE	+++ Ese
3) Intestine		+ Esdp	++ CHsp
4) Muscle		+ ChE	
5) Brain		+ ChE	
6) Eye		+ CE	

Rm: Relative mobility is calculated as a fraction of the distance migrated by the zone from the origin of a tracking dye. CE: Carboxylesterase. ChE: Cholinesterase. CHsp: Cholinesterase like enzymes. ER: Esterases resistant to inhibitors; ArE: Arylesterases; Esdp: Esterase sensitive to organophosphates and pCMB Ese: Esterases sensitive to Eserine alone; +++: High activity; ++: Moderate activity; Low activity; +: Very low activity.

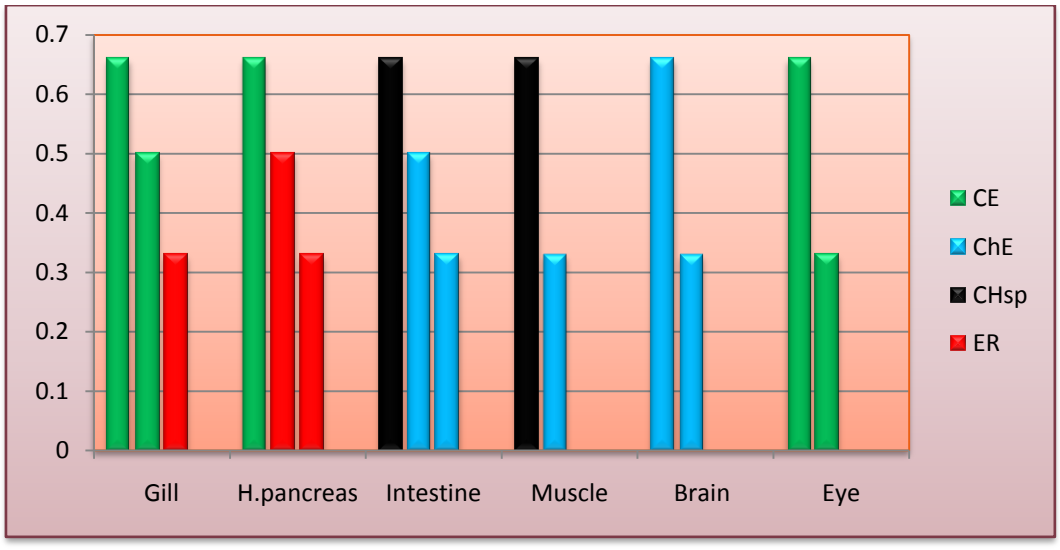


Figure 1. Tissue specific distribution of esterases in *Macrobrachium malcolmsonii*

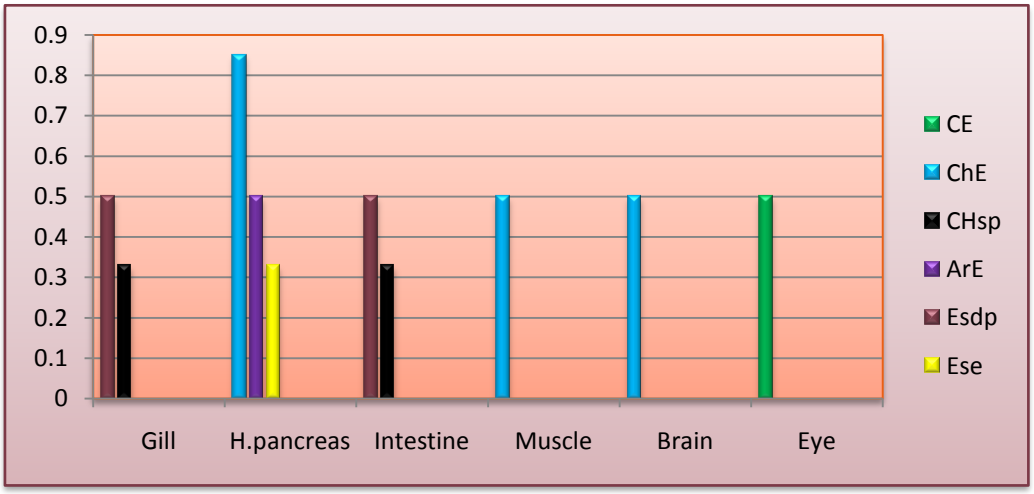


Figure 2. Tissue specific distribution of esterases in *Peneaus monodon*

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