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Tissue esterases polymorphism of *Macrobrachium rosenbergii* and *Penaeus indicus*

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Abstract
Comparative study of esterases polymorphism were studied in Macrobrachium rosenbergi and *Penaeus indicus* of two prawns belonging to fresh water and marine water respectively, Six tissues were studied viz; Gill, Hepatopancreas, Intestine, Muscle, Brain, and Eye. ER esterases are noticed in Gill, of both marine and fresh water prawns. CE esterases are noticed in all the tissues in fresh water and marine water prawns. CHsp esterases are present in intestine of both the prawns. But Esdp, ArE, ESE esterases are noticed in marine prawns only i.e. P. indicus where as in fresh water prawns this type of esterases are completely absent.

Keywords: electrophoresis, esterases, gill, hepatopancreas, intestine muscle, brain, and eye

Introduction
Esterases are the hydrolyze enzymes that splits esters into an acid and an alcohol. Two categories of such enzymes were recognized first by Lovenhart (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 and Nachlas, 1950) [14].

$$\text{ESTERS} \xrightarrow{\text{Esterases}} \text{Alcohol} + \text{Acids} + H_2O$$

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

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

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. (Rao *et al.*, 1998; Sharma *et al*., 2001; Bornscheucr *et al.*, 2002; Jaeger and eggert, 2002; Reetz 2002; maurer, 2004; Cammarota and Freire, 2006; Hasan *et al.*, 2006) [12, 15, 7, 13, 10, 2, 5]. 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 (Bornscheuer, 2002; Hasan *et al.*, 2006) [1, 5]. Their ability was to catalyze a variety of esterase without the aid of cofactors is an additional advantage (Bornscheuer, 2002) [1]. Esterases play a vital role in the metamorphosis of insects (Quan - You Yu *et al*., 2009) [11].

Meterils and Methods
Fresh water, prawns (*Macrobrachium rosenbergii*) 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 (*Penaeus indicus*) were collected from Vishakhapatnam 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 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 and Masters (1967) [6], Hart and Cook (1976) [4], Haritos and Salamastrikis (1982) [3] and Lakshmipathi and Reddy (1989) [8] 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:

1. 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 were not affected by any of the three inhibitors used.
5. Esdp Esterases: Enzymes, which were inhibited by pCMB and paraoxon.
6. Ese Esterases: Enzymes, which were inhibited by physostigmine alone.
7. CHsp Esterases: Enzymes, which were inhibited by paraoxon, physostigmine and pCMB.

Results

Macrobrachium malcolmsonii (H. *Milne Edwards*, 1844)

Gill

This tissue exhibited two active zones on the zymogram with Rm value. 56 and. 46. Out these two zones, the zone with Rm value. 56 is CE esterase and other zone with Rm value. 46 is ER esterase with moderate activity.

Hepatopancreas

Hepatopancreas exhibited three zones on the zymogram with Rm value. 96, 73 and. 66. The zone with Rm value. 96 is a CHsp esterase, and other zones with Rm value. 73 and. 66 are CE and ChE esterases respectively. The zones with Rm. 96 and. 73 exhibited moderate activity.

Intestine

There are three active esterase zones with Rm value. 96,. 66 and. 46. Among these, the zones with Rm value. 66 and. 46 were inhibited by paraoxon and Eserine. So they were classified as ChE esterase. While the zone with Rm. 96 is inhibited by only paraoxon so it was classified as CE esterases.

Muscle

Muscle exhibited only one zone with Rm value. 66 with CE esterase. They are inhibited by paraoxon alone with low activity.

Brain

Brain also exhibits only one zone on the zymogram with Rm value. 66 it is inhibited by paraoxon and Eserine. So it was classified as ChE esterase.

Eye

This tissue contains one zone on the zymogram with Rm value. 66. It is inhibited by paraoxon so it was classified as CE esterase. It exhibited moderate activity.

Table 1: Inhibitor sensitivity of individual esterase zones in *Macrobrachium rosenbergii*

Name of Tissue	Gill		Hepatopancreas			Intestine			Muscle	Brain	Eye
Rm values	.56	.46	.96	.73	.66	.96	.66	.46	.66	.66	.66
Activity	++	++	++	++	++	++	++	++	+	++	++
pCMB	+	+	-	+	+	+	+	+	+	+	+
Eserine	+	+	-	+	-	+	-	-	+	-	+
Paraoxon	-	+	-	-	-	-	-	-	-	-	-
Classification	CE	ER	CHsp	CE	ChE	CE	ChE	ChE	CE	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 2: Tissue specific distribution of esterases in *Macrobrachium rosenbergii*

Rm value Tissues	1	2	3	4	5
	.96	.73	.66	.56	.46
1) Gill				++ CE	++ ER
2) Hepatopancreas	++ CHsp	++ CE	++ ChE		
3) Intestine	++ CE		++ ChE		++ ChE
4) Muscle			+ CE		
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;

Penaeus indicus (H. Milne Edwards1837)

Scientific Classifi

Kingdom

Gill

There are four esterase zones on the zymogram with Rm values. 83, 66, 50 and. 33. Among these, the zone with Rm

value. 66 exhibited ER esterases. The zones with Rm value. 83 and. 33 are CE esterases. The remaining zone Rm. 50 exhibited Esdp esterases.

Hepatopancreas
This tissue exhibited four zones with Rm value. 83, 66, 50. and. 33. The zones with Rm. 66 exhibited CE esterases. While the zones with Rm value. 83. 50 and. 33 were Classified as CHsp and ArE, and Ese esterases respectively. Among these zones, the zone with Rm value. 50 and. 33 had higher activity and remaining zones are moderate activity.

Intestine
Intestine exhibited three active esterase zones on the zymogram with Rm value. 66, 50 &. 33. The zones with Rm value. 66 exhibited CE esterases and remaining zones with Rm value. 50 and. 33 were classified as Esdp and ChE esterases respectively. All the zones which are present in Intestine are exhibited moderate activity.

Muscle
There are three zones in this tissue, with Rm value. 83,. 50 and. 33. Among these, the zones with Rm value. 83 and. 50 are CHsp and ChE esterases respectively. While the zone with Rm value. 33 exhibits CE esterase. These all zones exhibit moderate activity.

Brain
Brain contains two active esterase zones with Rm value. 83 and. 33. Both of these zones are ChE esterases with moderate activity.

Eye
Eye exhibits three esterase zones on the zymogram with Rm value. 83. 50 and. 33. Out of these, the zones with Rm value. 50 and. 33 were exhibited CE esterases while other zone with Rm value. 83 is a ChE esterase.

Table 3: Inhibitor sensitivity of individual esterase zones in *Penaeus indicus* (Marine prawn)

Name of Tissue	Gill				Hepatopancreas				Intestine		Muscle			Brain			Eye		
Rm values	.83	.66	.50	.33	.83	.66	.50	.33	.66	.50	.33	.83	.50	.33	.83	.33	.83	.50	.33
Activity	+	++	++	+	++	++	+++	+++	++	++	++	++	++	++	++	++	++	+	++
pCMB	+	+	-	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+
Eserine	+	+	+	+	-	+	+	-	+	+	-	-	-	+	-	-	-	+	+
Paraoxon	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Classificatin	CE	ER	Esdp	CE	CHsp	CE	ArE	Ese	CE	Esdp	ChE	CHsp	ChE	CE	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 4: Tissue specific distribution of esterases in *Penaeus indicus*

Rm values Tissues	1	2	3	4
	.83	.66	.50	.33
1) Gill	+ CE	+ ER	+++ Esdp	+CE
2) Hepatopancreas	++ CHsp	++ CE	+++ ArE	+++ Ese
3) Intestine		++ CE	++ Esdp	++ ChE
4) Muscle	++ CHsp		++ ChE	++ CE
5) Brain	++ ChE			++ ChE
6) Eye	++ 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;

Fig 1A: *Macrobrachium rosenbergii* 123456 **Fig 1B:** *Penaeus indicus* 123456

Fig 1: 1-Gill, 2-Hepatopancreas, 3- Intestine, 4-Muscle, 5-Brain, 6-Eye

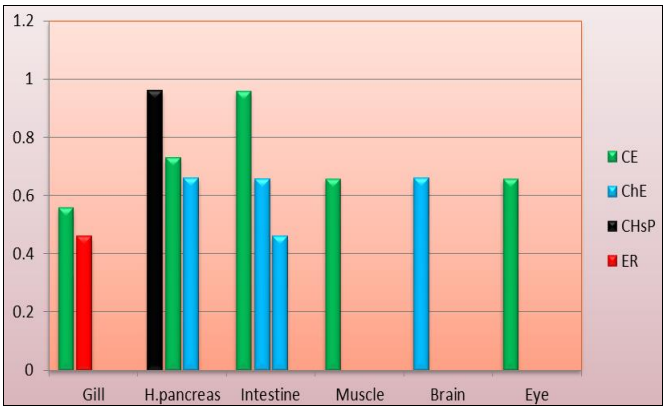

Fig 2: Tissue specific distribution of esterases in *Macrobrachium rosenbergii*

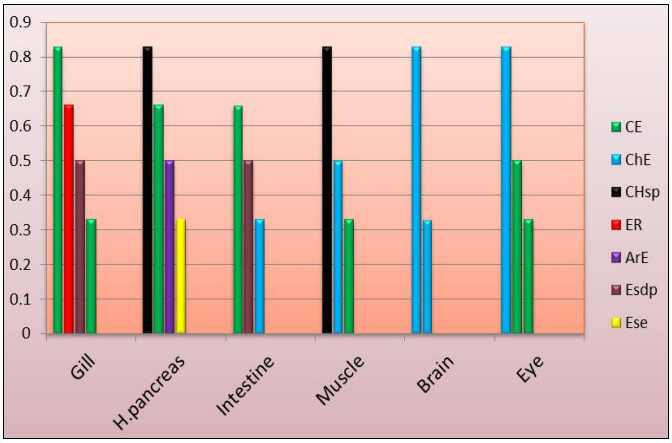

Fig 3: Tissue specific distribution of esterases in ***Penaeus indicus***

Based on the electrophoretic motilities of individual esterase zones, the *Macrobrachium rosenbergii*, exhibits five zones with Rm values. 96, 73, 66, 56 and. 46. Hepatopancreas and intestine exhibited three zones. While the gill tissue exhibited two zones. Muscle, brain and eye exhibit one esterase zone. The fast moving zones with Rm value. 96 was found in Hepatopancreas and intestine with CHsp and CE esterases respectively. The zone with Rm. 66 was found in five tissues with CE esterase in muscle and eye but remaining tissues exhibited ChE esterases. The zone with Rm. 46 was found in gill and intestine. In gill it is an ER esterase and in intestine it is ChE esterase. The zone with Rm. 73 and. 56 are found in Hepatopancreas and gill with CE esterases. CE esterases is predominant in *Macrobrachium rosenbergii*.

Esterases found in various tissues of *Penaeus indicus* can be grouped into four zones, with Rm values. 83, 66, 50, and. 33. Among the four zones, the zone with Rm. 50 was found in all the tissues except in brain. In gill and intestine it is Esdp esterase, in Hepatopancreas it is ArE esterase and in eye it is CE esterase, but in muscle it is ChE esterase. The fast moving zones with Rm. 83 was present in all tissues except in intestine. In gill it is CE esterase, in Hepatopancreas and muscle, it is CHsp esterase, but in brain and eye it is ChE esterase. The zone with Rm. 66 was found in gill, Hepatopancreas and intestine with ER, CE and CE esterases respectively. The zone with Rm. 33 was found in five tissues with ChE esterases in intestine and brain. CE esterases are present in gill, muscle and eye. While Ese esterase was found in Hepatopancreas.

The esterase pattern observed in the two species is highly species specific and tissue specific**.** *Macrobrachium rosenbergii* has five zones in all the tissues, Hepatopancreas and intestine have three zones, gill has two zones. Muscle, brain and eye exhibit one zone each. CE and ChE esterases are predominant. *Penaeus indicus* has four zones (Rm. 83, 66, 50 and.33) in all the tissues. Gill and Hepatopancreas have four zones. Intestine, muscle and eye have three zones and brain consists of two zones. In this prawn, ArE, Esdp and Ese esterases are specific esterases which are present in Hepatopancreas, gill and intestine. CE, ChE esterases are predominant in *Penaeus indicus*.

Among type of esterases observed, CE, ER, CHsp, and ArE esterases are principal contributors. CE esterases are found in gill tissue of fresh water prawns, *Macrobrachium rosenbergii* and whereas CE, ER and Esdp esterases are present in marine prawns i.e., *Penaeus indicus*. ArE and Ese esterases are noticed in Hepatopancreas of *Penaeus indicus*. But in fresh water prawns ER and CE esterases are principal contributors. Intestine has ChE esterases which are present in *Macrobrachium rosenbergii* and whereas Esdp esterases are noticed in and *Penaeus indicus*.

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