Tissue And Species Specific Distribution Of Esterases In Macrobrachium Rosenbergii And Penaeus Monodon

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.

Abstract

Tissue esterases polymorphism were studied in two prawns of Macrobrachium rosenbergii (fresh water prawns) and Penaeus monodon (marine water prawns)in six tissues viz; Gill, Hepatopancreas, Intestine, Muscle, Brain and Eye. Comparison were made between fresh water prawns ie Macrobrachium rosenbergiiwith marine water prawns ie Penaeus monodon across various tissues.In *Macrobrachium rosenbergii*, five esterase zones were identified, with the hepatopancreas and intestine showing the highest diversity. CE esterases were predominant in muscle and eye, while ChE esterases were common in other tissues. *Penaeus monodon* exhibited three esterase zones, with the Rm .50 zone present in all tissues, showing various esterases. Notable esterases in this species included ArE, Esdp, and Ese.

Keywords: Esterases, Electrophoresis, 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)

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). 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). 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 (Quan – You Yu *et al*., 2009).

MATERIALS AND METHODS

Prawns were collected from ponds (tanks) located within the radius of 60 kms from Kakatiya University Campus by netting with the help of local fishermen. 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 %, vii) 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), Hart and Cook (1976), Haritos and Salamastrikis (1982) and Lakshmipathi and 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:

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 rosenbergii

Gill: - This tissue exhibited two active zones on the zymogram with Rm value .56 and .46. Out thesetwo 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.

Based on the electrophoretic mobilities of individual esterase zones, the ***Macrobrachium rosenbergii,*** (Table-3.2.) Exhibits five zones with Rm values .96, .73, .66, .56and.46. Hepatopancreas and Intestine exhibited three zones. While the Gill tissue exhibit two zones. Muscle Brain and Eye exhibits 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 were found inHepatopancreas and Gill with CE esterases. CE esterases is predominant in ***Macrobrachium rosenbergii.***

Penaeus 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.

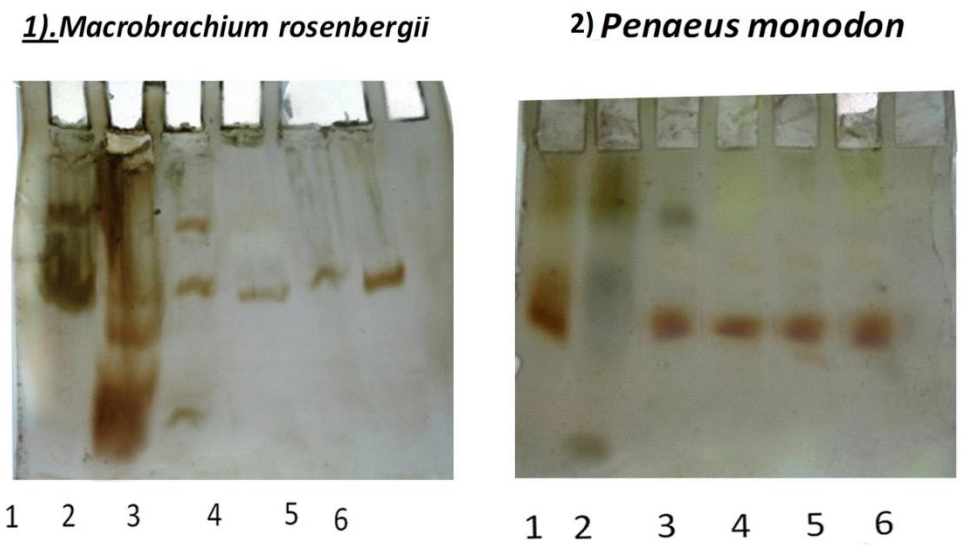
Eye: - Eye contain one zone on the zymogram with Rm .50, it is a CE esterase, because it is inhibited by paraoxon alone.

Based on relative mobilities of esterase zones found in the tissue of ***Penaeus monodon*** (Table-3.6) can be grouped into three zones with Rm values .85, .50 and .33 which were present in six tissues. The zone with Rm value .50 was found in all the tissue, in Gill and intestine it is Esdp esterases and in Hepatopancreas It is an ArE esterases. 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 esterases. The fast moving zone with Rm .85 was found in Hepatopancreas with ChE esterases. In Penaeus monodon, ArE Esdp and Ese specific esterases are found.

CONCLUSION

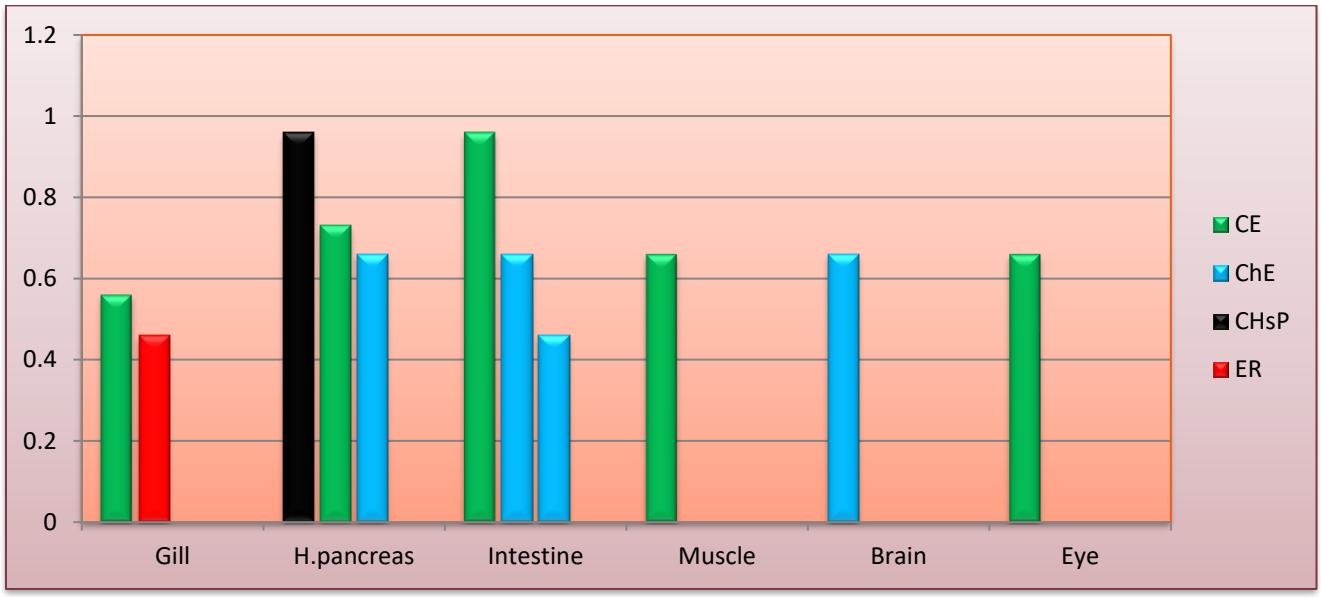
The study identified distinct esterase activity patterns in various tissues of *Macrobrachium rosenbergii(fresh water prawn)* and *Penaeus monodon*.(marine prawn)In *Macrobrachium rosenbergii*, five esterase zones (Rm .96, .73, .66, .56, and .46) were found, with the hepatopancreas and intestine showing the highest diversity. CE esterases were predominant, particularly in muscle and eye, while ChE esterases were common in other tissues.In *Penaeus monodon*, three esterase zones (Rm .85, .50, and .33) were identified across six tissues. The Rm .50 zone was present in all tissues, showing different esterases in each. ArE, Esdp, and Ese specific esterases were notable in this species.Overall, the study highlights tissue-specific esterase distributions and the predominance of certain esterases in both species, contributing valuable biochemical insights

PLATE-I



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

Tissue specific distribution of esterases in ***Macrobrachium rosenbergii***



Tissue specific distribution of esterases in ***Penaeus monodon***

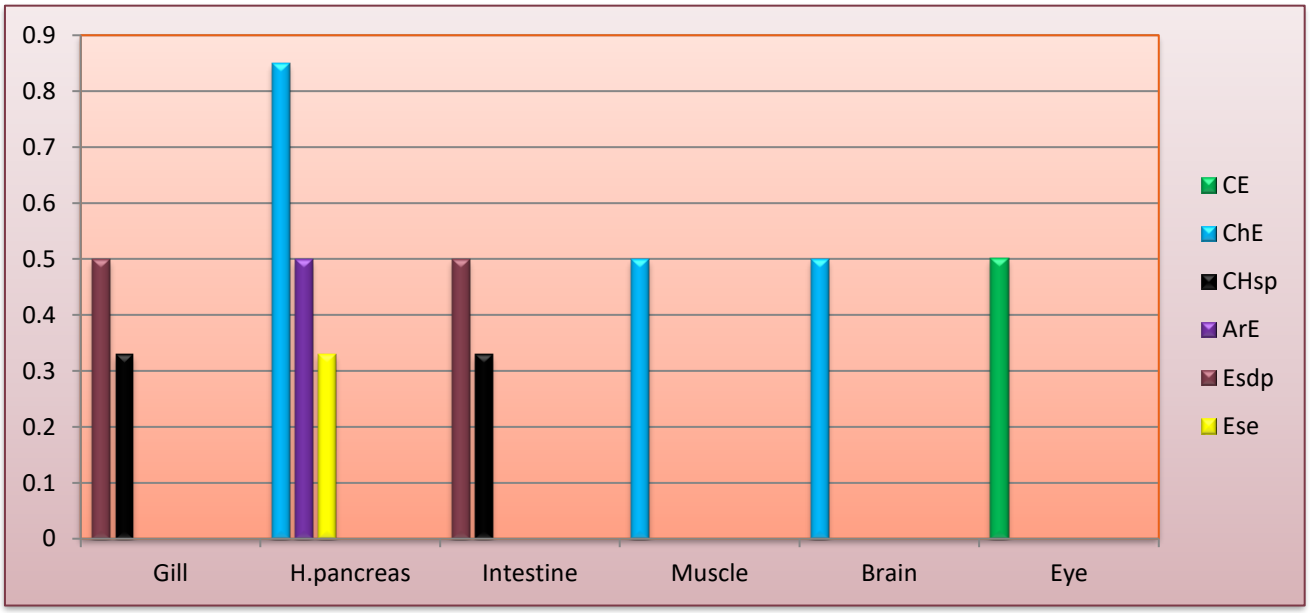


Table 1.1:- Inhibitor sensitivity of individual esterase zones in *Macrobrachium rosenbergii*

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

Table 1.2:- Inhibitor sensitivity of individual esterase zones in *Penaeus monodon* (Marine prawn)

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 pCMBEse = Esterases sensitive to eserine alone;

+++ = High activity; ++ = Moderate activity; += Low activity; + = Very low activity;

Table 1.3:- Tissue specific distribution of esterases in *Macrobrachium rosenbergii*

Rm values / 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		

Table 1.4:-Tissue specific distribution of esterases in *Penaeus monodon*

Rm values / Tissues	**1**	**2**	**3**
	.85	**.50**	**.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;

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