



TISSUE AND SPECIES SPECIFIC DISTRIBUTION OF ESTERASES IN LABEO ROHITA AND PUNTIUS SARANA OF CYPRINIFORMES ORDER

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Abstract; Tissue esterases polymorphism were studied in two fishes of *Labeo rohita* and *Puntius sarana* of cypriniformes fishes in six tissues viz; Gill, Liver, Intestine, Muscle, Brain and Eye. *Labeo rohita* and *Puntius sarana* exhibit distinct esterase profiles across various tissues. *Labeo rohita* shows five esterase zones with dominant CE and ER esterases. In contrast, *Puntius sarana* also has five zones, but ER and ChE esterases are more dominant,

Key words: Electrophoresis, esterases, Gill, liver, 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 (C₂-C₄) 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)

Esters-----Alcohol+Acid+H₂O

Alcohol+ Carboxylic acid ↔ Ester + water

R-OH + R-COOH..... R-COO-R +H₂O

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; Bornscheuer *et al.*, 2002; Jaeger and Eggert, 2002; Reetz 2002; Maurer, 2004; Cammarota and Freire, 2006; Hasan *et al.*, 2006). The high region and spatio 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 to catalyze a variety of esterases 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

Fishes 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. Fishes were immobilized by hitting them on the head and the tissues were dissected out of animals. Six tissues were selected for the study: gill, liver, 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) Liver - 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), Hart and Cook (1976), Haritos and Salamastrikis (1982) and Lakshmi pathi and Reddy (1989) on the basis of their sensitivity to 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 sulphhydryl 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

LABEO ROHITA

Gill: There are three esterase zones in the tissue with Rm values .50, .41 and .25 with low activity. Among these, the zones with Rm values .50 and .25 are ER esterases and other zone is CE esterases with low activity

Liver: Liver exhibited three zones with Rm values .50, .33 and .25 with moderate activity. Among three zones, the zone with Rm values .50 and .33 were CE esterases and .25 is ER esterase.

Intestine: This tissue exhibited three zones on the zymogram with Rm values .58, .50 and .33. Among these, the zones with Rm values .50 and .33 were ER esterases with moderate activity and the zone with Rm value .58 is Esdp esterases.

Muscle: Muscle exhibited two zones on the zymogram with Rm values .41 and .33. The zone with Rm .41 is ChE esterase and another zone is CHsp esterase.

Brain: There are three zones in the Brain with Rm values .50, .41 and .25. Among these, the zone with Rm values .50 and .25 were classified as choline esterases. The zone with Rm value .41 is ArE esterase.

Eye: The eye consisting of two active zones on the zymogram with Rm values .41 and .33. Both of them are carboxyl esterases with moderate activity.

Esterase found in various tissues of *Labeo rohita* (Table 1.1 and 1.3) exhibited five zones (EST 1, 2, 3, 4, 5) with Rm value .58, .50, .41, .33 and .25 respectively. Among the five zones of esterases, the zone with Rm values .50, .41 and .33 exhibit in four tissues. The zone with Rm value .50 was found in gill, liver, intestine and brain. In gill and intestine it is an ER esterase, liver and brain exhibit CE and ChE esterases respectively. The zone with Rm .41 was found in gill, muscle, brain and eye with CE esterase in gill and eye. In muscle it is a ChE esterases, in brain it is an ArE esterase. The zone with Rm value .33 was found in liver, intestine, muscle and eye. In liver and eye, it is CE esterase but in muscle and intestine have CHsp and ER esterases are found respectively. The zone with Rm value .25 was found in gill, liver, and brain. In gill and liver it is ER esterases but in brain ChE esterase is found. The zone with Rm value .58 is found intestine with Esdp esterase. The esterase patterns observed in *Labeo rohita* are CE and ER esterases are dominant among all the tissues and followed by other esterases.

PUNTIUS SARANA

Gill: - These are four active esterase zones on the zymogram with Rm values .75, .66, .50 and .33 respectively. Among these, the zone with Rm value .50 was inhibited by Paraoxon and Eserine so it was classified as ChE esterases. While the zone with Rm values .75 and .66 were classified as ER esterases and Rm value .33 is CHsp esterase.

Liver: - Liver exhibited four zones with Rm values .75, .66, .50 and 33. The zones with Rm values .75, .66 was not inhibited any of the inhibitors used so they were classified into ER esterases and .33 was inhibited by paraoxon and Eserine So it was classified as ChE esterases and the zone with Rm value .50 was classified into CE esterases.

Intestine: -This tissue exhibited four active zones with Rm values .86, .66, .50 and .33. The zones with Rm values .86 and .66 were classified as ER and Esdp esterases respectively but the zone with Rm values .50 and .33 were inhibited by Paraoxon and Eserine so they were classified as ChE esterases. All zones are exhibited higher activity except Rm.86 zone.

Muscle: -Muscle exhibited three active zones on the zymogram with Rm values .66, .50 and .33. The zones with Rm values .50 and .66 were classified as CHsp esterases, while the zone with Rm value .33 was carboxyl esterases. All the zones exhibited moderate activity.

Brain: - There are three esterase zones in this tissue with Rm values .75, .50, and .33. The zones with Rm values .75, .50 and .33 were classified as ER, Esdp and ArE esterases respectively with moderate activity

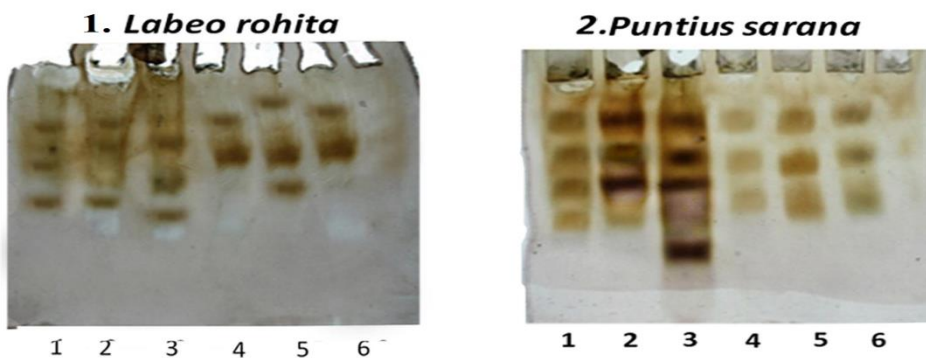
Eye: - This tissue exhibited three zones on the zymogram with Rm values .75, .50 and .33 with moderate activity. The zone with Rm value .75 is a CHsp esterase and with Rm value .50 is a Carboxyl esterase while the zones with Rm value .33 is a ChE esterase.

Based on relative mobilities, the esterase zones are found in the tissues of *Puntius sarana* (Table 1.2 and 1.4) and can be grouped into five zones with Rm values .86, .75, .66, .50 and .33. Each tissue has its own characteristic patterns. The zones with Rm values .50 and .33 are present in all the tissues. The zone with Rm value .50 is a ChE esterase which is found in gill and intestine, CE esterase is found in liver and eye, but in brain and muscle Esdp, CHsp esterases are found. The zones with Rm.33 exhibit ChE esterase in liver, intestine and eye. But in gill, brain and muscle exhibit CHsp, ArE and CE esterases respectively. The zones with Rm .75 and .66 exhibits in four tissues. The zone with Rm value .75 is ER esterases in gill, liver and brain but in eye it is CHsp esterase. The zone with Rm value .66 is an ER esterase in gill and liver. But intestine and muscle exhibit Esdp, CHsp esterases respectively. The zone with Rm value .86 is a fast moving zone which was found in intestine with ER esterase. Among the various tissues, gill, liver and intestine exhibit four zones and followed by remaining tissues (3 zones each). Among the type of esterase, ER esterase is predominant in almost all the tissues and followed by the ChE esterases. But only one ArE esterase is found in brain with fast moving zones.

CONCLUSION

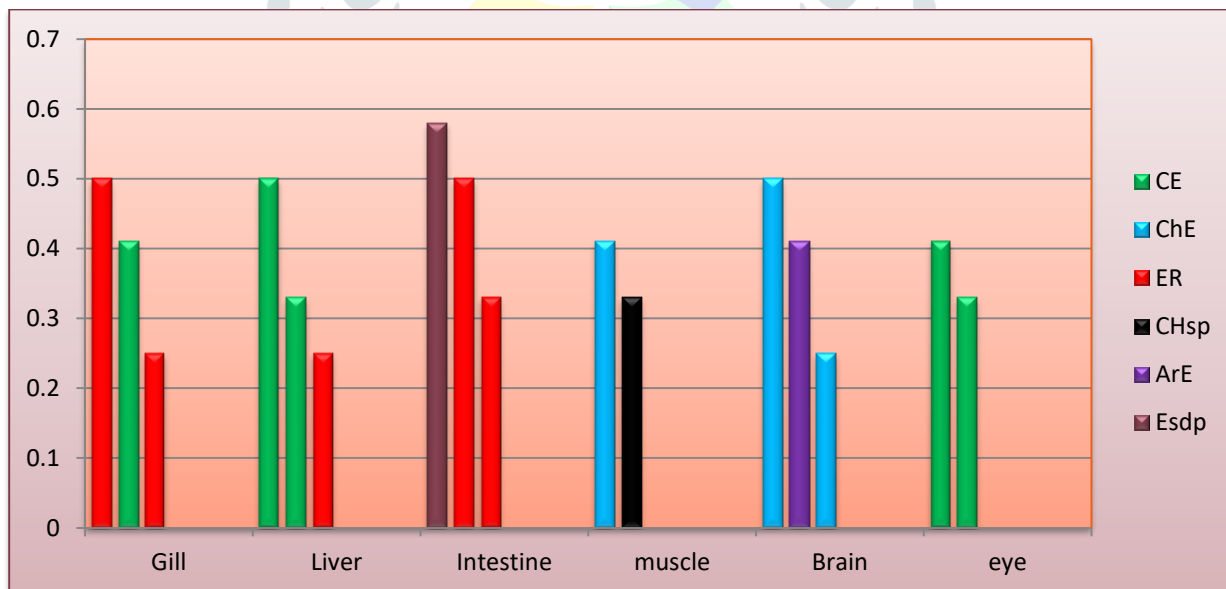
Labeo rohita and *Puntius sarana* exhibit distinct esterase profiles across various tissues. *Labeo rohita* shows five esterase zones (Rm values .58, .50, .41, .33, .25) with dominant CE and ER esterases, and activity levels ranging from low to moderate. The zones with Rm values .50, .41, and .33 appear in multiple tissues, highlighting the prevalence of these esterases. In contrast, *Puntius sarana* also has five zones (Rm values .86, .75, .66, .50, .33), but ER and ChE esterases are more dominant, with activity levels generally higher, including a unique fast-moving zone (.86) in the intestine. The zones with Rm values .50 and .33 are consistently found in all tissues of *Puntius sarana*, marking a distinct difference in esterase distribution and activity between the two species.

PLATE -I



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

Tissue specific distribution of esterases in *Labeo rohita*



Tissue specific distribution of esterases in *Puntius sarana*

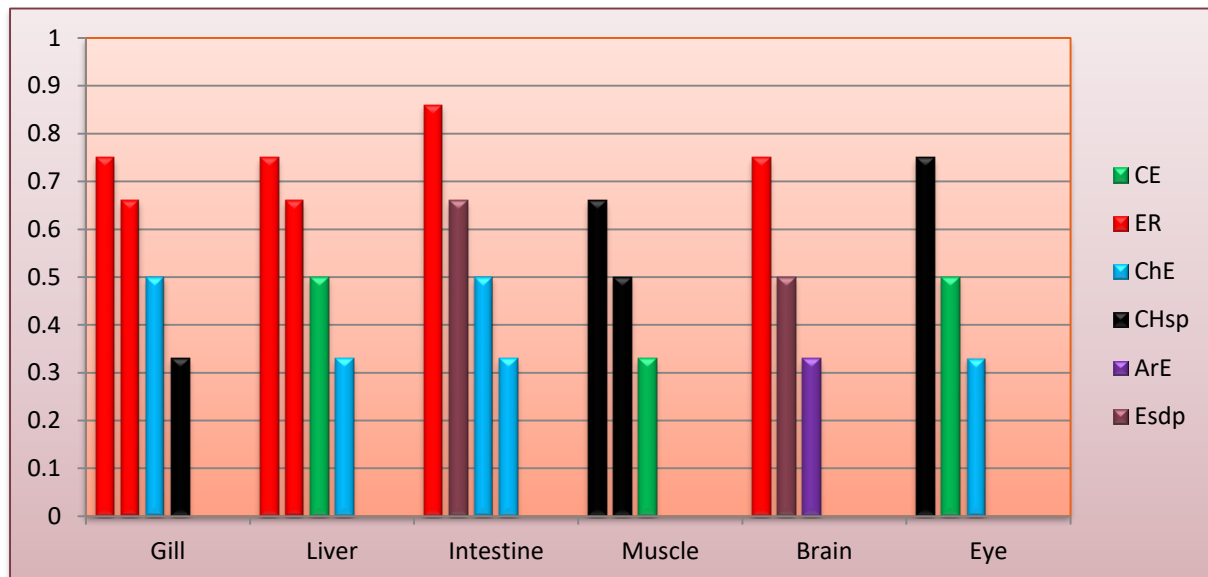


Table 1.1:- Inhibitor sensitivity of individual esterase zones in *Labeo rohita*

Name of Tissue	Gill			Liver			Intestine			Muscle		Brain			Eye	
Rm values	.50	.41	.25	.50	.33	.25	.58	.50	.33	.41	.33	.50	.41	.25	.41	.33
Activity	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++
pCMB	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+
Eserine	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+
Paraoxon	+	-	+	-	-	+	-	+	+	-	-	-	+	-	-	-
Classification	ER	CE	ER	CE	ER	Esdp	ER	ER	ER	ChE	CHsp	ChE	ArE	ChE	CE	CE

Table 1.2:- Inhibitor sensitivity of individual esterase zones in *Puntius sarana*

Name of Tissue	Gill				Liver				Intestine				Muscle		Brain			Eye			
Rm values	.75	.66	.50	.33	.75	.66	.50	.33	.86	.66	.50	.33	.66	.50	.33	.75	.50	.33	.75	.50	.33
Activity	++	++	++	++	++	++	++	++	++	+++	++	++	++	++	++	++	++	++	++	++	++
pCMB	+	+	+	-	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-	-	-
Eserine	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	+	+	+	-	+	-
Paraoxon	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-
Classification	ER	ER	ChE	CHsp	ER	ER	CE	ChE	ER	Esdp	ChE	ChE	CHsp	CHsp	CE	ER	Esdp	ArE	CHsp	CE	ChE

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 1.3:- Tissue specific distribution of esterases in *Labeo rohita*

Tissues	Rm values				
	1	2	3	4	5
	.58	.50	.41	.33	.25
1) Gill		+ ER	+ CE		+ ER
2) Liver		++ CE		++ CE	++ ER
3) Intestine	++ Esdp	++ ER		++ ER	
4) Muscle			++ ChE	++ CHsp	
5) Brain		++ ChE	++ ArE		++ ChE
6) Eye			++ CE	++ CE	

Table 1.4:- Tissue specific distribution of esterases in *Puntius sarana*

Tissues	Rm values				
	1	2	3	4	5
	.86	.75	.66	.50	.33
1) Gill		++ ER	++ ER	++ ChE	++ CHsp
2) Liver		++ ER	+++ ER	+++ CE	++ ChE
3) Intestine	++ ER		+++ Esdp	+++ ChE	+++ ChE
4) Muscle			++ CHsp	++ CHsp	++ CE
5) Brain		++ ER		++ Esdp	++ ArE
6) Eye		++ CHsp		++ CE	++ ChE

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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