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Original Research Article

Tissue esterase polymorphism of *Cyprinus Carpio* and *Puntius Sarana* of cypriniformes order

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Keywords

Electrophoresis; esterases; Gill; liver; intestine;

A B S T R A C T

Tisuue esterase polymorphism were studied in two fishes of *Cyprinus carpio* and *Puntius sarana* of cypriniformes fishes in six tissues viz; Gill, Liver, Intestine, Muscle, Brain and Eye. ER esterases are noticed in gill, liver and but CHsp and ChE esterases are noticed in muscle and brain. While eye exhibited CE esterases in both fish. One zone of ArE esterases are noticed in brain of both fishes.

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

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 %, 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:

Carboxylesterases (CE): These esterases were sensitive to inhibition by the organophosphate but were not affected by physostigmine or pCMB.

Arylesterases (ArE): They were sensitive to inhibition by sulphydryl Agent pCMB and were not affected by paraoxon or physostigmine.

Cholinesterases (ChE): Enzymes, which were inhibited by paraoxon and physostigmine.

ER Esterases: Enzyme which were not affected by any of the three inhibitors used.

Esdp Esterases: Enzymes, which were inhibited by pCMB and paraoxon.

Ese Esterases: Enzymes, which were inhibited by physostigmine alone.

CHsp Esterases: Enzymes, which were inhibited by paraoxon, physostigmine and pCMB.

Results and Discussion

Cyprinus Carpio

Gill

There are three zones of esterases in Gill with Rm values .66, .50, .25. Among these, the zone with Rm value .66 and .25 are ER esterases and the zone with Rm

value .50 was CE esterase with moderate activity.

Liver

Liver contains three active zones on the zymogram with Rm values .66, .50 and .25. Out of these, the zone with Rm value .50 was classified into CE esterases and Rm value .25 was classified as ChE esterase. The zone with Rm value .66 was not inhibited by any of the inhibitors used so it was classified as ER esterases.

Intestine

This tissue exhibited four zones with Rm values .75, .66, .50 and .25, with Rm .75 and .66 was classified as Ese, CHsp esterases respectively. Rm value .50 and .25 is ChE esterases. The zone with Rm value .50 exhibited higher activity and other three zones with moderate activity.

Muscle

Muscle exhibited three zones on the zymogram with Rm values .50, .41 and .33 with moderate activity. The zone with Rm value .50 and .33 were classified in to CHsp esterases. While the zone with Rm value .41 was classified into ChE esterase.

Brain

There are three zones with Rm values .66, .50, .33. The zones with Rm values .66, .50, .33 were classified into CHsp, CE and ChE esterases with moderate activity.

Eye

Eye exhibited three active zones with Rm values .66, .50 and .33. The zones with Rm values .66 and .50 were CHsp and CE esterases and the zone with Rm value .33 was ChE esterase with moderate activity.

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 0.50 and 0.33 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.

Table.1.1 Inhibitor sensitivity of individual esterase zones in *Cyprinus carpio*

Name of Tissue	Gill			Liver			Intestine				Muscle			Brain			Eye		
Rm values	.66	.50	.25	.66	.50	.25	.75	.66	.50	.25	.50	.41	.33	.66	.50	.33	.66	.50	.33
Activity	++	++	++	++	++	+++	++	+++	+++	++	++	++	++	++	++	++	++	++	++
pCMB	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	+	-	+	+
Eserine	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	+	-
Paraoxon	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Classification	ER	CE	ER	ER	CE	ChE	Ese	CHsp	ChE	ChE	CHsp	ChE	CHsp	CHsp	CE	ChE	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.1.2 Tissue specific distributions of esterases in *Cyprinus carpio*

Rm values / Tissues	1	2	3	4	5	6
	.75	.66	.50	.41	.33	.25
1) Gill		++ ER	++ CE			++ ER
2) Liver		++ ER	++ CE			+++ ChE
3) Intestine	++ Ese	+++ CHsp	+++ ChE			++ ChE
4) Muscle			++ CHsp	++ ChE	++ CHsp	
5) Brain		++ CHsp	++ CE		++ ChE	
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;

Table.1.3 Inhibitor sensitivity of individual esterase zones in *Puntius sarana*

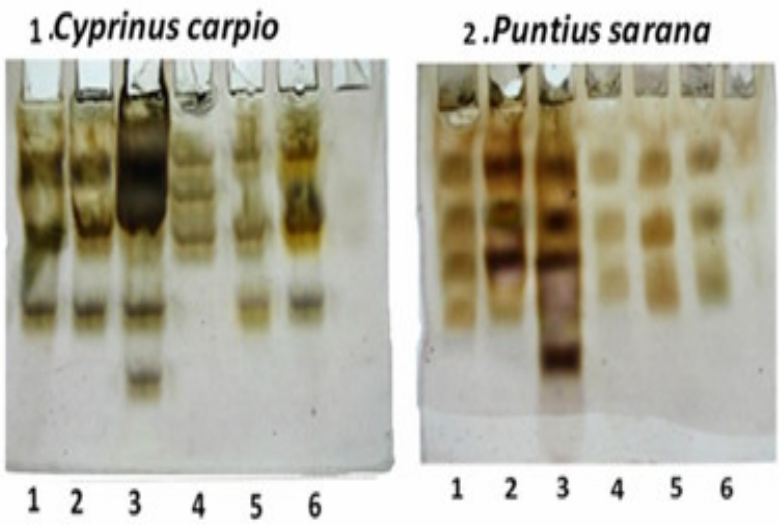
Name of Tissue	**Gill**				**Liver**				**Intestine**				**Muscle**			**Brain**			**Eye**		
Rm values	.75	.66	.50	.33	.75	.66	.50	.33	.86	.66	.50	.33	.66	.50	.33	.75	.50	.33	.75	.50	.33
Activity	++	++	++	++	++	+++	+++	++	++	+++	+++	+++	++	++	++	++	++	++	++	++	++
pCMB	+	+	+	-	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-	+	+
Eserine	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	+	+	+	-	+	-
Paraoxon	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-
Classification	ER	ER	Ch E	CH sp	ER	ER	CE	Ch E	ER	Esd p	Ch E	Ch E	CH sp	CH sp	CE	ER	Esd p	Ar E	CH sp	CE	Ch E

Table.1.4 Tissue specific distribution of esterases in *Puntius sarana*

Rm values / **Tissues**	**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;

Figure.1



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

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

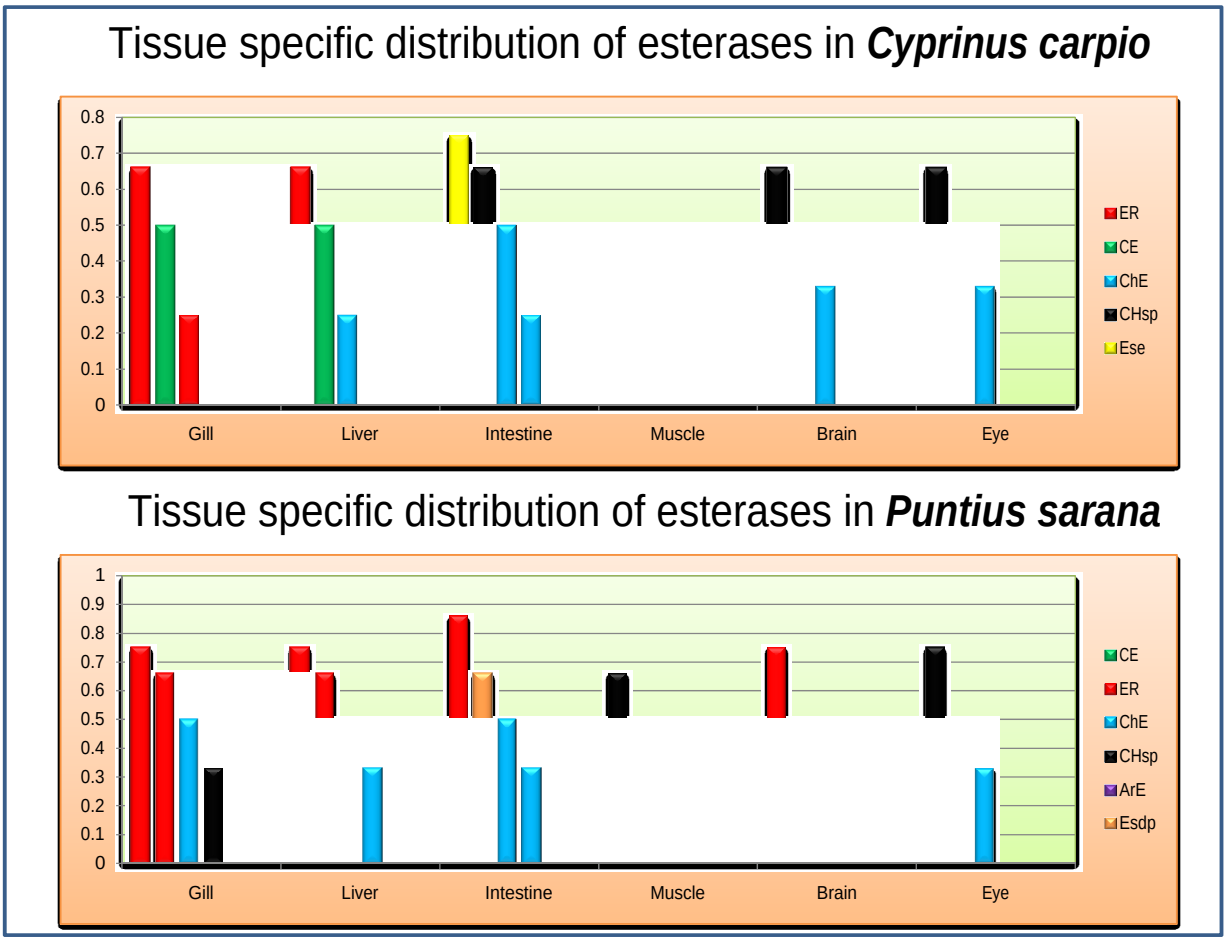
Eye

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.

Esterases found in various tissues of *Cyprinus carpio* (Table 1.2)are grouped into six zones, with Rm .75, .66, .50, .41, .33, and .25 respectively. Among six zones, the zones with Rm values .66 and .50 are found in all tissues. The zone with Rm value .66 exhibited ER esterases in gill and liver. The remaining tissues exhibit CHsp esterases. The zones with Rm value .50 exhibit CE esterases in gill, brain, and eye and in liver. The intestine exhibit ChE esterase, while muscle exhibit CHsp esterase.

The zone with Rm .33 was exhibited in muscle, brain and eye with ChE esterase in brain and eye. But muscle exhibit CHsp esterase. The zone with Rm value.25 was found in three tissues, viz., gill, liver and intestine. It is ChE esterase in liver and intestine, but in gill it is an ER esterase. The zone with Rm value .75 was found in intestine which is Ese esterase. The zone with Rm value.41 was found only in muscle with ChE esterase. Out of six tissues examined, five tissues are exhibit three zones. Among the types of esterases, ChE and CHsp esterases are predominant. Only one Ese esterases are found in intestine with fast moving zones.

Figure.2



Based on relative mobilities, the esterase zones are found in the tissues of *Puntius sarana* (Table 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.

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