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Tissue esterases polymorphism of amphipnous cuchia and mastacembelus armatus

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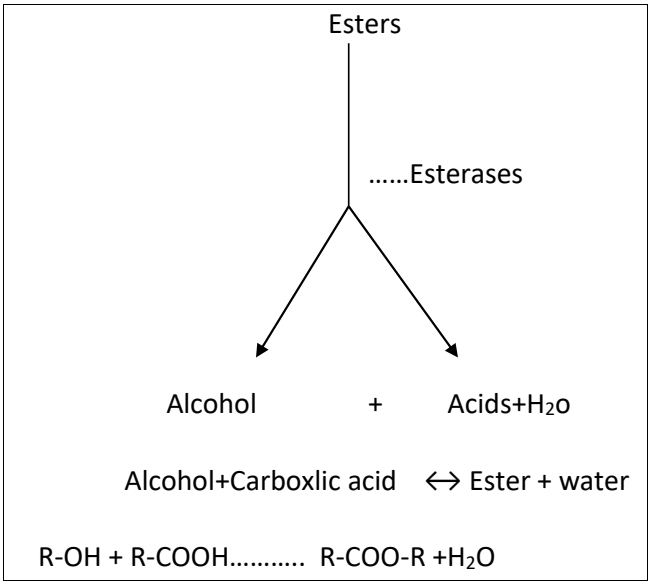
Abstract
Tisuue esterases polymorphism were studied in two fishes of *Amphipnous cuchia* and *Mastacembelus armatus* of fishes belonging to different orders ie synbranchiformes and Mastocembeliformes. six tissues of esterases patterns were studied *viz*; Gill, Liver, Intestine, Muscle, Brain and Eye. ChE esterases are predominant in amphinous cuchia in all tissues excepts in liver and CE esterases are predominant in all tissues of Mastacembelus armatus. CHsp esterases are noticed in two fishes of liver tissue. ER estrases are noticed in gill liver, intestine and muscle in Amphinous cuchia and whereas in *Mastacembelus armatus* ER esterases are noticed in gill intestine and brain etc.

Keywords: 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 (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]



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, 1, 7, 13, 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]. Tissue esterases polymorphism of marcrobhachium rosenbergi and penaeus indicus (vimala *et al* 2018) [18] Their ability was to catalyze a variety of esterase without the aid of cofactors is an additional advantage (Bornscheuer, 2002) [1]. Tissue estrases of lamellidens corrianus fresh water mussel was studied (Swapna *et al* 2014) [16]. Esterases play a vital role in the metamorphosis of insects (Quan – You Yu *et al*., 2009) [11]. tissue specific distribution of esterases are studied in Cyprinus Carpio and puntius sarana of cypriniformes order (vimala *et al* 2014) [16]

Meterils and methods

The adult 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) [6], Hart and Cook (1976) [9], Haritos and Salamastrikis (1982) 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

Amphipnous cuchia (Hamilton, 1822)

Gill

This tissue contain four esterase active zones on the zymogram with Rm values0.66,0.58,0.33 and0.25, out of these, the zones with Rm value0.66 and0.25 were inhibited by paraoxon and eserine. So they were classified as ChE esterase. The zone with Rm value0.58 and0.33 were classified as ER and CE esterases respectively. All the zones were exhibited higher activity.

Liver

Liver exhibited four esterase zones with Rm value0.66,0.58,0.33 and0.25. Among these, the zone with Rm value0.58 and0.33 were inhibited by Paraoxon alone. So they were classified as CE esterase. While the other two zones with Rm value0.66 and0.25 were classified ER and CHsp esterase respectively.

Intestine

There are four active esterase zones were present on the zymogram with Rm value0.66,0.58,0.33 and0.25. The zone with Rm value0.33 and0.25 were classified as ChE esterases with moderate activity while the zone with Rm value0.66 and0.58 were classified CE esterase and ER esterases respectively.

Muscle

Muscle contains three esterase zones on the zymogram with Rm value0.66,0.33 and0.25. Among these, the zone with Rm value0.66 and0.25 were classified as ER and ChE esterases with moderate activity. The zone with Rm value0.33 was inhibited by only paraoxon. So it was classified as CE esterase with low activity.

Brain

This tissue exhibited three zones with Rm value0.66,0.41 and0.33. The zones with Rm value0.33 and0.41 were classified as ChE esterases. While the zone with Rm value0.66 was inhibited by only paraoxon. So it was classified as CE esterase with moderate activity.

Eye

Eye exhibited three active esterase zones, with Rm value0.66,0.41 and0.33 with higher activity. The zones with Rm value0.41 and0.33 were inhibited by paraoxon and eserine. So it was classified as ChE esterases. While the zone with Rm value0.66 was classified as CE esterase.

Mastacembelus armatus (Lacepede 1800)

Gill

There are three zones were present on the zymogram with Rm value0.75,0.50,0.33, with ER, ChE, and CE esterases respectively. Among these, the zone with Rm value.75, 50 exhibited higher activity while other zone exhibited lower activity.

Liver

Liver tissue consisting of three esterase zones with Rm value0.75,0.50, and0.33. Among these, the zones with Rm value0.50 and0.33 were inhibited by paraoxon alone. So they were classified as CE esterases. While the zone with Rm0.75 exhibit CHsp esterase with higher activity.

Intestine

This tissue contains three active zones on the zymogram with Rm value0.75,0.50 and0.33 with ChE, ER and CE esterases respectively. The zone with Rm value0.75 exhibited higher activity and other remaining zones were exhibited moderate activity.

Muscle

There are three active esterase zones with Rm value0.75,0.50 and0.33. Among these, the zones with Rm value0.50 and0.33 were inhibited by Paraoxon and eserine. So they were classified as ChE esterase while the zone with Rm value0.75 was classified as CE esterases.

Brain

Brain tissue exhibited only two zones with Rm value0.75 and0.50. Among these, the zone with Rm value.75and.50 was classified as ER and CE esterases respectively with moderate activity.

Eye

This tissue also exhibited two active esterase zones on the zymogram with Rm value0.75 and0.50. Among these, the zone with Rm value0.75 exhibits moderate activity and other zone with lower activity both of these zones are CE esterases

Plate 1

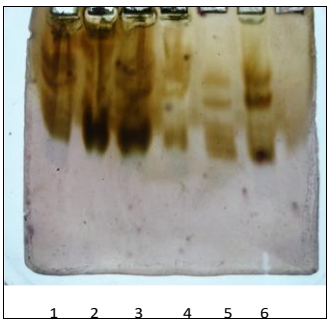


Fig 1: Amphipnous cuchia

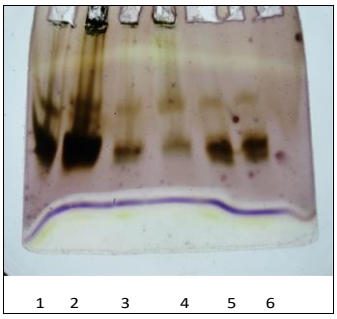


Fig 2: Mastecembelus armatus

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

Table 2. 5: Inhibitor sensitivity of individual esterase zones in *Amphipnous cuchia*

Name of Tissue	Gill				Liver				Intestine				Muscle			Brain			Eye		
Rm values	.66	.58	.33	.25	.66	.58	.33	.25	.66	.58	.33	.25	.66	.33	.25	.66	.41	.33	.66	.41	.33
Activity	+++	+++	+++	+++	++	++	++	++	++	++	++	++	++	+	++	++	+	+	++	++	++
pCMB	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Eserine	-	+	+	-	+	+	+	-	+	+	-	-	+	+	-	+	-	-	+	-	-
Paraoxon	-	+	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
Classification	ChE	ER	CE	ChE	ER	CE	CE	CHsp	CE	ER	ChE	ChE	ER	CE	ChE	CE	ChE	ChE	CE	ChE	ChE

Table 2.7: Inhibitor sensitivity of individual esterase zones in *Mastacembelus armatus*

Name of Tissue	Gill			Liver			Intestine			Muscle			Brain		Eye	
Rm values	.75	.50	.33	.75	.50	.33	.75	.50	.33	.75	.50	.33	.75	.50	.75	.50
Activity	+++	+++	+	+++	++	+	+++	++	++	++	++	++	++	++	++	+
pCMB	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Eserine	+	-	+	-	+	+	-	+	+	+	-	-	+	+	+	+
Paraoxon	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Classification	ER	ChE	CE	CHsp	CE	CE	ChE	ER	CE	CE	ChE	ChE	ER	CE	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 2.6: Tissue specific distribution of esterases in *Amphipnous cuchia*

Rm values / Tissues	1	2	3	4	5
	.66	.58	.41	.33	.25
1) Gill	+++ ChE	+++ ER		+++ CE	+++ ChE
2) Liver	++ ER	++ CE		++ CE	++ CHsp
3) Intestine	++ CE	++ ER		++ ChE	++ ChE
4) Muscle	++ ER			++ CE	++ ChE
5) Brain	++ CE		+ ChE	+ ChE	
6) Eye	+++ CE		+++ ChE	+++ ChE	

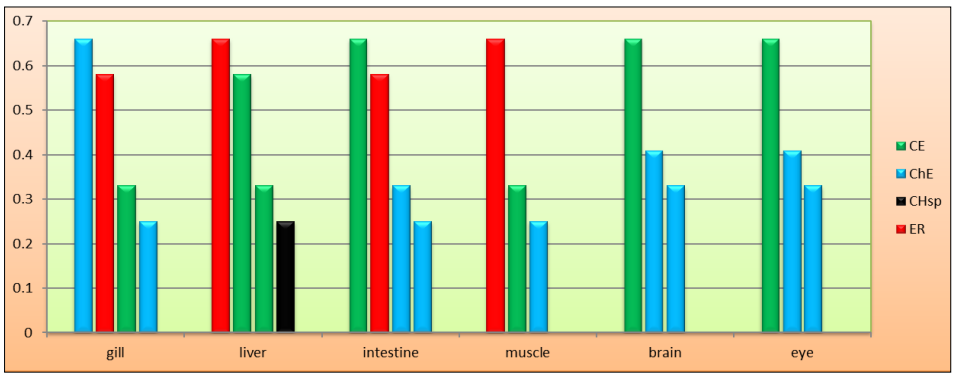


Fig 13: Tissue specific distribution of esterases in *Amphipnous cuchia*

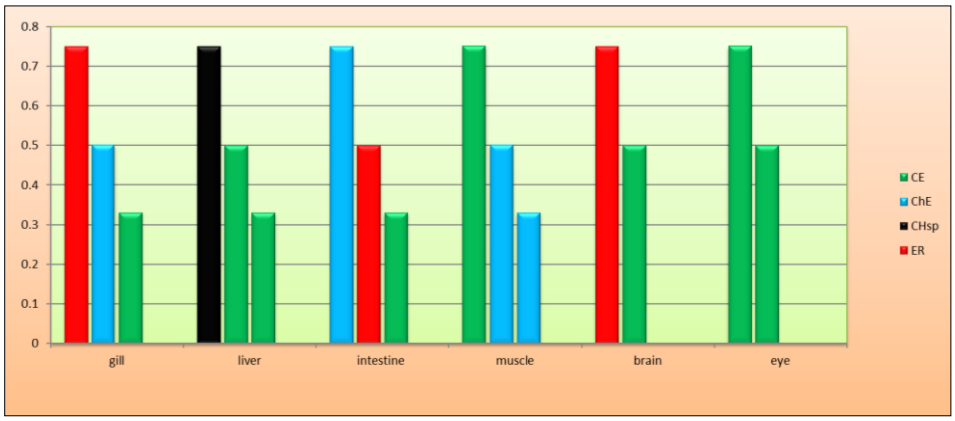


Fig 14: Tissue specific distribution of esterases in *Mastacembelus armatus*

Conclusions

Amphipnous cuchia (Table-2.6) exhibited five zones with Rm values0.66,0.58,0.41,0.33 and0.25 in all the six tissues. The zone with Rm value0.66 was found in all the tissues. It is a ChE esterase in gill and it is CE esterases in intestine, brain and eye. But muscle and liver exhibited ER esterases. The zone with Rm value0.58 was found in three tissue *viz*., gill, liver, and intestine with ER esterase in gill and intestine, but in liver it is CE esterase. The zone with Rm value0.41 was found in brain and eye with ChE esterases. The zone with Rm0.33 was present in all the tissues with moderate activity. It is CE esterase in gill, liver and muscle. But it is ChE esterase in intestine, eye and brain. The zone with Rm value0.25 was found in gill, liver, lntestine and muscle. It is ChE esterase in gill, intestine and muscle. But in liver it is CHsp esterase.

Mastacembelus armatus (Table- 2.8) consist of three zones with Rm values0.75,0.50 and0.33. The zone with Rm value0.75 was found in all tissues. It is ER esterases in gill and brain, CHsp esterase in liver, ChE esterase in intestine, and CE esterase in muscle and eye. The second zone with Rm value0.50 is also present in all the tissues with different activity. ChE esterases are found in gill and muscle. While CE esterases are found in liver, brain and eye. But in intestine it is ER esterase. The third zone with Rm value0.33 is found in four tissues *viz*., gill, liver, intestine, and muscle. Gill, liver and intestine exhibited CE esterases and in muscle it is ChE esterase.

The two fishes of different orders exhibits highly tissue specific and species specific in esterase patterns. in Amphinous cuchia belongs to order synbranchiformes and Mastacembelus armatus belongs to mastacembeliformes order. Among the two fishes ampinous cuchia exhibits CHE estrases are predominanant and present in all the tissues except in liver and CE estrases are present in all the tissues next to CHE esterases. ER esterases are present in only gill liver intestine and muscle. whereas in Mastecembelus armatus exhibits CE esterase are predominant in all tissues and CHE esterases are present in gill, intestine and muscle only. CHsp esterases are present in two fishes of liver tissue. But in CHsp esterases are present in liver in both fishes.

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