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TISSUE ESTERASES POLYMORPHISM OF TILAPIA MOSSAMBICA AND NOTOPTERUS NOTOPTERUS

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Abstract: Tisuue esterases polymorphism were studied in two fishes of Tilapia mossambica of Perciformes order and Notopterus notopterus of Osteoglossiformes order, six tissues were selected viz; Gill, Liver, Intestine, Muscle, Brain and Eye. In Tilapia mossambica ,CE esterases are predominant and present in all the tissues except muscle and eye and ChE esterases are noticed in muscle and eye. but In Notopterus notopterus, CHsp esterases are more dominant in all tissues except in muscle but this CHsp esterases are noticed common in both the fishes of muscle.

Index Terms **- Electrophoresis, esterases, Gill, liver, intestine, muscle brain, and eye**

I. 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)

Esters----------alcohol+Acid+H2O

Alcohol+Carboxlic acid ↔ Ester + water

R-OH + R-COOH…….. 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). 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).

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

II. MATERIALS 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), 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.

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

III. RESULTS

3.1 *Tilapia mossambica* (Peters, 1852)

Gill: - There are two zones on the zymogram with Rm values.96 and .75 with moderate activity. Among these two zones, the zone with Rm value .75 was classified as the ER esterase and the zone with Rm value .96 was classified as CE esterase.

Liver: - This tissue exhibited three active zones with Rm values .96, .75 and .66. The zones with Rm values .96 and .75 were classified as CE esterases with higher activity. While the zone with Rm value .66 was an ER esterase with moderate activity.

Intestine: -Intestine exhibited three zones with Rm values .96, .75 and .66. The zone with Rm value .75 was classified as CHsp esterase with higher activity, while the zones with Rm value .96 and .66 were classified as CE and ER esterases respectively with moderate activity.

Muscle: - This tissue contains only two zones with Rm values .75 and .66 with moderate activity. Among these, the zone with Rm .75 was classified as CHsp esterase and while zone with Rm value .66 was classified as ChE esterase.

Brain: - Brain exhibited two zones with Rm values .75 and .66. Among these, the zone with Rm value .75 was classified as CE esterase while the other zone with Rm value .66 was classified as ChE esterase with moderate activity.

Eye: - This tissue exhibited two active zones on the zymogram with Rm values .75 & .66 with moderate activity. Both of them are inhibited by paraoxon and eserine. They were classified as ChE esterases.

3.2 Notopterus notopterus (Pallas, 1769)

Gill: - This tissue exhibited three active esterase zones on the zymogram with Rm values .75, .58 and .33. The zones with Rm values .58 and .33 were inhibited by eserine and paraoxon. So they were classified as ChE esterase while other zone with Rm value .75 was classified as ER esterase with moderate activity.

Liver: - There are four active esterase zones with Rm values .83, .75, .66 and .33. Among these, the zones with Rm values .75 and .66 were classified as CE and ER esterases with moderate activity. While the other two zones with Rm values .83 and .33 were classified as ER and ChE esterases with moderate activity.

Intestine: - The Intestine contains three active esterase zones with Rm values .75, .66 and .33 with CE, ChE and ER esterases respectively. Among these, the zones with Rm values .66 and .33 were higher activity while the other zone with Rm value .75 exhibited moderate activity.

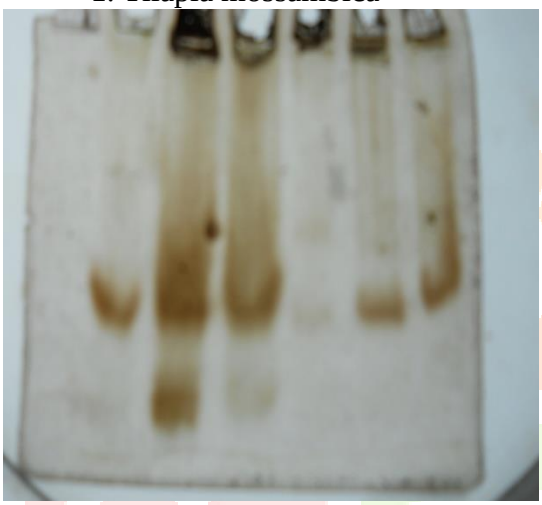
Muscle:-There are two active esterase zones on the zymogram with Rm values .75 and .33 with CE and CHsp esterases respectively. Both of zones exhibited moderate activity.

Brain:-Brain exhibited two active esterase zones with Rm value .75 and .58. Among these, the zone with Rm value .75 was not inhibited by any of the inhibitor used. So it was classified as ER esterase and other zone with Rm value .58 was classified as ChE esterase.

Eye: - This tissue contains two active zones with Rm value .75 and .58. The zone with Rm value .75 was inhibited by only paraoxon. So it was classified as CE esterase. Other zone with Rm value .58 was inhibited by paraoxon and eserine. So they were classified as ChE esterase with moderate activity.

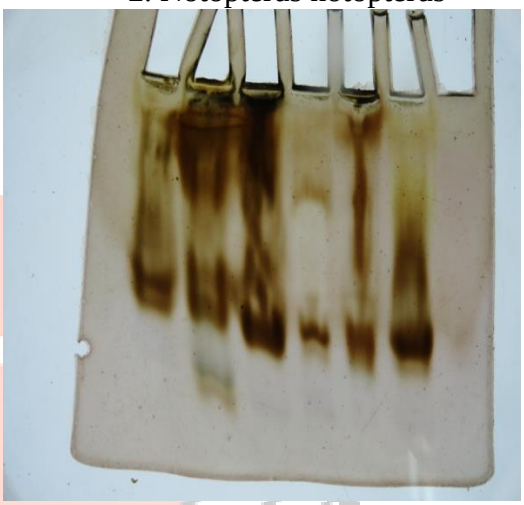
ESTERASES PLATE

1. Tilapia mossambica



1 2 3 4 5 6

2. Notopterus notopterus



1 2 3 4 5 6

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

Table 3.1:- Inhibitor sensitivity of individual esterase zones in *Tilapia mossambica*

Name of Tissue	Gill		Liver			Intestine			Muscle		Brain		Eye	
Rm values	.96	.75	.96	.75	.66	.96	.75	.66	.75	.66	.75	.66	.75	.66
Activity	+	++	++	+++	++	+	+++	++	+	+	+++	++	++	+
pCMB	+	+	+	+	+	+	-	+	-	+	+	+	+	+
Eserine	+	+	+	+	+	+	-	+	-	-	+	-	-	-
Paraoxon	-	+	-	-	+	-	-	+	-	-	-	-	-	-
Classification	CE	ER	CE	CE	ER	CE	CHsp	ER	CHsp	ChE	CE	ChE	ChE	ChE

Table 3.2:- Tissue specific distribution of esterases in *Tilapia mossambica*

Rm values / Tissues	1	2	3
	.96	.75	.66
1) Gill	+ CE	+ ER	
2) Liver	++ CE	+++ CE	++ ER
3) Intestine	+ CE	+++ CHsp	++ ER
4) Muscle		+ CHsp	+ ChE
5) Brain		+++ CE	++ ChE
6) Eye		++ ChE	+ ChE

Table 3.3: - Inhibitor sensitivity of individual esterase zones in *Notopterus notopterus*

Name of Tissue	Gill			Liver				Intestine			Muscle		Brain		Eye	
Rm values	.75	.58	.33	.83	.75	.66	.33	.75	.66	.33	.75	.33	.75	.58	.75	.58
Activity	+++	++	++	++	++	++	++	+++	+++	++	+++	++	+++	++	+++	++
pCMB	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Eserine	+	-	-	+	+	+	-	+	-	+	+	-	+	-	+	-
Paraoxon	+	-	-	+	-	+	-	-	-	+	-	-	+	-	-	-
Classification	ER	ChE	ChE	ER	CE	ER	ChE	CE	ChE	ER	CE	CHsp	ER	ChE	CE	ChE

Table 3.4:- Tissue specific distribution of esterases in *Notopterus notopterus*

Rm values / Tissues	1	2	3	4	5
	.83	.75	.66	.58	.33
1) Gill		+++ ER		++ ChE	++ ChE
2) Liver	++ ER	++ CE	++ ER		++ ChE
3) Intestine		+++ CE	+++ ChE		+++ ER
4) Muscle		+++ CE			++ CHsp
5) Brain		+++ ER		++ ChE	
6) Eye		+++ 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
Tissue specific distribution of esterases in ***Tilapia mossambica***

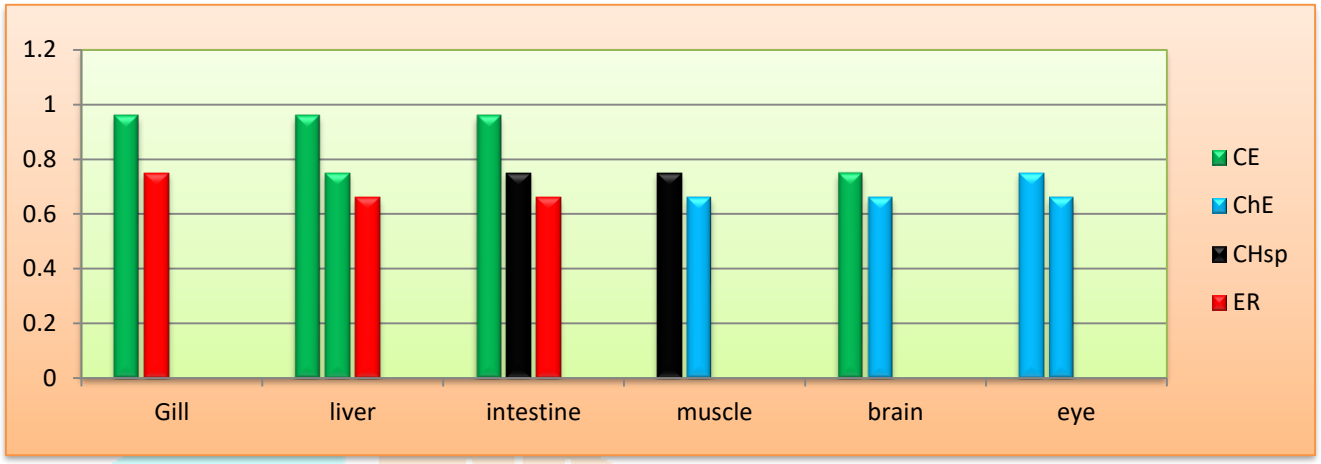
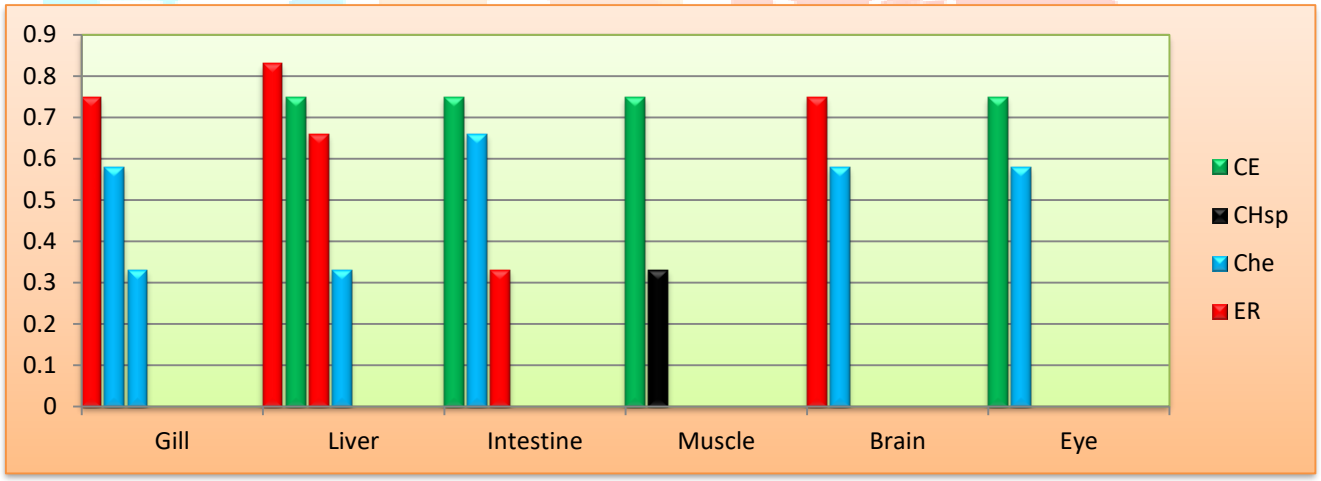


Figure. 2
Tissue specific distribution of esterases in ***Notopterus notopterus***



IV.CONCLUSION

Tissue specific distribution of esterases in *Tilapia mossambica* (Table -3.2) exhibited only three zones with Rm values .96, .75, and .66. Among these, the zone with Rm value .96 was present in gill, liver and intestine with CE esterases. The zone with Rm value .75 is present in all the tissues, with CE esterases in liver and brain, while in muscle and intestine it is CHsp esterase, gill and eye exhibit ER and ChE esterases respectively. The zone with Rm value .66 was also present in five tissues with ER esterases in liver, and intestine excepting gill,. ChE esterases are present in muscle, brain and eye.

Tissue specific distribution of esterases in *Notopterus notopterus* is presented in Table- 3.4. *Notopterus notopterus* has five zones in all the tissues, with Rm values .83, .75, .66, .58 and .33. The zone with Rm value .83 is exhibited in only one tissue i.e liver with ER esterase. And the zone with Rm value .75 was found in all the tissues. In liver, intestine, muscle and eye it is CE esterase and in gill and brain it is ER esterases. The zone with Rm value .66 was found in two tissues viz., liver and intestine. It is an ER esterase in liver and

ChE esterase in intestine. The zone with Rm value .58 is exhibited in three tissue viz., gill, brain and eye. It is ChE esterases in gill, brain and eye. The zone with Rm value .33 was found in four tissues viz., gill, liver, intestine and muscle. It is ChE esterase in gill and liver. But intestine and muscle exhibit ER esterase and CHsp esterase respectively**. In Tilapia mossambica ,CE esterases are predominant and present in all the tissues except muscle and eye** and ChE esterases are noticed in muscle and eye. but **In Notopterus notopterus, CHsp esterases are more predominant in all tissues** except in muscle but this CHsp esterases are noticed common in both the fishes of muscle and ER esterases are noticed in gill liver and intestine of both the fishes

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