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COMPARATIVE STUDY OF ESTERASES IN TWO FRESH WATER FISHES *(HETEROPNEUSTES FOSSILIS* AND *LABEO ROHITA)*

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ABSTRACT

Tissue esterase polymorphism was studied in two freshwater fishes, *Heteropneustes fossilis* and *Labeo rohita*, in five different tissues, namely gill, liver, intestine, muscle, and brain. *Heteropneustes fossilis* and *Labeo rohita* exhibited distinct esterase profiles across the tissues examined. *Heteropneustes fossilis* exhibited four esterase zones, whereas *Labeo rohita* showed three esterase zones with different Rm values. In *Heteropneustes fossilis*, EST-1 was deeply stained (+++) in the gill and brain tissues only, while EST-2 was deeply stained (+++) in the liver and intestine tissues. In *Labeo rohita*, EST-1 was deeply stained (+++) only in the gill tissue, whereas EST-2 was deeply stained (+++) in the liver, intestine, and muscle tissues.

Keywords: *Electrophoresis, esterases, gill, liver, intestine, muscle, brain*.

INTRODUCTION

Esterases are hydrolytic enzymes that split esters into an acid and an alcohol. Two categories of these enzymes were first recognized by Lovenhart (1906). Enzymes that hydrolyze the esters of short-chain (C_2–C_4) fatty acids are classified as esterases, whereas those that hydrolyze long-chain fatty acid esters (>C_8) are classified as lipases (Seligman & Nachlas, 1950; Lunavath et al., 2013).

Reaction:
Esters + H_2O → Alcohol + Acid
Alcohol + Carboxylic acid ↔ Ester + Water
R–OH + R–COOH ↔ R–COO–R + H_2O

Esterase enzymes are involved in several important physiological processes, including nervous impulse transmission, reproduction, development, detoxification, and tolerance to xenobiotics. They also serve as valuable biomarkers for environmental pollution and have been widely used as genetic markers in various organisms. In recent decades, these enzymes have attracted considerable industrial interest because of their applications in food processing, detergents, fine chemicals, wastewater treatment, biodiesel production, pharmaceutical industries, and bioremediation (Rao et al., 1998; Sharma et al., 2001; Bornscheuer et al., 2002; Jaeger & Eggert, 2002; Reetz, 2002; Maurer, 2004; Cammarota & Freire, 2006; Hasan et al., 2006; Swapna et al., 2024).

The high regio- and stereospecificity of these enzymes makes them useful in the kinetic resolution of optical isomers for the synthesis of optically pure compounds in pharmaceutical and chemical industries (Bornscheuer et al., 2002; Hasan et al., 2006). Their ability to catalyze a wide variety of ester hydrolysis reactions without the requirement of cofactors is an additional advantage (Bornscheuer et al., 2002). Esterases also play a vital role in insect metamorphosis (Quan-You Yu et al., 2009).

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MATERIALS AND METHODS

Fishes were collected from ponds (tanks) located within a radius of 60 km of the Kakatiya University campus using nets with the assistance of local fishermen. They were immediately transported to the laboratory in plastic buckets containing water and acclimatized under laboratory conditions for one week in aquaria. The fishes were fed natural plankton collected from their native habitats.

The fishes were immobilized by a blow to the head, and the tissues were dissected immediately. Six tissues, namely gill, liver, intestine, muscle, brain, and eye, were selected for the study. Tissues from three large fishes or six small fishes were pooled, weighed to the nearest milligram, and homogenized in 0.01 M Tris-HCl buffer (pH 7.5) containing 0.9% NaCl. The concentrations of tissue homogenates were as follows:

- Gill – 10%
- Liver – 10%
- Intestine – 10%
- Muscle – 20%
- Brain – 10%
- Eye – 10%

The homogenates were placed in ice-jacketed centrifuge tubes and centrifuged at 2,000 rpm for 10 min in a clinical centrifuge at room temperature. The supernatants were mixed with equal volumes of 20% sucrose containing 0.05% bromophenol blue as the tracking dye. An aliquot (0.1 mL) of the mixture was loaded onto the gel for electrophoretic separation of esterase isozyme patterns.

Esterases were classified according to the procedures of Holmes and Masters (1967), Hart and Cook (1976), Haritos and Salamastrakis (1982), and Lakshmipathi and Reddy (1989), based on their

Table 1. Electrophoretic banding patterns showing the intensity variation of esterase isozymes in different tissues of *Labeo rohita* (Stained with α-naphtyl acetate)

Tissues	EST-1	EST-2	EST-3
Rm Values	**(0.6 ± 0.05)**	**(0.4 ± 0.05)**	**0.3 ± 0.05)**
Gill	**+++**	**++**	**++**
Liver	**++**	**+++**	**++**
Intestine	**++**	**+++**	**+**
Muscle	**++**	**+++**	**+**
Brain	**+**	**-**	**+**

+ indicates faintly stained; ++ indicates medium staimed; +++ indicates deeply stained

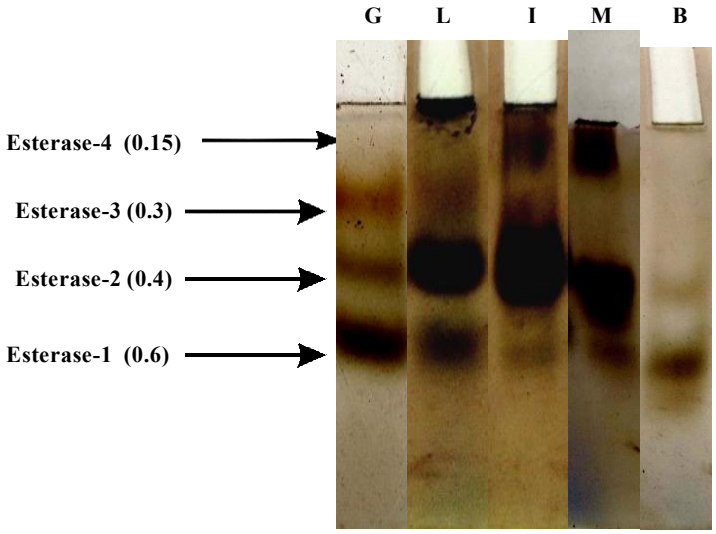

Figure-1. Comparative study of electrophoretic patterns of esterases in different tissues of *H. fossils* *(G=Gill, L-Liver, I-Intestine, M-Muscle, B-brain)*

sensitivity to specific inhibitors. Physostigmine (carbamate), p-chloromercuribenzoate (pCMB), and paraoxon (organophosphate compound) were used as inhibitors.

The classification scheme was as follows:

1. **Carboxylesterases (CE):** Inhibited by paraoxon but not by physostigmine or pCMB.
2. **Arylesterases (ArE):** Inhibited by pCMB but not by paraoxon or physostigmine.
3. **Cholinesterases (ChE):** Inhibited by both paraoxon and physostigmine.
4. **ER Esterases:** Not inhibited by any of the three inhibitors.
5. **Esdp Esterases:** Inhibited by both pCMB and paraoxon.
6. **Ese Esterases:** Inhibited only by physostigmine.
7. **CHsp Esterases:** Inhibited by paraoxon, physostigmine, and pCMB.

RESULTS AND DISCUSSION

Heteropneustes fossilis

Gill: Three esterase zones were observed with Rm values of 0.50, 0.41, and 0.25, all exhibiting low activity. The zones with Rm values of 0.50 and 0.25 were identified as ER esterases, whereas the zone with an Rm value of 0.41 was classified as a CE esterase.

Liver: Three esterase zones were detected with Rm values of 0.50, 0.33, and 0.25, showing moderate activity. The zones with Rm values of 0.50 and 0.33 were identified as CE esterases, while the zone with an Rm value of 0.25 was classified as an ER esterase.

Intestine: Three esterase zones were observed with Rm values of 0.58, 0.50, and 0.33. The zones with Rm values of 0.50 and 0.33 were identified as ER esterases with moderate activity, whereas the zone with an Rm value of 0.58 was classified as an Esdp esterase.

Muscle: Two esterase zones were detected with Rm values of 0.41 and 0.33. The zone with an Rm value of 0.41 was identified as a ChE esterase, whereas the zone with an Rm value of 0.33 was classified as a CHsp esterase.

Brain: Three esterase zones were observed with Rm values of 0.50, 0.41, and 0.25. The zones with Rm values of 0.50 and 0.25 were identified as cholinesterases (ChE), whereas the zone with an Rm value of 0.41 was classified as an ArE esterase.

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.

Three Esterase are found in various tissues of Labeo rohita (Table 2) exhibited three zones (EST 1, 2, 3) with Rm value .58, .50, .and 41, respectively. Among the three 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.

The classification of esterases into various groups has been based on their substrate specificities and sensitivity to inhibition. Typically, esterases that are inhibited by organophosphate compounds are categorized as carboxyl esterases, as noted by Holmes and Master (1967), Haritos and Salamastrakis (1982), Namthabad et al (2014) and Reddy et al. (1989). Holmes et al. (1968) utilized substrate specificity and inhibitor sensitivity to classify the electrophoretically separated esterases present in the tissues of various vertebrates.

Table 2. Electrophoretic banding patterns showing the intensity variation of esterase isozymes in different tissues of *Labeo rohita* (Stained with α-naphtyl acetate)

Tissues	EST-1	EST-2	EST-3
Rm Values	(0.6 ± 0.05)	(0.4 ± 0.05)	0.3 ± 0.05)
Gill	+++	++	++
Liver	++	+++	++
Intestine	++	+++	+
Muscle	++	+++	+
Brain	+	-	+

+ indicates faintly stained; ++ indicates medium staimed; +++ indicates deeply stained

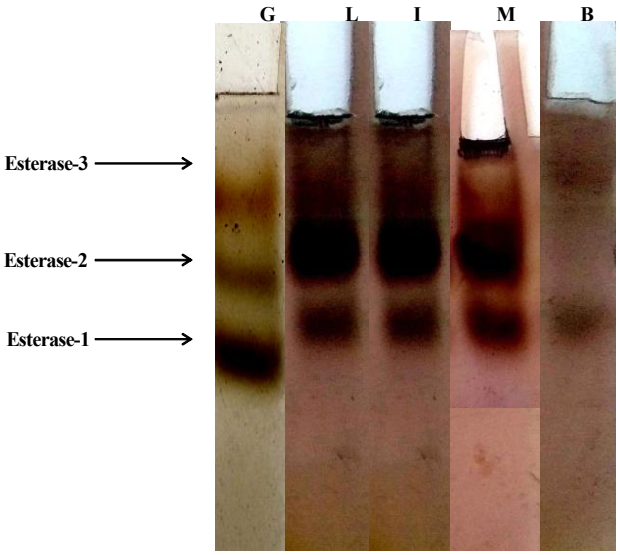

Figure-2. Comparative study of electrophoretic patterns of esterases in different tissues of *Labeo rohita* *(G=Gill, L-Liver, I-Intestine, M-Muscle, B-brain)*

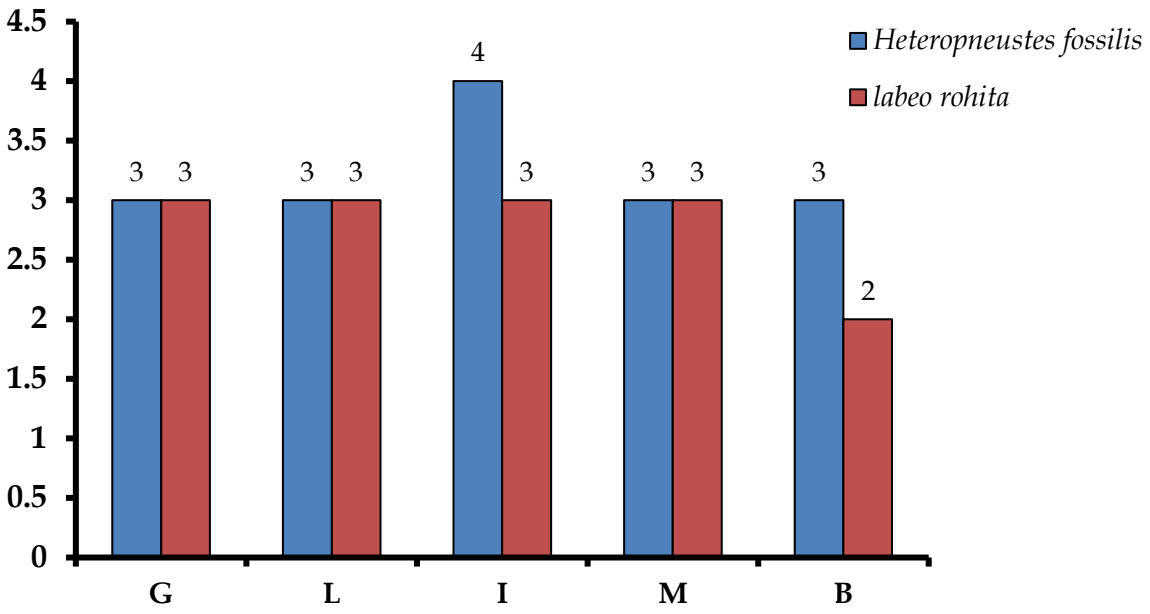

Figure-3. Graphical Representation of Esterase bands variations in *Hateropneustes fossilis* and *Labeo rohita*

Previous studies have indicated that both vertebrate and invertebrate esterases display a significant degree of polymorphism. Similar inhibition patterns have been observed in the esterases of fish, as well as in other organisms such as crustaceans, insects, mollusks, and amphibians, as reported by Bheem Rao (2018), Swapna Ravinder Reddy (2015, 2017), Venkaiah et al. (2013), and Pranavi et al. (2012). However, the investigations surrounding esterases remain ambiguous. Notably, bufodienoloids, which are found in the skin and glandular secretions of toads, exist in multiple conjugate forms.

CONCLUSION

Heteropneustes fossilis and *Labeo rohita* fishes exhibit distinct esterase profiles across various tissues. *Heteropneustes fossilis* shows 4 esterase zones, (Rm values .58, .50, .41, .33) *Labeo rohita* shows 3 esterase zones (Rm values .60, .40, .030) with different intensity levels like from low, moderate, deeply stained. marking a distinct difference in esterase distribution and activity between the two species. In *Heteropneustes fossilis* EST-1 is deeply stained(+++) in gill and brain tissues only.EST-2 is deeply stained (+++) in liver and intestine tissues only. In *Labeo rohita* EST-1 is deeply stained(+++) only in gill tissue. EST-2 is deeply stained (+++) in liver, intestine and muscle tissues.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

Funding -NA.

Ethics Statement - NA

Informed Consent - NA

Data Availability
All data generated or analyzed during this study are included within this published article.

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