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Electrophoretic Banding Pattern of Esterase Isozymes In Fresh Water Fish *Labeo rohita*

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ABSTRACT

Labeo rohita is most preferred indigenous edible species in most of the countries and the most abundant species in our fresh water rivers, paddy fields and other water channels. Fishes are the excellent models for monitoring environmental contamination in aquatic system. The In our present investigation electrophoretic banding patterns of tissue specific esterases in various tissues i.e. gill, liver, intestine, muscle and brain were investigated in fresh water fish *Labeo rohita.* The results revealed that the electrophoretic esterase banding patterns varied in different tissues i.e. gill, liver, intestine, muscle and brain of fish *Labeo rohita*. Esterase Isozyme patterns were separated on thin layer 1.5 mm (thickness) polyacrylamide gels (SDS-7.5%) and stained with α – naphthyl acetate used as substrate. Three different esterase bands were detected and named as Est-1, Est-2 and Est-3 with different relative motilities (Rm) such as 0.6± 0.05; 0.4± 0.05; 0.3± 0.05. All the three esterase bands were present in all tissues i.e. gill, liver, intestine, muscle and brain. Among the all tissues gill and liver tissue exhibited deeply staining of Est-1; Est-2 and Est-3(+++). Intestine tissue shown Est-1 deeply stained (+++) whereas Est-2 and Est-3 were median deep stained (++). Muscle tissue showed Est-1 and Est-2 were deeply stained (+++) whereas Est-3 was median deep stained (++). Brain tissue exhibited Est-2 was deeply stained(+++) whereas Est-1 and Est-3 were median deep stained(++).Studies on esterases of fish and other organisms revealed similar type of patterns of esterases were noticed in one or other tissue of all the animals.

Keywords: *Labeo rohita*, Esterase Isozymes, Polyacrylamide gels, α-naphthylacetate, Electrophoretic banding patterns. Relative mobilites. (Rm)

INTRODUCTION

Labeo rohita is most preferred indigenous edible species in most of the countries and the most abundant species in our fresh water rivers, paddy fields and other water channels (K. Somaiah *et al*., 2014).

Fish is a good source of protein, lipid and also vitamins particularly Vit-'A' hence becomes a valuable form of food for growing population and can also play an important role on checking malnutrition and undernourishment in developing countries such as India. The fish is rich in essential amino acids and can complement or fulfill the overall protein quality that is required in mixed diet (Lailith Pathak *et al*., 2015; Louka, *et al*., 2004; Dempson, *et al*., 2004). Fish can be used as an excellent model for monitoring environmental contamination affected by water pollution (G. R. Scott *et al*., 2004; S. C. S. Shinde, 2007). Esterases are multifunctional, lipid hydrolyzing enzymes occurring in multiple forms and capable of separation by electrophoresis (King 1974). They may be used as bio-indicators to monitor pollutants in the environment (Vanda et al. 2003). Esterase enzyme may be of particular importance because fish utilize lipid/fat as their main nutritional source rather than carbohydrates and protein especially during the later developmental stages (Baglole et al., 1998).

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Esterases comprise a diverse group of enzymes catalyzing the hydrolysis of organic esters. In a wide variety of organisms, they have been used as important gene markers (Augustinsson, 1961; Holmes and Whitt, 1970; Choudhury, 1972; Matteo et al., 1973; Vedbrat and Whitt, 1975; Massaro et al., 1975; Gurrapu et al, 2016). Aldridge (1953) classified the esterases into Esterases-A and Esterases-B by using the Organophosphate inhibitors. The Esterases –A are inhibited by organophosphates (OPs), Ex: Arom and Aryl esterases. B-Estearses are sensitive to inhibition by OP compounds. Ex: Aliesterases and Cholinesterases. Holmes and Masters, 1967; Holmes *et al*., 1968 in to four groups depending upon substrate sensitivity and specificity, they are :

(1) Carboxylesterases (E.C.3.1.1.1=Aliesterases).
(2) Arylesterases (E.CC.3.1.1.2=Aromesterases).
(3) Acetylesterases (E.C.3.1.1.6=C esterases).
(4) Cholinesterases (Acetyl cholinesterase E.C.3.1.1.7 and

Pseudocholiesterases E.C.3.1.1.8). Esterase isozymes are one of the lipid hydrolyzing enzymes which have a great significance in the field of genetics and toxicology (Callaghan et al., 1994) and can be separated by electrophoresis due to having different isoelectric points. An organism may develop resistance to insecticides by producing large amount of specific esterases which either break down the insecticide molecules or bind to it so tightly that it cannot function [R. S. Holmes].

As the electrophoretic banding patterns of esterases of different tissues show species specific variation it can be successfully used for the identification of fish species (Shengming, H et al., 1998) These enzymes appear to be playing a critical role in offering resistance to insecticides [S. H. P. P. Karunaratne] and used as a bioindicator to measure the toxic potency of pesticide residues usually applied in agriculture. The residual effect of pesticide in aquaculture specifically in fish which in-turn cause death of fish (Debnath 1978), (Sahib et al., 1980), (Begum et al., 2008)

The present research work revealed that the electrophoretic banding patterns of esterase isozymes in fresh water fish *Labeo rohita* were varied in different tissues i.e. gill, liver, intestine, muscle, brain of the fish i.e. *Labeo rohita.*

MATERIALS AND METHODS

The adult fishes (weighed about 50-70g) were collected from ponds(tanks) located within the radius of 60kms from Kakatiya University campus by netting with the help of local fishermen. They were immediately brought to the laboratory in plastic buckets and acclimatized to laboratory condititions 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 from the animals. Five tissues were selected for the study i.e. gill, liver, intestine, muscle and brain. The tissues from (adult fishes) six individuals were collected from ice jacketed containers. After collecting the tissues blotted to free from blood clots and other adherent tissues and weighed to the nearest milligram and were homogenized in 0.01N 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%. The homogenates were centrifuged at 2000 rpm for 10 min on a clinical centrifuge at room temperature. The supernatant were mixed with equal volumes of 20% sucrose solution containing 0.05% bromophenol blue as the tracking dye. An aliquot of 0.1 ml of this mixture was used for loading the sample on to the separating gel for separation of esterase patterns. (Holmes RS, Masters CJ.1967, Reddy. M.T. and Lakshmipathi, V. 1988.). Esterase patterns were separated on thin layer 1.5mm (thickness) polyacrylamide gels (7.5%). The gel mixture was prepared according to Clark-1959. Gelling was allowed to 45 min, after loading the samples on the gels, the samples were laid with electrode buffer on gel plates were connected to the electrophoretic tank. Tris (0.05M), glycine (0.38M) buffer (PH=8.3) was used as the electrode buffer. A constant current of 50 volts for the first 15 min followed by 150 volts for the rest of the run was supplied during the electrophoresis. The electrophoretic run was terminated when the tracking dye migrated to the distance of 5cm from the origin. Esterases were visualized on the gels by adapting the staining procedures of (Raju and Venkaiah 2013; Bheem Rao et al., 2018; Shankar et al., 2019). They were stained for esterase activity with α- naphthyl acetate as substrate. (Reddy. M.T. and Lakshmipathi, V. 1988)

RESULTS & DISCUSSION

Electrophoretic banding patterns of Esterase isoenzyme banding pattern of gill tissue showed three esterase isoenzymes with Rm value 0.6 ±0.05(Est-1), 0.4 ±0.05(Est-2) and 0.3± 0.05(Est-3) respectively. Est-1, Est-2 and Est-3 were deeply stained (+++).

Liver

Liver tissue exhibited 03 esterase isoenzymes, i.e. Est-1(Rm value0.6± 0.05); Est-2 (Rm value 0.4± 0.05) and Est -3(Rm value 0.3± 0.05) respectively. Est-1 and Est-3 were deeply stained (++). Est -2 was medium deep stained.

Intestine

Intestine tissue showed 03 zones of Esterase isoenzymes i.e. Est-1,Est-2 and Est-3 .Est-1 with Rm value 0.6± 0.05 was deeply stained (+++) .While Est-2 with Rm value 0.4± 0.05 and Est-3 with Rm value 0.3± 0.05 were medium deep stained(++).

Muscle

Muscle tissue shown 03 bands of Esterase isoenzymes .Est-1 with Rm value 0.6± 0.05 and Est-2 with Rm value 0.4± 0.05 was deeply stained (+++). Whereas Est-3 with Rm value 0.3 0.05 was medium deep stained. (++)

Brain

Brain tissue exhibited 03 Esterase isoenzymes. Est-2 with Rm value 0.4 ± 0.05 was deeply stained (+++), while Est-1 with Rm value 0.6 ± 0.05 and Est-3 with Rm value 0.3± 0.05 were medium deep stained (++).

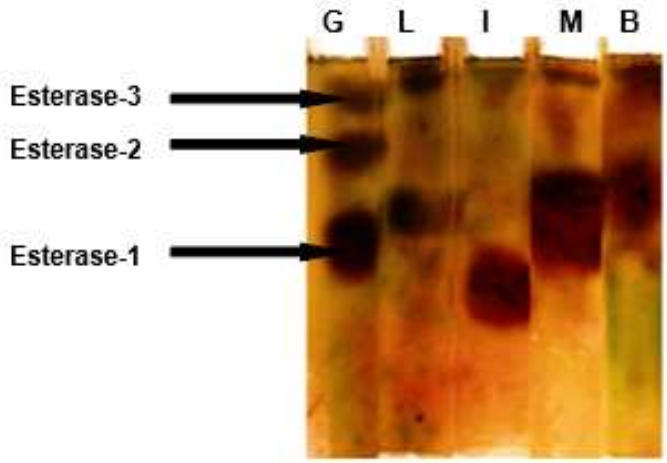

Figure-1. Comparative esterase isozyme banding patterns in different tisssues of *Labeo rohita* stained with α-naphtyl acetate .
Lane-1 G-Gill, Lane -2:L- liver, Lane-3:I--intestine, Lane-4:M- muscle, Lane-5:B- barin

In the present study the three electrophoretic banding patterns of esterase isozymes were identified i.e. Esterase-1, Esterase-2 and Esterase-3 in the different tissues i.e. gill, liver, intestine, muscle and brain of L.rohita.Est-1, Est-2 and Est-3 are present in all tissues. Est-1 is deeply stained (+++) in gill, liver, intestine, muscle tissues and medium deep stained (++) in brain tissue. Est-2 is deeply stained (+++) in gill, liver, muscle, brain tissues and is moderately stained (++) in intestine tissue. Est-3 intensity is deeply stained (+++) in gill, liver tissues and medium deep stained (++) in intestine, muscle and brain tissues. Gill and liver tissue exhibited all the three esterase isozyme banding pattern which were deeply stained (+++).Intestine tissue showed Est-1 is deeply stained (+++) where as Est-2 and Est-3 were medium deep stained (++). Muscle tissue showed Est-1, Est-2 banding pattern is deeply stained (+++) and Est-3 is medium deep stained (++). Brain tissue exhibited Est-2 deeply stained (+++) where as the Est-1 and Est-3 were medium deep stained (++).

Esterase enzymes are multiple forms of a single enzyme which have different iso-electric points and therefore can be separated through electrophoresis. Electrophoretic studies were done extensively on various tissues of different animals from which it reveals that the enzyme exists in multiple molecular forms and functions (Abdur Rashid 2012).

Table-1.Electrophoretic banding patterns showing the intensity variation of esterase isozymes in different tissues of Labeo rohita (Stained with α-naphtyl acetate) ++ indicates medium deep stained,+++ indicates deeply stained

Est (Rm value) Tissue	Est-1(0.6±0.05)	Est-2(0.4±0.05)	Est-3(0.3±0.05)
Gill	+++	+++	+++
Liver	+++	++	+++
Intestine	+++	++	++
Muscle	+++	+++	++
Brain	++	+++	++

Six esterase bands in *Oreochromis niloticus* (R.A.Begum et al., 2010) and in Channel cat fish (K.Knowls 1968) and seven esterase bands in blunt snout lorean fish (L.Sifa et al., 1993). Three esterase bands were reported in *Channa punctatus* (Venkateswara Rao et al., 2020). Four esterase bands investigated in fresh water fish *Heteropneustes fossilis* (Bheemrao et al., 2018). Effect of Chloropyrifos on brain and muscle tissue of fresh water fish Heteropneustes fossilis investigated three esterase isozyme bands. (Shankar et al., 2019). Tissue specific esterase isozyme variations in *Clarius batrachus, Clarius garipinus*, cat fish, toad and *Hypophthalmicthyes molitrix* were reported (Md.Abdur Rashid 2013., Md.Mustafa 2013). Venkaiah et al., 2006, Rama Seshaiah et al., 1984, Md.Abdur Rashid et al., 2012). Esterase isozyme banding patterns in different tissues of stinging catfish showed similarities and dissimilarities in their staining intensities as well as in the occurrence of number of bands in both the larvae and adults (R.A.Begum et al., 2011).The tissue and species specific distribution of esterases were earlier reported from two catfishes and toad (Shahjahan R.M et al., 2008, Venkaiah et al., 2006). Tissue esterase patterns of muscle and brain of *Channiforms* and *Perchiforms* were reported (Rajaiah et al., 2010). Different forms of esterases found in different tissues of *Punctius sophore* was analyzed (Hawajahan et al., 2016, Ghajala 2016). Number of esterase bands may vary from species to species. As for example, three, four, five, six, seven and eight esterase bands were found in *Poecilia reticulata* (Ahmaed et al, 2011), *Heteropneustes fossilis* (Begum RA et al., 2011), *Oreochromis niloticus* (Shajahan RM et al., 2008), *Ictalurus punctatus* (Knowles et al, 1968), *Megalobrama amblycephala* (Sifa L et al., 1993) and in *O. aureus* (Hungtuo F et al.,1993) respectively.

Conclusion

The present study reports that Expression of tissue specific esterase isozymes showed differential banding pattern that could be used in toxicological study and also could be used for the development of molecular markers for the identification of different species of fish.

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Conflicts of Interest

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

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