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Research Article

Comparative Study Of Electrophoretic Patterns Of Esterases In Various Tissues Of Fresh Water Cat Fish *Heteropneustes Fossilis* (Bloch

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ABSTRACT

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The present study was carried out to investigate the comparative study of electrophoretic patterns of esterases extracted from various tissues i.e. gill, liver, intestine, muscle and brain of fresh water cat fish *Heteropneustes fossilis* (Bloch). The qualitative analysis of esterase isozymes were examined on 7.5% native polyacrylamide gel electrophoresis (PAGE) stained with α -naphthyl acetate as substrate. Altogether 4 esterase bands were named as Est-1 (0.6 ± 0.05), Est-2 (0.4 ± 0.05), Est-3 (0.3 ± 0.05), Est-4 (0.15 ± 0.05) were observed with different relative mobility. Est-2, Est-3, Est-4 were found in gill, muscle, brain and liver where as all the four were found in intestine. Among the four esterases Est-3 is found to be more abundant in all the tissues tested with the highest intensity found in liver followed by intestine, gill, brain and muscle. Thus our present investigation reveals that all the four tissues of *H. fossilis* is rich in esterases.

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INTRODUCTION:

The stinging cat fish *Heteropneustes fossilis* (Bloch) is locally called as Ingilayee or Marpujella. It is an important air sac catfish indigenous to many Asian countries (1). It inhabits in fresh water and able to tolerate brackish water too. It is very popular not only for its good taste but also highly nutritional and medicinal point of view. Popularity of this species for cultivation is also high due to extreme hardiness, fast growing and ability to survive in poorly oxygenated water (2).

Esterases are a group of hydrolytic enzymes occurring in multiple forms with broad substrate specificity. Esterases comprise a diverse group of enzymes catalyzing the hydrolysis of organic esters (3). Esterases (Est., 3.1.1.2) are ubiquitous in living organisms. Several esterases have been isolated from various tissues of microbes, plants, and animals and investigated for their biochemical properties (4-6).

Esterase enzymes are multiple forms of a single enzyme, which have different iso-electric points and therefore can be separated by electrophoresis. Electrophoretic studies were done extensively on various tissues of different animals from which it reveals that the enzyme exists in multi molecular forms and functions (7). 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 (8). Esterases also used as bio indicators to measure the toxic potency of pesticide residues usually applied in agriculture (9). The residual effect of pesticides in agriculture especially in fish is very high which in turn cause death of fish particularly, after the rainy season (10,11). Taking this into consideration some enzymological work has been under taken in fresh water cat fish with special reference to esterases.

MATERIAL AND METHODS:

The adult fishes (weighed about 50-70g) were collected from ponds (tanks) located within the radius of 60 kms from Kakatiya university campus by netting with the help of local fisher men. They were immediately brought to the laboratory 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 immobilised 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 dissected 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 homogenised in 0.01N Tris.HCl buffer ($P^H = 7.5$) 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 10min 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 0.1ml of this mixture was used for loading the sample directly on to the separating gel for separation of esterase patterns. 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 45min, after loading the samples on to the gel, the samples were over laid with electrode buffer and gel plates were connected to the electrophoretic tank. Tris (0.05M), glycine (0.38M) buffer ($P^H=8.3$) was used as the electrode buffer.

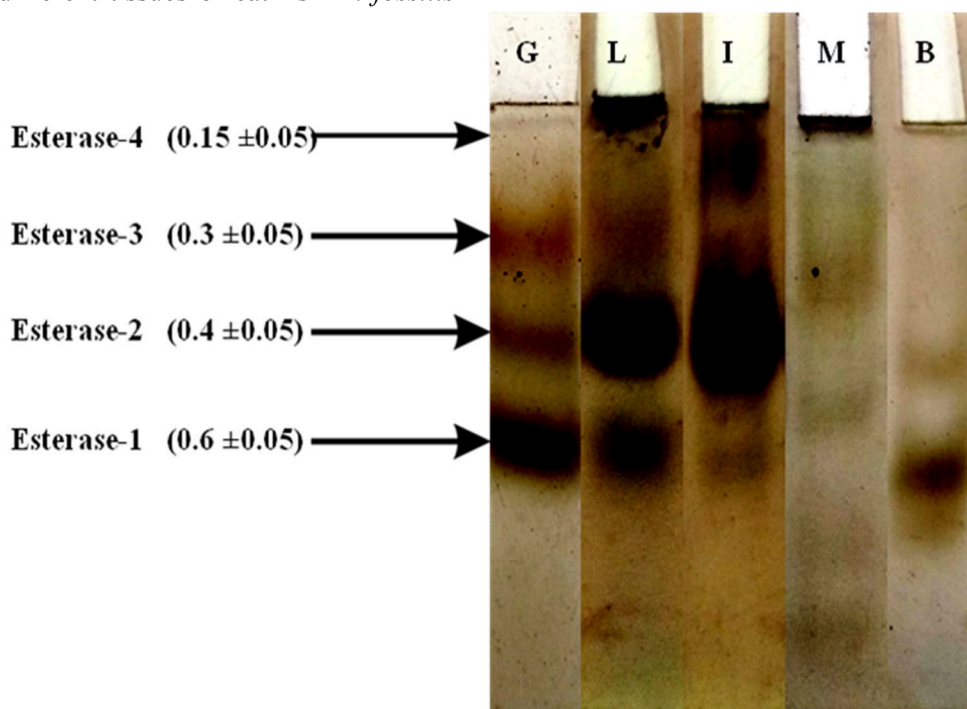
A constant current of 50 volts for the first 15min followed by 150 volts for the rest of the run was supplied during electrophoresis. The electrophoretic run was terminated when the tracking dye migrated to the distance of 5cm from the origin. Esterases were visualized on the gels by adopting the staining procedures of Venkaiah & Laksmipathi 2006 (12), Raju & Venkaiah 2013 (13). They were stained for esterase activity with α -naphthyl acetate as substrate.

RESULTS & DISCUSSION:

The result obtained on the comparative study of electrophoretic patterns of esterases in various tissues of fresh water cat fish *H. fossilis* are presented in figure1 and table 1. The details about the relative mobility of individual esterase isozyme zones are presented in table α -naphthyl acetate was used as

substrate to score the intensity of esterase on 7.5% native polyacrylamide gels. The results showed differences at different tissues of cat fish *H. fossilis*

which were followed by visibility of the zones in electrophoresis.



**Fig.1. Comparative esterase isozyme banding patterns in different tissues of *H. fossilis* Stained with α -naphthyl acetate Lane-1 Gill, Lane-2 Liver; Lane-3 Intestine, Lane-4 Muscle, Lane-5 Brain
 → mark represents esterase band with number and Rm values**

Est value \ (Rm)	Est-1 (0.6±0.05)	Est-2 (0.4 ±0.05)	Est-3 (0.3±0.05)	Est-4 (0.15±0.05)
Tissue				
Gill	+++	++	+	-
Liver	++	+++	+	-
Intestine	++	+++	+	+
Muscle	++	+	+	-
Brain	+++	++	+	-

Tab.1. Electrophoretic banding patterns showing the intensity variation of esterase isozymes in different tissues of *H.fossilis* (Stained with α -naphtyl acetate)+ indicates faintly stained; ++ indicates medium staimed; +++ indicates deeply stained

Four esterase(Est-1, Est-2, Est-3, Est-4) bands were observed in different tissues of fresh water cat fish and presented in figure 1, Table. The detailed results of which are described as below

Gill

There are three active esterase zones on the zymogram Est-2, Est-3, Est-4 with Rm values 0.4 ±0.05, 0.3±0.05, 0.15 ±0.05 respectively. Est-1 was deep stained (+++), Est-2 was medium stained (++) and Est-3) was faint stained (+).

Liver

The liver exhibited 3 esterase zones with Rm values Est-1. 0.6 ± 0.05 , Est-2. 0.4 ± 0.05 , Est-3. 0.3 ± 0.05 respectively out of which Est-2 was deeply stained (+++), Est-1 was medium stained (++) and Est-3 was faint stained (+).

Intestine

All together four esterase bands (Est-1, Est-2, Est-3, Est-4) with Rm values 0.6 ± 0.05 , 0.4 ± 0.05 , 0.3 ± 0.05 , 0.15 ± 0.05 , were found in intestine of which Est-2 was deep stained (+++), Est-1 was medium stained (++) and Est-3, and Est-4 were faint stained (+).

Muscle

Muscle exhibited only 3 esterase bands Est-1, Est-2 and Est-3 on the zymogram with Rm values 0.6 ± 0.05 , 0.4 ± 0.05 , 0.3 ± 0.05 were found in the muscle Est-1 (++) was medium stained and Est-2 (++) , Est-3 (+) were faint stained.

Brain

3 esterase bands Est-1, Est-2, Est-3 were observed with Rm values 0.6 ± 0.05 , 0.4 ± 0.05 , 0.3 ± 0.05 in cat fish among Est-1(+++) was deeply stained Est-2 (++) was medium stained and Est-3 (+) was faint stained. Highest relative mobility value was 0.6 ± 0.05 (Est-1) close to the anode (+), slowest relative mobility was 0.15 ± 0.05 (Est-4) on the cathode (-) (Fig.1 and Table 1).

In the present study Est-1 and Est-2 were deeply stained with α -naphthyl acetate gill, liver, intestine and brain. But Est-1 was medium stained in liver, intestine and muscle. Est-2 in muscle Est-3 in all the tissues were faintly stained. Est-1, Est-2, Est-3, Est-4 were present in all of the tissues. Est-1, Est-2, Est-3 were found to be present in all the tissues analysed i.e gill, liver, intestine, muscle and brain.

The tissue and species specific distribution of esterases were earlier reported from two cat fishes and the toad (12, 5). Tissue esterase patterns of muscle and brain of Channiforms and Perchiforms were reported (14). Non specific esterase isozyme after electrophoresis was used to identify the species of *Anabas* and *Clarius* (15-17). Different forms of esterases found in different tissues of *Puntius sophore* were analyzed (18).

Gajala et al 2016 (19) reported the effect of Triazophous on esterase activity and protein contents of liver, kidney, brain, blood and muscle of *Catla*

catla, *Labeo rohita* and *Cirrinus mrigala*. Tissue specific esterase isozyme variations in *Clarias batrachus* and *C. gariapinus* were also studied (20,21).

CONCLUSION:

The present study reports that the variability of patterns of esterase isozymes describes electromorphs of an individual. Representing expression of tissue specific esterase isozymes, which showed differential banding patterns that, could be used in toxicological study. It can be concluded that the tissue wise variation in the banding patterns of esterase may be used for the development of genetic molecular markers.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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