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Original Research Article

Electrophoretic Banding Patterns of Esterase Isozymes in Fresh Water Fish *Channa punctatus*

Dr. M. Venkateswara Rao[1], *Dr. Y. Venkaiah[2]

Author's Affiliation:
[1]Assistant Professor, Dept. of Zoology, SR & BGNR Degree College, Khammam, Telangana 507002, India.
E-mail: venkatmandalapu18@gmail.com
[2]Associate Professor of Zoology, Department of Zoology, Kakatiya University,
Warangal, Telangana State 506009, India
E-mail: venkaiahyanamala07@gmail.com

***Corresponding author:**
Dr. Y. Venkaiah
Associate Professor of Zoology, Department of Zoology, Kakatiya University, Warangal, Telangana State 506009, India
E-mail: venkaiahyanamala07@gmail.com

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ABSTRACT:
Esterase enzymes catalyse the formation and breakdown of carboxylic acid esters of alcohols. The present work aimed to study the electrophoretic banding patterns of esterase isozymes in fresh water fish *Channa punctatus* The results revealed that the electrophoretic esterase banding patterns varied in different tissues i.e. gill, liver, intestine, muscle, and brain of fish *c. puctatus* Esterase patterns were separated on thin layer 1.5mm (thickness) polyacrylamide gels (SDS-7.5%) and stained with α-naphthyl acetate used as substrate. Three different esterase bands were detected and named as Esi-1; Est-2; and Est-3; with different relative mobilities such as 0.6 ±0.05; 0.4±0.05; 0.3±0.05**.** All the three esterase bands were found in all tissues i.e. gill, liver, intestine, muscle and brain. Among the three esterases Est-1 is found in all the tissues. Est-2and Est-3 were found in all the tissues. Studies on esterases of fishes and other organisms revealed similar type of patterns of esterase were noticed in one or the other tissue of all the animals.

Keywords: α-naphthylacetate, *Channa punctatus*, Electrophoretic banding patterns, Esterase.

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INTRODUCTION

Esterase enzymes catalyse the formation and breakdown of carboxylic acid esters of alcohols. Aldridge (1953) classified the esterase 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.

Electrophoretic techniquues and application of histochemical staining helps into detection of enzymes on the gels. The identification of tissue enzymes of various animals tissues has become more rapid. (Smithies, O. 1955, 1959; Hunter, R.L. & Markert, C.L 1957). With the help of histochemical staining techniques and using α-naphthyl acetate as a substrate, the tissue specific esterase enzyme patterns of several organisms were identified. These enzymes occur in multiple molecular forms and shown more heterogeneity in tissue and

species distribution (Masters, C,J. and Holmes, R.S. 1974&1975).

Holmes and Masters (1967&1968) classified esterases on zymogram into four categories depending upon substrate specificity andsensitivity to various inhibitors. Carboxylesterases (E.C.3.1.1.1=Aliesterases), Arylesterases (E.C.3.1.1.2=Aromesterases), Acetyl esterases (E.C.3.1.1.6=C esterases), Cholinesterases (including Acetyl cholinesterase E.C.3.1.1.7 and pseudocholinesterases E.C.3.1.1.8).

Some investigators (Hart, N.H. & Cook, M. 1976; Verma, A.K. & Frankel, J.S., 1980; Horitos & Salamastrakis 1982; Lakshmipathi, V. & Reddy, T. M. 1989, 1990) have identified the deviations in the sensitivity of fish esterases according to Holmes and Masters classification, and these authors have classified the fish esterases into four types. Viz : ER-esterases they are resistant to all the inhibitors, Ese - esterases-they are inhibited by Eserine only, Esdp esterases-they are inhibited by OP and pCMB both, CHsp esterases (Cholinesterases like enzymes)-they are inhibited by all three inhibitors.

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

MATERIALS & 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 icejacketed 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 (PH=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 (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.

RESULTS

The results obtained from the electrophoretic banding patterns of Esterases of various tissues incontrol of *C. punctatus* are depicted in Table 1 and Figure. 1. α-naphthylacetate is used as substrate to score the intensity of Esterase on 7.5% native polyacrylamide gel. The esterase activity of the different bands obtained and arbitrarily measured based on eye estimation of staining intensity, categorized into deep stained (DS), medium deep stained (MDS) and faint stained (FS) in different test tissues of *C. punctatus*.

Gill

The electrophoretic esterase isoenzyme banding pattern of gill tissue showed three active zones of Esterase i.e. Est-1, Est-2 and Est-3 with Rm values, 0.6±0.05, 0.4±0.05 and

0.3±0.05 respectively. Esterase-1, Est-2 were deeply stained (+++) while Est-3 was moderately stained (++).

Liver

Liver tissue showed three zones of esterase isoenzymes with Rm value 0.6 ±0.05, 0.4± 0.05 and 0.3 ±0.05 respectively. Est-1, Est-2 and Est-3 were highly stained (+++).

Table 1: Electrophoretic banding patterns showing the variation of intensity of Esterase isoenzymes in various tissues of *Channa punctatus*

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

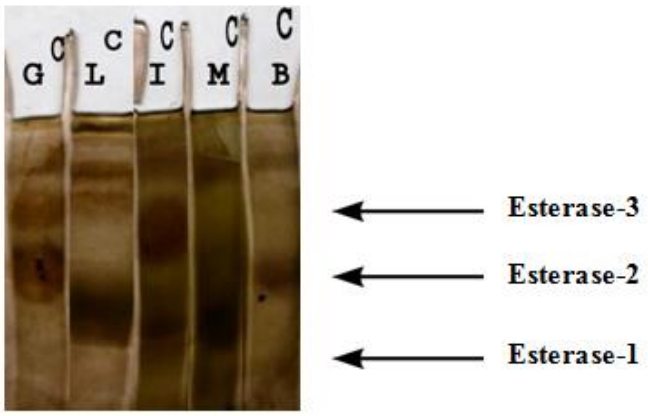

Figure 1: Esterase band intensity of various tissues of *Channa punctatus*

Intestine

Intestine tissue exhibited three esterase isoenzyme zones with Rm value 0.6± 0.05, 0.4 ±0.05 and0.3± 0.05 respectively. In which Est-1 and Est -2 were deeply stained (+++), while Est-3 was stained moderately (++).

Muscle

Muscle tissue showed three zones of esterase with Rm value 0.6± 0.05, 0.4± 0.05 and 0.3± 0.05 respectively. Est-1 was deeply stained (+++) while Est-2 and Est-3 were moderately stained (++).

Brain

Brain tissue exhibited three esterase isoenzymes with Rm value 0.6± 0.05, 0.4± 0.05 and 0.3 ±0.05respectively. Est-1 and Est-2 were deeply stained (+++) while Est-3 were moderately stained (++).

Est-1 with Rm value 0.6± 0.05 has highest relative mobility value which is close to anode (+), where as Rm value 0.3± 0.05 has relative lowest mobility value which close to cathode (-).

DISCUSSION

In the present study among the three esterases Est-1 is found in all the tissues and to be more abundant with deeply stained (+++). The intensity of Est-2 were deeply stained (+++) in gill, liver, intestine and brain tissues and moderately stained in muscle tissue. The Est-3 were deeply stained (+++) in liver tissue, and moderately stained (++) in gill, intestine, muscle and brain tissues. The liver tissue showed in all the three esterases zones (i.e., Est-1; Est-2; Est-3) and were deeply (+++) stained. In Est-3 esterases zone the gill. Muscle, intestine, and brain were moderately

(++) stained. Est. -2 were moderately stained (++) in muscle tissue. The muscle tissue shown in Est-1 was deeply stained (+++).

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. Esterases (EST, 3.1.1.1) are ubiquitous in living organisms. Several esterases have been isolated from various tissues of microbes, plants and animals and investigated for their biochemical properties.

Shankar *et al*., 2019; Bheem Rao.T *et al*., 2018 studied on esterase patterns of fish *H. fossilis* tissues and revealed similar type of inhibition patterns. Studies on tissue esterase polymorphism of *Cyprinus Carpio* and *Puntius Sarana* of cypriniformes order by V. Vimala and (V. Rajaiah, 2010 & 2014) reported that among the various tissues, gill, liver and intestine exhibit four zones and followed by remaining tissues (3 zones each). Among the type of esterase, ER esterase is predominant in almost all the tissues and followed by the ChE esterases. But only one ArE esterase is found in brain with fast moving zones.

The study of A. Gopalakrishnan *et al.,* (1997) concluded that electrophoretic pattern of soluble esterases of *L. rohita* and *C. mrigala* showed species-specific differences. The basic profile of esterases can be useful in detecting genetic introgression and polymorphism in these cyprinids.

Quantitative and qualitative changes in the levels of activity of esterases are studied in five stages of developing embryos of the freshwater crab *Barytelphusa guerini.* Esterase activity levels increase enormously as development progresses. Only two of the seven zones of esterases are present in all five stages. These bands show an increase in their activity in stages 111-V. Heat denaturation studies indicated an increase in the thermal stability of the enzyme as development progresses (V. Lakshmipatahi and M. Sujatha, 1991).

Distribution of different kinds of esterases indicates that CE esterases were present in all the channiformes fishes. But CE esterases were not noticed in Perciformes fishes in brain tissue. ArE esterases were found in all the Perciformes fishes but ArE esterases were not noticed in Channiformes fishes indicated that channiformes order is distinct from perciformes order fishes (V. Rajaiah,V. Vimala, K. Vasumathi Reddy and T. Ravinder Reddy, 2010).

Although the biological functions of these enzymes and the natural substrates that they catalyze these little understood, they are known to play a role in the xenobiotic metabolism and hydrolyze or detoxify several foreign chemicals entering into the cells.

Various authors reported that the different number of esterase fractions in the gut spectrum of different breeds of B. *mori*. Esterase isozyme exhibited higher level of polymorphism in vertebrates and invertebrates. Gillespie and Kojima (1968) reported a relationship between the level of polymorphismand metabolic enzymes such as esterases. The differences in fractions of esterase may be due to the degree of genetic heterogeneity.

CONCLUSION

The present study reports that the variability of patterns of esterase isozyme describes the electromorphs of an individual. It can be conclude that each tissue has specific esterase banding pattern which may be used for the development of genetic molecular marker for proper identification of fish species.

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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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