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Tissue Specific Esterase Isozyme Banding Pattern in Nile Tilapia (*Oreochromis niloticus*)

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Abstract: The electrophoretic banding pattern of esterase isozymes were examined in eighteen different tissues of Nile tilapia after staining with α and β naphthyl acetate as substrate. The tissues were anterior-, mid-, tail-muscle (ventral and tip region), buccal muscle, stomach, fore-, mid-, hind-gut, liver, gill, heart, kidney, eye (lens), eye (iris), fore-, mid- and hind- brain. Altogether five bands named as Est-$1^{1.4}$, Est-$2^{1.1}$, Est-3^{1}, Est-$4^{0.62}$ and Est-$5^{0.25}$ were observed different relative mobility. Est-1 and Est- 5 denote the fastest and slowest band. Est-3 was present in all the tissues. All five bands were expressed in liver and stomach. Some of the esterase bands showed tissue and substrate specificity, where Est-1 was in fore-, mid- and hind- brain, Est-2 in hind gut, Est-5 in stomach, gill and heart was stained only with α naphthyl acetate. None of the band was expressed with β naphthyl acetate only.

Key words: esterase, isozyme, *Oreochromis*, tissue

Introduction

Oreochromis niloticus (Linnacus, 1758) commonly known as Nile tilapia is one of the most widely cultured species all over the world. Among tilapias, Nile tilapia, was first introduced into Bangladesh in 1970 (Hussain, 2004). Usually tilapia is capable of taking a wide range of food materials from tiny plankton to macrophytes and grows well on artificial feeds. When kept in captivity they can reproduce easily and will tolerate a wide range of environmental stress and are resistant to diseases. Overall performance of Nile tilapia and other fast growing tilapias have come to be known as "aquatic chicken". The large scale production of fries of Nile tilapia is being carried out in private sectors without considering genetic background. The genetic status of Nile tilapia is needed to be analyzed.

Isozymes are multiple forms of a single enzyme, which often have different isoelectric points and therefore can be separated by electrophoresis. Electrophoretic studies were done extensively on the different tissues of various animals from which it reveals that the enzyme exit in multi molecular forms and functions (Markert & Moller, 1959). As the electrophoretic banding patterns of esterases of different tissues show species-specific variation it could be successfully used for the indentification of fish species (Shengming *et al.*, 1988). Esterase isozymes are one of the lipid-hydrolyzing enzymes, possess high significance in genetics and toxicology (Callaghan *et al.*, 1994; Markert & Moller, 1959). The banding pattern of esterases appears to be genetically controlled and therefore it has been used to estimate the genetic distance among different populations or the distance between populations (Turner, 1973). Esterases are also used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture. The residual effect of pesticide in aquaculture specifically in fish is high which in tern cause death of fish particularly, after the rainy season (Debnath, 1978; Sahib & Rao 1980).

Considering the above facts, it is essential to understand the genetic status in terms of esterase variability. The paper deals in polymorphic pattern of esterase isozymes in different body tissue of the Nile tilapia *O. niloticus*.

Material and Methods

Sample collection: The adult fish samples were collected from "M/S AL-AMIN MATSHA KHAMAR", Narayangang in 1st May of 2007. The fishes were caught and taken live to the Laboratory of Animal Genetics and Molecular Biology, Department of Zoology, University of Dhaka, in plastic bags which was filled with water. The length of the specimen was 12cm, width 3.5cm and weight 22gm. The tissues studied were anterior muscle, mid muscle, tail muscle (ventral region), tail muscle (tip region), buccal muscle, stomach, foregut, midgut, hindgut, liver, eye (lens), eye (iris), gill, heart, kidney, forebrain, mid brain and hind brain. Altogether eighteen different tissues were removed and put into eppendorf tube as stock samples and were kept in a deep freeze at -80^0c.

Gel preparation: 7.5% gel was prepared using 7.5 ml of Acrylamide-bisacrylamide (30:0.8) (Bio Basic Inc); 6.0 ml of 5X of TBE buffer; 0.150 ml of 10% ammonium persulphate (AMPS) (Fluka Biochemica); 0.140 ml of TEMED and 16.025 ml of water.

Sample preparation: 0.015gm of each tissue sample was taken in a seperate eppendorf tube and squashed through electronic micro pester (Sigma) in 40 µl of lx Tris-Borate (MERCK) – EDTA (Sigma) buffer. 40 µl of Bromophenol blue (MERCK) in 1x TBE solution was added to each sample. The samples were centrifuged at 12000 rpm for 12 minutes at 25^0C, and 15 µl of supernatant was used in every slot of the gel.

Electrophoresis of gel: 7.5% Polyacrylamide gel electrophoresis (PAGE) was carried out to analyze. The gel was run at 120v constant voltage for at least 1 hour and 20 min until the tracking dye (bromophenol blue) reaches bottom of the gel. Then the gel was recovered by dismantling the cassettes/glass-plates sinking in water very cautiously.

Staining: Elevation of esterase banding patterns was done in the presence of the two substrates, α and β naphthyl acetate in different tissues following basically the technique described by Johnson & Denniston (1964) and Steiner & Joslyn (1964). The gel was then taken in a staining tray and 30 ml of substrate mixture was poured onto it. It was kept in this mixture for 15 minutes at 25°C. After 15 minutes, substrate mixture was out poured and 30 ml of fast blue RR solution was added to the gel, it was incubated at 37°C for 25 minutes. Photograph of the gel was taken by a digital zoom lens camera laying the gel on a white background (Samsung Kenox-SHD lens, 3.2 Mega pixels).

Scoring of bands: Bands were scored from the stained gel as the highest and lowest mobility of the band were numbered (Webb, 1964).

Measuring total protein concentration by Lowry test: The principle behind the Lowry method is to determine protein concentration. Folin Ciocalteau was used as reagent solution, Bovin Serum Albumin (BSA) was used as a standard protein and optical density was measured at 660 nm wavelength. Dunn (1992) suggests concentrations ranging from 0.10 - 2 mg of protein per ml.

Results and Discussion

Altogether five esterase bands namely, Est-1, Est-2, Est-3, Est-4 and Est-5, were found in Nile tilapia (*O. niloticus*) (Table 1 and Fig. 1). Eight esterase bands were found in brain, eye, heart and liver tissues of *O. aureus* (Hongtuo *et al.*, 1993). Seven esterase bands were found in Sword tail fish (*Xiphophorus helleri)* (Ahuja *et al.* 1977). According to the previous Mendelian inheritance studies on these esterase loci, each of the bands corresponds to one allele (Stordeur, 1976).

Relative mobility of each band was determined by comparing with the most frequent band 3 selected as a standard and it was present in almost all the tissues. The relative mobility of the bands were 1.40±.04, 1.10±.04, 1.0±.04, 0.62±.04, 0.25±.04 respectively, showed in Table 1. The highest relative mobility value was 1.40±.04 (Est-1) close to the anode (+). Slowest relative mobility was 0.25±.04 (Est-5) the cathode (-) (Table 1).

These esterase bands had tissue and substrate specific expression. All the bands were expressed when stainedwith both α and β naphthyl acetate as substrate fewer bands were observed with only one substrate and most of the bands was α specific. The bands also showed an intensity variation among different tissues. The black α specific bands are shown in plate AB and β specific red bands are shown in plate CD. Both α and β staining bands are in plate EF. (Fig. 1)

Table 1. Electrophoretic banding pattern showing the intensity variation of esterase isozymes in different tissues of Nile tilapia (*Oreochromis niloticus*) scored from α and β naphthyl acetate

Tissue	Est-1 1.40 ±.04	Est-2 1.10 ±.04	Est-3 1.00 ±.04	Est-4 0.62 ±.04	Est-5 0.25 ±.04
Anterior muscle			++		+
Mid muscle			++		+
Tail muscle (vent region)			++		+
Tail muscle (tip region)			++		+
Buccal muscle			++		++
Stomach		+++	+++	++	++*
Fore gut		+++	+++	++	
Mid gut		+++	+++	++	
Hind gut		+++*	+++	++	
Liver	+	+++	+++	+++	
Kidney			+++	++	
Gill			+++	++	++*
Heart			+++	++	+*
Eye (lens)			+++	+	
Eye (iris)			++	+	
Fore brain	+*		+		
Mid brain	++*		+++		
Hind brain	++*		+++		

'+', '++' and '+++' marks denote faintly, medium and deeply stained in substrate, respectively.
'*' mark denotes mentioned esterase band was absent in β naphthyl acetate.

In the liver tissue of Nile tilapia (*O. niloticus*) maximum four namely Est-1, Est-2, Est-3 and Est-4 esterases were observed. Some esterase bands showed substrate specific expression. All four esterases were expressed only in α naphthyl acetate. However, three esterase bands (Est-2, 3 and 4) were found when stained with β naphthyl acetate as substrate. In Sword tail fish (*X. helleri)* the liver exhibited five esterases with strong enzyme activity (Ahuja *et al.*, 1977). Strong enzymatic activity was also found in the liver of the present study (Table 2 and Fig. 2).

Three esterase bands Est- 2, 3 and 4 were observed in both α and β naphthyl acetate in fore, mid and hind gut of the present study. Exception was observed in hind gut when stained with β naphthyl acetate where Est- 2 was absent (Table 1 and Fig. 1). The anterior portion of intestine is richer in esterase activity than that of the posterior portion as regards the number of bands concern. Among different part of intestine Hirji and Courtney (1983) found strong enzymatic activity in the upper and middle portion of the intestine whereas weak in the lower intestine of the perch fish *P. fluvitalis*.

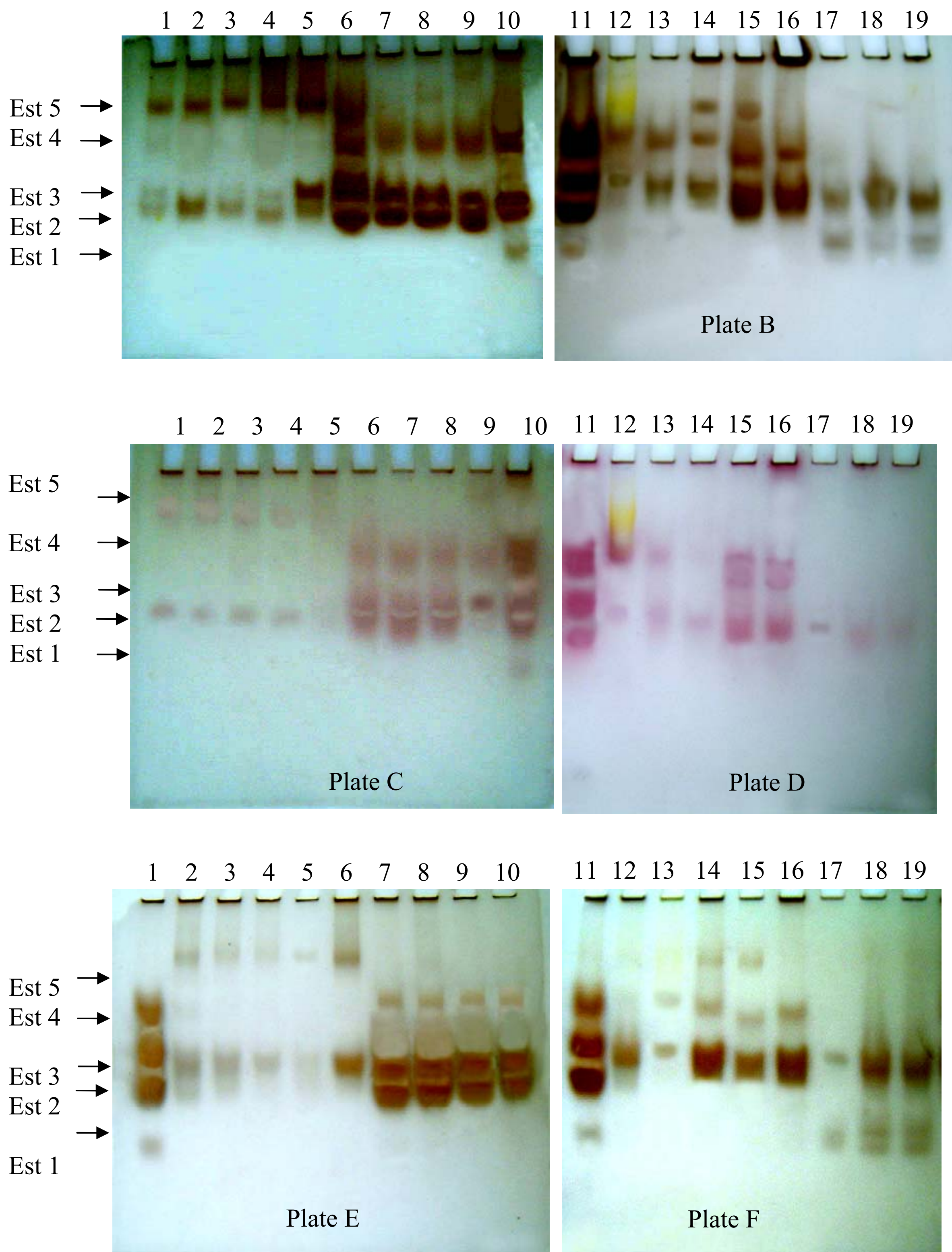

Fig1. Esterase isozyme pattern in different tissues of Nile tilapia (*Oreochromis niloticus*) stained in α naphthyl acetate as substrate (A and B plates), β naphthyl acetate as substrate (C and D plates) and both α and β napthyl acetate as substrate (E and F plates). Where lane (1) denotes tissue from anterior muscle; (2) mid muscle; (3) Tail muscle, ventral region; (4) Tail muscle, tip region; (5) Buccal muscle; (6) stomach; (7) Foregut; (8) Midgut; (9) Hindgut; (10) Liver; (11) liver; (12) Eye; (13) Eye black portion; (14) Gill; (15) Heart; (16) Kidney; (17) Fore brain; (18) Mid brain; (19) Hind brain. Arrow indicates position of esterase bands.

Table-2. Electrophoretic pattern showing variation in the intensity of bands scored from α and β naphthyl acetate and optical density (OD) values of total protein as recorded in Lawry test in different tissues of Nile tilapia (*Oreochromis niloticus*)

Tissue	Est-1	Est-2	Est-3	Est-4	Est-5	OD
Muscle			++		++	0.130
Stomach		+	++	++	+	0.145
Foregut		+++	+++	+++		0.100
Hindgut		+++	+++	++		0.072
Liver	++	+++	+++	+++		0.174
Kidney			+++	+		0.147
Heart			+++	+++	++	0.083
Gill			+++	++	+++	0.155
Eye			+++	+++		0.193
Brain	+		+++			0.093

+, ++, and +++ denote faintly, medium and deeply stained in substrate, respectively.

Two esterase bands Est-3 and Est-5 were found in anterior, mid tail and buccal muscles. Esterase was more actives in α naphthyl acetate than that of the β naphthyl acetate in all of the mentioned three muscles. Teixeira *et al.* (2005) found six of esterases in the skeletal muscle of three species of peacock bass (*Cichla monoculus, C. temensis* and *Cichla* sp.). Nile tilapia showed lower variability than peacock bass interns of esterase isozyme in different muscles.

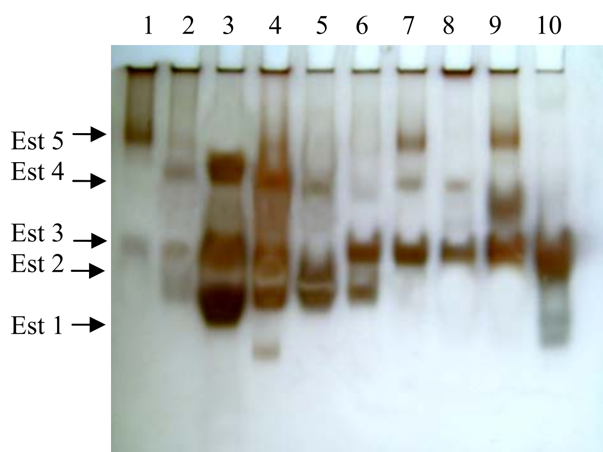

Fig 2. Esterase isozyme pattern in different tissues of Nile tilapia (*Oreochromis niloticus*) stained both in α and β naphthyl acetate as substrate for lowry test. Where lane (1) denotes Muscle; (2) Stomach; (3) Foregut; (4) Liver; (5) Hindgut; (6) Eye; (7) Gill; (8) Kidney; (9) Heart; (10) Brain. Arrow indicates position of esterase bands.

Many researchers observed frequently the high activity of esterase in the brain of different species (Brestkin *et al.*, 1975). In the present study two esterase bands were found in all the three parts of the brain (fore, mid and hind) where Est-1 which was faintly stained and Est-3 medium deep stained in α naphthyl acetate (Table 2 and Fig. 2). Only one esterase band Est-3 was found in β naphthyl acetate in all of the three parts of the brain.

In stomach four esterase bands namely Est-2, Est-3, Est-4 and Est-5 were observed in α naphthyl acetate (Table 1 and Fig. 1). However, in β naphthyl acetate Est-5 was absent and three esterase bands were observed. Three bands (Est-3, Est-4 and Est-5) were also observed in kidney, heart and gill tissues for esterase in both α and β naphthyl acetate (Table 1 and Fig. 1). Except in the gill where Est-5 was absent only in β naphthyl acetate. Two esterase bands Est-3 and Est-4 were found in both lens and black portion of the eye in both α and β naphthyl acetate. In kidney, darkly stained Est-3 in both α and β naphthyl acetate indicate high esterase activities (Table 2 and Fig. 2). Above results indicate that tissue specific differences observed in the banding pattern of esterase. Significant banding difference in esterase was also noticed in the kidney and heart of *Carassius* sp. that shows a similar trend in esterase diversity occurs among different fish (Shengming *et al.*, 1988).

Among eighteen observed tissues in the present study the esterase activity was most abundant in liver and stomach. Mentioned two organs have a relation with food digestion. Liver esterases could be associated with digestion and metabolism of different esters e.g. fat, cholesterol etc. (Jones & Brancoft, 1986; Sastry, 1974). Of the present study less esterase activity was observed in the eye and all muscles. Eye and muscles have a different physiological function in contrast with digestion and metabolism. Present study revealed that Est-3 was most abundant and most of the tissues contained it.

Banding pattern of esterases of different tissues has a good potential used in the identification of species. Al-Amin *et al.* (2005) reported that isozyme banding pattern of the intestine could be used for identification of two species of *Pangasius* (*P. sutchi* and *P. pangasius*). Between the two species the intestine of *P. sutchi* and *P. pangasius* possesses 4 and 6 bands, respectively. Furthermore, two species of *Anabas* (*A. testudineus* and *A. oligolepis*) was identified using esterase bands of liver, kidney, skeletal muscle, heart and egg (Ramaseshaiah & Dutt, 1984). The findings in the present study may be extended to use as genetic marker in various fields of physiology, taxonomy and toxicology in Nile tilapia (*O. niloticus*).

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