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Tissue Specific Esterase Isozyme Variation In *Clarias batrachus* And *C. gariepinus*

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Esterase isozymes of *Clarias batrachus* and *C. gariepinus* were studied on 7.5% Polyacrilamide Gel Electrophoresis (PAGE) stained with α and β naphthyl acetates as substrates to see the tissue specific variation of this enzyme from nineteen different tissues. Maximum six esterase bands (Est-$1^{1.52\pm0.01}$, Est-$2^{1.26\pm0.01}$, Est-$3^{0.99\pm0.01}$, Est-$4^{0.67\pm0.02}$, Est-$5^{0.33\pm0.01}$ and Est-$6^{0.17\pm0.02}$) were observed in Asian catfish (*C. batrachus*) where as African catfish (*C. gariepinus*) shown only four (Est-$1^{1.43\pm0.02}$, Est-$2^{1.22\pm0.03}$, Est-$3^{1.02\pm0.02}$ and Est-$5^{0.33\pm0.02}$). Localization of esterase isozymes were observed in different tissues of both Asian and African catfishes that indicated the switch on and off of specific allele based on the physio-chemical condition of corresponding tissues. Relatively higher concentration of esterase isozymes were found in digestive tissues. High number of esterase bands was found in Asian catfish which seems to indicate the higher allelic variation than in African catfish.

Keyword: Electrophoresis, Esterase isozymes, Tissue specificity, *Clarias batrachus*, *C. gariepinus*.

1. Introduction

Isozyme pattern showed pronounced differentiation in many organisms including fish [1, 2, 3] that could be used to estimate the genetic variation between different populations of fish species [4], also used to develop a genetic sexing system [5]. They are still amongst the quickest and cheapest marker systems to develop, and remain an excellent choice for projects that only need to identify low levels of genetic variation. Esterase isozyme is one of the lipid-hydrolyzing enzymes which have a great significance in the field of genetics and toxicology [6] and can be separated by electrophoresis at 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 [7]. This enzyme may be of particular importance because fish utilize lipid/fat as their main nutritional source rather than carbohydrate and protein especially during the later developmental stages [8].

The walking catfish, *Clarias batrachus,* is a species of freshwater air breathing catfish found primarily in Southeast Asia, so named for its ability to "walk" across the dry land, to find food or suitable environments. Walking catfish have a strong potential to be a pest due to their ability to migrate across land, and the fact that they feed on almost anything. The albino variety of this species has been quite popular in the aquarium fish industry, mostly as a curiosity [9]. Over its native range, however, it is cultured as a food fish [10, 11]. On the other hand, *C. gariepinus* belonging to family Clariidae is the native species of Africa. The species has drawn attention of aqua culturists because of its biological attributes that include

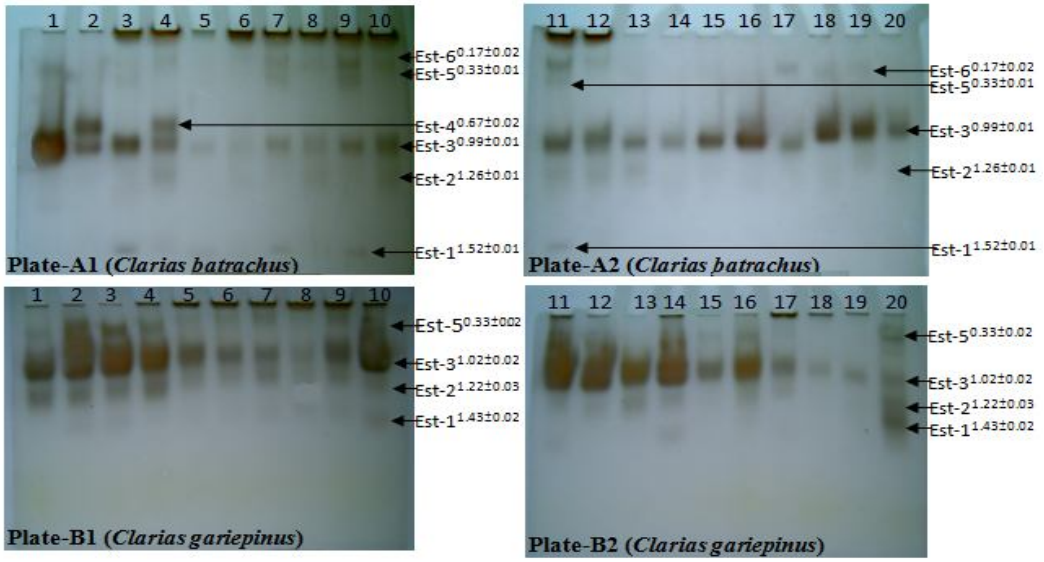
faster growth rate, resistance to diseases and possibility of high stocking density. Fish is the main source of animal protein (more than 80%) that is consumed by the people of Bangladesh [12]. Present attempt was taken to find out tissue specific esterase isozyme banding pattern in different tissues of Asian (*C. batrachus*) and African catfish (*C. gariepinus*).

2. Materials and Methods

Samples were collected from New Market, Dhaka, Bangladesh (original source-unknown) and the experiments were conducted in the laboratory of Genetics and Molecular Biology, Department of Zoology, University of Dhaka. Nineteen different tissues namely Anterior, Mid, Tail (in ventral region), Tail (in tip region), Buccal and Pelvic muscle, Fore, Mid and Hind gut and brain, Liver, Stomach, Kidney, Gill, Heart, Eye (lens, black portion) were taken (~ 0.016 g) from both species to observe the tissue specific esterase isozyme variability. Each tissue was squashed in TBE buffer (40 µl) and aliquots from each sample (15 µl) were loaded on the separate gel slots for electrophoresis after centrifuged at 12500 rpm for 15 min. The entire technique for polyacrylamide gel electrophoresis (PAGE) was followed as that of Shahjahan et. al.[13] and the electrophoretic bands of esterase isozymes resulting from stained gel with α and β naphthyl acetates were assigned to increasing numbers based on decreasing mobility following Richardson[14]. The experiment was repeated to standardize the result with different specimens. As there were no significant variations in each repetition, only one repetition was subjected to analysis.

3. Results

Six (Est-1, Est-2, Est-3, Est-4, Est-5 and Est-6) and four (Est-1, Est-2, Est-3 and Est-5) esterase bands were observed in Asian (*C. batrachus*) and African (*C. gariepinus*) catfishes in order (figure 1, table 1), the detail results of which are described below:



[Figure 1, Table 1]

Figure 1: Esterase isozyme banding pattern in different tissues of *Clarias batrachus* (Plate-A) and *C. gariepinus* (Plate-B), where lane denotes 1-Liver, 2-Anterior muscle, 3-Mid muscle, 4-Tail muscle (in ventral region), 5-Tail muscle (in tip region), 6-Buccal muscle, 7-Stomach, 8-Foregut, 9-Midgut, 10-Hindgut, 11-Liver, 12-Kidney, 13-Gill, 15-Eye (lens), 16-Eye (black portion), 17-Pelvic muscle, 18-Fore brain, 19-Mid brain and 20-Hind brain.
"→" mark represents esterase band with number.

3.1 Liver:

Altogether, five esterase bands (Est-1, Est-2, Est-3, Est-5 and Est-6) were found in the liver of Asian catfish of which Est-1 & Est-6 were medium stained, Est-2 & Est-5 were faint stained and Est-3 was deep stained. On the other hand, altogether four esterase bands (Est-1, Est-2, Est-3 and Est-4) were found in the same tissue of African catfish of which Est-1 was faint stained, Est-2 & Est-4 were medium stained and Est-3 was deep stained.

Table 1: Electrophoretic banding pattern showing the intensity variation of esterase isozymes (scored from α and β naphthyl acetates stained gels) in different tissues of *Clarias batrachus* and *C. gariepinus*.

Isozymes	**Est- 1**		**Est- 2**		**Est- 3**		**Est- 4**		**Est- 5**		**Est- 6**		**T1**	**T2**
Sample Name	Cb	Cg	Cb	Cg	Cb	Cg	Cb	Cg	Cb	Cg	Cb	Cg	Cb	Cg
1-Liver	-	-	F	M	D	D	F	-	F	F	-	-	67	50
2-Anterior muscle	-	F	-	M	D	D	D	-	-	D	-	-	33	67
3-Mid muscle	M	F	F	M	D	D	-	-	F	D	-	-	67	67
4-Tail muscle (in ventral region)	F	-	M	D	M	D	M	-	-	M	F	-	83	50
5-Tail muscle (in tip region)	F	-	-	M	F	D	-	-	-	F	-	-	33	50
6-Buccal muscle	-	-	-	M	F	M	-	-	-	-	-	-	17	33
7-Stomach	F	F	F	M	M	M	-	-	F	-	F	-	83	50
8-Foregut	-	F	F	M	M	M	-	-	-	-	F	-	50	50
9-Midgut	F	F	F	M	M	M	-	-	M	-	M	-	83	50
10-Hindgut	-	F	F	M	M	D	-	-	F	-	F	-	67	50
11-Liver	M	F	F	M	D	D	-	-	F	M	M	-	83	67
12-Kidney	-	-	F	F	D	D	-	-	-	F	F	-	50	50
13-Gill	-	-	F	M	M	D	-	-	-	-	-	-	33	33
14-Heart	-	F	F	F	M	D	-	-	-	M	-	-	33	67
15-Eye (lens)	-	-	-	-	D	M	-	-	-	-	-	-	17	17
16-Eye (black portion)	-	-	-	F	D	D	-	-	-	-	-	-	17	33
17-Pelvic muscle	-	-	-	F	M	M	-	-	-	-	F	-	33	33
18-Fore brain	-	-	F	-	D	M	-	-	-	-	F	-	50	17
19-Mid brain	-	-	F	-	D	M	-	-	-	-	F	-	50	17
20-Hind brain	-	M	-	M	M	M	-	-	-	M	-	-	17	67
C1	30	45	65	85	100	100	15	0	30	45	50	0	48.3	45.9

(-, F, M and D denote absent, faint, medium and deep stained bands; Cb and Cg stand for *C. batrachus* and *C. gariepinus* respectively; T1 and T2 represent the frequency (%) of esterase bands (out of six and four bands) present in a certain tissue of above mentioned species in order; C1 personates the frequency (%) of each esterase band in each species)

3.2 Anterior Muscle:

Only two esterase bands (Est-3 and Est-4) were found in the anterior muscle of Asian catfish both of which were deep stained whereas, four esterase bands (Est-1, Est-2, Est-3 and Est-4) were found in the same tissue of African catfish of which Est-1 was faint stained, Est-2 was medium stained and Est-3 & Est-4 were deep stained.

3.3 Mid Muscle:

Altogether, four esterase bands (Est-1, Est-2, Est-3 and Est-5 in Asian catfish and Est-1, Est-2, Est-3 and Est-4 in African catfish) were found in the mid muscle of both species. Est-1 of Asian catfish was medium stained whereas; it was faint stained in African catfish. Contrary to Est-1, Est-2 was faint stained in Asian catfish whereas; it was medium stained in African catfish. Est-3 was deep stained in both species. Est-5 of Asian

catfish was faint stained; Est-4 of African catfish was deep stained.

3.4 Tail Muscle (ventral region):
Altogether, five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-6) were found in the tail muscle (ventral region) of Asian catfish of which Est-1 & Est-6 were faint stained and rest of all were medium stained. On the other hand, only three esterase bands (Est-2, Est-3 and Est-4) were found in African catfish of which Est-4 was medium stained and Est-2 & Est-3 were deep stained.

3.5 Tail Muscle (tip region):
Only two (Est-1 and Est-3) and three (Est-2, Est-3 and Est-4) esterase bands were found in the tail muscle (tip region) of Asian catfish and African catfish accordingly. Bands of the Asian catfish were faint stained whereas; bands were faint, medium and deep stained in African catfish in order.

3.6 Buccal Muscle:
Only one (Est-3) and two (Est-2 and Est-3) esterase bands were present in the buccal muscle of Asian catfish and African catfish accordingly. Band of the Asian catfish was faint stained whereas; bands were medium stained in African catfish.

3.7 Pelvic Muscle:
Only two esterase bands were found in the pelvic muscle of Asian (Est-3 and Est-6) and African (Est-2 and Est-3) catfishes of which Est-3 was medium stained in both species whereas, Est-2 and Est-6 were faint stained.

3.8 Foregut:
Altogether three esterase bands were found in the foregut of Asian (Est-2, Est-3 and Est-6) and African (Est-1, Est-2 and Est-3) catfishes. In Asian catfish, Est-3 was medium stained and Est-2 & Est-6 were faint stained. In African catfish, Est-1 was faint stained and Est-2 & Est-3 were medium stained.

3.9 Midgut:
Altogether five esterase bands (Est-1, Est-2, Est-3, Est-5 and Est-6) were found in the midgut of Asian catfish of which Est-1 & Est-2 were faint stained, rest of all were medium stained. Whereas, only three esterase bands (Est-1, Est-2 and Est-3) were found in African catfish of which Est-1 was faint stained and Est-2 & Est-3 were medium stained.

3.10 Hindgut:
Altogether four (Est-2, Est-3, Est-5 and Est-6) and three (Est-1, Est-2 and Est-3) esterase bands were found in the hindgut of Asian and African catfishes. All the bands of Asian catfish were faint stained except Est-3 that was medium stained. Oppositely, faint, medium and deep stained bands were observed in African catfish in order.

3.11 Forebrain and Midbrain:
Altogether three esterase bands (Est-2, Est-3 and Est-6) were found in the forebrain and midbrain of Asian catfish of which Est-2 & Est-6 were faint stained and Est-3 was deep stained. On the other hand, only one medium stained band (Est-3) was present in African catfish in both tissues.

3.11.1 Hindbrain:
Only one band (Est-3) was present in the hindbrain of Asian catfish, whereas, four esterase bands (Est-1, Est-2, Est-3 and Est-4) were found in the hindbrain of African catfish. All of the bands were medium stained.

3.11.2 Eye (lens):
Only one band (Est-3) was present in the eye (lens) of Asian and African catfishes which was deep stained in first species and medium stained last one.

3.11.3 Eye (black portion):
Only one (Est-3) and two (Est-2 and Est-3) esterase bands were present in the eye (black portion) of Asian and African catfishes. Est-2 was faint stained and Est-3 was deep stained in both species.

3.12 Stomach:
Altogether, five esterase bands (Est-1, Est-2, Est-3, Est-5 and Est-6) were found in the stomach of Asian catfish, all of which were faint stained except Est-3 that was medium stained. On the other hand, only three esterase bands (Est-1, Est-2 and Est-3) were found in African catfish of which Est-1 was faint stained and Est-2 & Est-3 were medium stained.

3.13 Kidney:
Altogether, three esterase bands were found in the kidney of Asian (Est-2, Est-3 and Est-6) and African (Est-2, Est-3 and Est-4) catfishes. Est-3 of both species was deep stained, whereas other bands were faint stained.

3.14 Heart:
Only two esterase bands (Est-2 and Est-3) were found in the heart of Asian catfish of which Est-2 was faint and Est-3 was medium stained. On the other hand, four esterase bands (Est-1, Est-2, Est-3 and Est-4) were found in African catfish of which Est-1 & Est-2 were faint stained, Est-3 was deep stained and Est-4 were medium stained.

3.15 Gill:
Only two esterase bands (Est-2 and Est-3) were found in the gill of both Asian and African catfish of which Est-2 was faint and Est-3 was medium stained showing no significant differences.

4. Discussion
Polyacrylamide gel was not solid but made of a labyrinth of tunnels through a meshwork of fibers. As a result, the collections of proteins of any given size tended to move through the gel at the same rate, even if they did not take exactly the same tunnels to get through. The charge density on the proteins would cause smaller molecules to move more quickly through the gel's pores. The technique of polyacrylamide gel electrophoresis (PAGE) enabled us to determine the degree to which individual genes were polymorphic in a natural population [15]. Mendelian inheritance studies on these esterase loci showed that each of the bands corresponds to one allele [16]. Maximum six esterase bands were observed in Asian catfish where as African catfish shown only four that might be due different species of the present study. Eight, seven, six and five esterase bands were observed in the different tissues of *Oreochromis aureus* [17], *Megalobrana mblycephala* [18], *Ictalurus punctatus* [19] and of *Pangasius hypophthalmus*[20] accordingly. As the electrophoretic pattern of esterases of different tissues showed species specific variation, it could be successfully used for the identification of fish species [21], but needs further experimentation (running the sample into separate wells of same gel, same time). Although, current results may not represent the exact phenomena to identify the catfishes based on isozymic differences, but it represented a nearly exact experiments because the experiments were done maintaining the same gel concentration (7.5%) and same running time (~1 hour) on same iso-electric points (300 mA, 120 V). Tissue specific expression of esterases were also found in *Platypoecilus maculates, Xiphophorine helleri* and their F1 hybrid, where seven esterase zones were identified [22].

From the present investigation, it was found that the expression of esterase bands were tissue specific both in Asian and African catfishes. It is important to note that certain bands were absent in one tissue, while the other bands were present in other tissues that showed the localization of this enzyme in different tissues. Intensity of the same band also varied from tissue to tissue and species to species. Maximum number of bands was observed in the liver (5) and minimum in eye lens (1). Comparing the expression pattern of isozymes from different tissues of Asian and African catfishes, it was found that same tissue of them showed differential expression (Table 1).
Relatively higher concentration of esterase isozymes (in terms of occurrence and intensity) were found in the digestive tissues (liver, stomach, gut etc.) (Figure 1) resembled to that reported by Shahjahan et. al. [13] in *Oreochromis niloticus* and Begum et. al. [23] in *Heteropneustes fossilis*. Hirj and Courtney [24] found strong enzymatic activity in the upper and middle portion of the intestine where as weak in the

lower intestine of the perch fish *Perca fluvitalis*. Similar result was also observed in the digestive system of freshwater fishes [25]. Specific allele in specific tissues shows higher esterase activity due to biological need of that tissue specific function as for example digestive esterases, neuro tranmmiting esterases etc. Witzemann and Bousted[26] investigated that the location and function of the various esterases could vary from tissue to tissue and depend on the physiological demands of each system. Staining intensity may also be a parameter but in present study we take less attention on it as it need further experimentation (total protein estimation before running the gel).

Est-2 and Est-3 were frequently present in most of the tissues (65 % & 100% and 85% & 100% in Asian and African catfishes respectively). There was no significant difference in the gill tissue of Asian and African catfishes.

In average, esterase activity was higher in African catfish than that of Asian catfish that could be due to differential environmental conditions where they lived. Fore and mid brain of Asian catfish shown relatively higher esterase activity whereas, hind brain of African catfish was more active. The reason behind the fact was unknown and need further intensive study. Many researchers observed frequently the high activity of esterases in the brain of different species [27].

Frequency of esterase bands was found to be higher in Asian catfish (48.3%) which seems to indicate the higher allelic variation than African catfish (45.9%) (table 1). Differences in the number of bands between species may have underlying mechanisms regulating the esterase-related processes [28]. Distinctive esterase pattern from individuals made the determination of resistance status more efficient and much more precise [29].

5. Conclusion

Esterase studies are essential to know the genetic make up of a species for any successful conservation effort. Present study may be used for the development and application of molecular markers as for example species identification, selection and toxicological status.

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