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Comparison of esterase banding pattern in some selected tissues of *Macrognathus aculeatus* and *Mastacembalus armatus*

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ABSTRACT

Electrophoretic banding pattern of esterases from ten different tissues was compared between *Macrognathus aculeatus* and *Mastacembalus armatus* on polyacrilamide gels (7.5%) stained with α and β naphthyl acetates, where altogether, five esterase bands (Est-$1^{1.5}$, Est-$2^{1.13}$, Est-3^{1}, Est-$4^{0.63}$ and Est-$5^{0.5}$) were observed. The expressions of esterase bands appeared to have tissue specific as well as species specific distribution. Comparative study revealed that esterase isozymes banding pattern in different tissues of the studied fishes has both similarity and dissimilarity in their staining intensity, in the occurrence of the number of bands and also in the expression of specific allele. Frequency of bands was comparatively higher in *M. aculeatus* (62%) than that of *M. armatus* (46%). Tissues that showed pronounced differentiation viz. gut, stomach, eye etc. were selected as marker tissues for identification.

INTRODUCTION

Electrophoretic investigation of genetic marker such as proteins and enzymes especially allozymes and isoenzymes have been decisive in determining the taxonomic and population status of many organisms (Ferguson, 1980; Rashid, 2012). Isozymes which are the products of allelic genes are the multiple forms of single enzyme that differ in amino acid sequences but catalyze the same chemical reaction. To identify isozymes, a crude protein extract is made by grinding tissues with an extraction buffer and the components of extract are separated according to their size and charge by gel electrophoresis. Esterases are complex of lipid hydrolyzing enzyme that show a striking multiplicity of forms and function in many species and exhibit differential pattern of expression from tissue to tissue in many organisms including fish (Ferguson, 1980). Esterases play an important role in maintaining normal physiology and metabolism, detoxifying various drugs and environmental toxicants in living systems and are increasing important for chemical synthesis in industry. *Macrognathus aculeatus*, the lesser spiny eel is an edible fish available throughout the south-east Asia. *Mastacembelus armatus*, leopard spiny eel is not

only a popular aquarium fish but also a food fish. They are common during the tropical summer months and dwell in canals, lakes and other floodplain areas during the flood season. Although, it is possible to identify the studied species with naked eye or based on simple morph metric measurements, but study on molecular marker conformed their identity. As the electrophoretic pattern of esterases of different tissues showed species specific variation, it could be successfully used for the identification of fish species (Shengming et al., 1988). Hence, an attempt was taken to develop an isozyme based molecular marker.

MATERIALS AND METHODS

The adult fish samples were collected from Malibag fish market, Dhaka and were transported to the laboratory of Genetics and Molecular Biology, University of Dhaka, with ice cool packs. The specimens were then dissected to collect the desired amount (~0.016 g) of ten different tissues viz. liver, kidney, eye, stomach, fore_, mid_ and hind_gut, anterior_, mid_ and tail_muscle. Each tissue was then 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 electrophoresis was done on the continuous supply of 120 V and 300 mA. The entire technique for polyacrylamide gel electrophoresis (PAGE) was followed as that of Shahjahan et al., (2008) 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 (1986). The experiment was repeated to standardize the result with different specimens. As there was no significant variation in each repetition, only one repetition was subjected to analysis.

RESULTS AND DISCUSSION

Attempts were made to have a comprehensive picture of esterase isozyme variation from different squashed tissues of the studied species in terms of switch on or off of the specific allele and also the intensity variation, the result of which were as follows (Figure 1, Table 1)

Liver

Three (Est-2, Est-3 and Est-5) and two (Est-3 ans Est-5) esterase bands were observed in the liver of *M. aculeatus* and *M. armatus* respectively. Est-2 was faintly stained whereas Est-3 and Est-5 were moderately stained in *M. aculeatus* and deeply stained in *M. armatus*. Four esterase bands were observed in the same tissue of *Notopterus chitala* while only two bands in *N. notopterus* (Begum et al., 2012a). Differential expressions of these isozymes were also observed in the genus *Clarias* where four and five bands were found in *C. gariepinus* and *C. batrachus* respectively (Rashid and Rahman, 2013).

Kidney

Three esterase bands were found both in *M. aculeatus* (Est-2, Est-3 and Est-5) and *M. armatus* (Est-3, Est-4 and Est-5) that were moderate to deep stained. Est-2 and Est-4 were unique to above mentioned species in order. Three esterase bands were found in the same tissue of both *C. batrachus* and *C. gariepinus* (Rashid and Rahman, 2013).

Stomach

Moderate to deep stained, three (Est-2, Est-3 and Est-5) and two (Est-3 and Est-4) esterase bands were also observed in the stomach of *M. aculeatus* and *M. armatus* respectively where Est-2 and Est-4 were unique to above mentioned species in order. Five and three esterase bands were found in *C. batrachus* and *C. gariepinus* (Rashid and Rahman, 2013) respectively, whereas both *N. notopterus* and *N. chitala* exhibited two bands (Begum et al., 2012a).

Fore gut

Three (Est-1, Est-3 and Est-5) and two (Est-2 and Est-3) esterase bands were observed in the fore gut of *M. aculeatus* and *M. armatus* respectively. All the bands of *M. aculeatus* were deeply stained whereas moderate to deep stained in *M. armatus*. Three esterase bands were found in the same tissue of both *C. batrachus* and *C. gariepinus* (Rashid and Rahman, 2013) while single band was detected both in *N. chitala* and *N. notopterus* (Begum et al., 2012a).

Mid gut

Four (Est-1, Est-2, Est-3 and Est-5) and three (Est-3, Est-4 and Est-5) esterase bands were observed in the mid gut of *M. aculeatus* and *M. armatus* respectively. Est-1 and Est-2 of *M. aculeatus* were faintly stained while Est-3 and Est-5 were deeply stained. On the other hand, Est-3, Est-4 and Est-5 of *M. armatus* were deep, medium and faintly stained in order. Five and three esterase bands were found in the same tissue of *C. batrachus* and *C. gariepinus* (Rashid and Rahman, 2013) accordingly. Whereas, one and two esterase bands were detected in *N. notopterus* and *N. chitala* (Begum et al., 2012a) respectively.

Hind gut

Four (Est-1, Est-2, Est-3 and Est-5) and two (Est-3 and Est-4) esterase bands were observed in the hind gut of *M. aculeatus* and *M. armatus* respectively. Est-1 and Est-2 of *M. aculeatus* were faint and moderately stained accordingly while; Est-3 and Est-5 were deeply stained. On the other hand, Est-3 and Est-4 of *M. armatus* were deep and moderately stained respectively. Four and three esterase bands were observed in the same tissue of *C. batrachus* and *C. gariepinus* accordingly (Rashid and Rahman, 2013).

Eye

Moderate to deep stained, three (Est-2, Est-3 and Est-5) and two (Est-3 and Est-4) esterase bands were also observed in the eye of *M. aculeatus* and *M. armatus* respectively where Est-2 and Est-4 were unique to above mentioned species in order. One and two bands were found in *N. chitala* and *N. notopterus* (Begum et al., 2012a) respectively. One and two bands were also found in the separate study on *Heteropneustes fossilis* (Begum et al., 2011) and *O. niloticus* (Shahjahan et al., 2008) accordingly.

Anterior muscle

Anterior muscle of both *M. aculeatus* and *M. armatus* showed two esterase bands (Est-3 and Est-4). In *M. aculeatus* bands were faintly stained and in *M. armatus* bands were moderately stained. Single band was observed in *N. notopterus* and *N. chitala* (Begum et al., 2012a), whereas two and four bands in *C. batrachus* and *C. gariepinus* accordingly (Rashid and Rahman, 2013).

Mid muscle

In the mid muscle of *M. aculeatus* three bands namely Est-1, Est-2 and Est-5 were observed of which Est-1 and 2 were faintly stained and Est-5 were deeply stained. In *M. armatus* two esterase bands (Est-3 and Est-4) were found, both of which were faintly stained. Four esterase bands were observed in the same tissue of both *C. batrachus* and *C. gariepinus* (Rashid and Rahman, 2013).

Tail muscle

Tail muscle of *M. aculeatus* showed three esterase bands (Est-1, Est-2 and Est-5) of which Est-1 and 2 were faintly stained and Est-5 was moderately stained. Three esterase bands (Est-1, Est-3 and Est-4) were also found in *M. armatus* where, Est-1 was faintly stained while; Est-3 and Est-4 were moderately stained. Only one esterase band was found in *N. notopterus* and *N. chitala* (Begum et al., 2012a) while, two and three bands were observed in *C. batrachus* and *C. gariepinus* in order (Rashid and Rahman, 2013).

Non specific esterase isozymes after electrophoresis were used to identify two species of *Anabas* (Ramaseshaiah and Dutt, 1984), *Clarias* (Begum et al., 2012b) and *Pangasius* (Amin et al., 2005) based on relative mobility and presence or absence of certain band. Staining intensity might also be a good parameter but in present study we have taken less attention on it as it need further experimentation (i.e., equivalence of total protein before loading into gel slots). Both species showed five esterase bands but their expression pattern might differ from one another. Five esterase bands were also observed from the different tissues of *Oreochromis niloticus* (Shahjahan et al., 2008) and of *Pangasius hypophthalmus*

(Begum et al., 2008) but the banding pattern varied from tissue to tissue. Number of bands may or may not vary among the species. As for example six and four esterase bands were observed in *Clarias batrachus* and *C. gariepinus* accordingly (Rashid and Rahman, 2013) while, four bands were found both in *N. notopterus* and *N. chitala* (Begum et al., 2012a).

High number of esterase bands was found in *M. aculeatus* (62%) than that of *M. armatus* (46%) which seems to indicate the higher allelic variation in *M. aculeatus* (Table 1). Similar result was also observed in the tissue specific esterase isozyme study of *Clarias batrachus* and *C. gariepinus* where *C. gariepinus* showed higher allelic variation (Rashid and Rahman, 2013). Differences in the number of bands between species may have underlying mechanisms regulating the esterase related processes (Lima-Catelani et al., 2004). Distinctive esterase pattern from individuals made the determination of resistance status more efficient and much more precise (Zhou et al., 2002).

Maximum four bands were found in the mid and hind gut of *M. aculeatus* whereas most of the tissues showed two or three bands absent in one tissue, while it was present in other tissues.

As for example, Est-1 was common in tail muscle, Est-2 in mid gut, Est-3 in all the tissues except mid and tail muscle, Est-4 in anterior muscle and Est-5 in liver, kidney and mid gut of both species. The location and function of the various esterases could vary from tissue to tissue and depend on the physiological demands of each system (Witzemann and Bousted, 1981). Three esterase bands were observed in fore gut, mid muscle, stomach and eye of *M. aculeatus*, while only two bands in *M. armatus*. Mid and hind gut of *M. aculeatus* showed four esterase bands while three and two in *M. armatus* accordingly. Relatively, higher esterase activity was observed in the mid gut in comparison with fore and hind gut which could be due to digestive function of this enzyme (Lima-Catelani et al., 2004). Hirj and Courtney (1983) 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*. Specific allele in specific tissues showed higher esterase activity due to biological need of that tissue specific function (Rashid and Rahman, 2013).

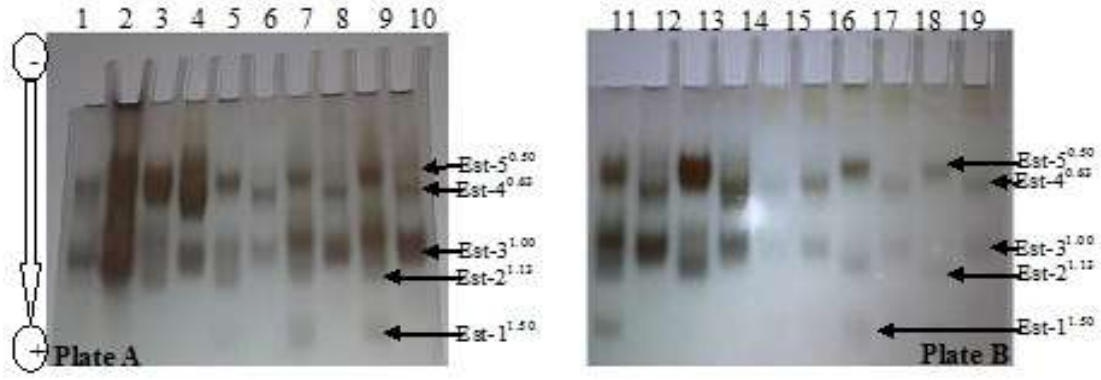

Fig.1: Comparative esterase isozyme banding pattern in different tissues of *Macrognathus aculeatus* and *Mastacembalus armatus* stained with both α and β naphthyl acetates by using 7.5% PAGE. Where odd and even number represent sample of *M. aculatus* and *M. armatus* respectively. "→" mark represents esterase band with number and relative mobility (in superscript). Lane 1, 2-Liver; 3, 4-Kidney; 5, 6-Stomach; 7, 8-Fore gut; 9, 10-Mid gut; 11, 12-Hind gut; 13, 14-Eye; 15, 16-Anterior muscle; 17, 18-Mid muscle and 19, 20-Tail muscle.

Est-3 was prominent band (90%) among all selected tissues of both species, while Est-1 showed the lowest frequency (30%) which indicated that each allele might have underlying mechanisms regulating the esterase related processes (Lima-Catelani et al., 2004). Certain band was also common in all the studied tissues of *H. fossilis* (Begum et al., 2011), *O. niloticus* (Shahjahan et al., 2008) and of *P. hypophthalmus* (Begum et al., 2008).

CONCLUSION

Expression of tissue specific esterase isozymes showed differential banding pattern that could be used in rapid and inexpensive identification of studied species. To get more conclusive result, it is recommended to check the variability in terms of sex, age and geographical distribution. Current study could be useful for extended workers of toxicological study and to develop molecular markers.

Table 1: Electrophoretic banding pattern showing the intensity variation of esterase isozymes (scored from napthyl acetate stained gels) in different tissues

Tissues	Species	Est-$1^{1.50}$	Est -$2^{1.13}$	Est-$3^{1.00}$	Est-$4^{0.63}$	Est-$5^{0.50}$	T1	T2
Liver	M1	-	+	++	-	++	60	50
	M2	-	-	+++	-	+++	40	
Kidney	M1	-	++	++	-	+++	60	60
	M2	-	-	++	+++	+++	60	
Stomach	M1	-	++	++	-	+++	60	50
	M2	-	-	++	++	-	40	
Foregut	M1	+++	-	+++	-	+++	60	50
	M2	-	+++	++	-	-	40	
Midgut	M1	+	+	+++	-	+++	80	70
	M2	-	-	+++	++	+	60	
Hind gut	M1	+	++	+++	-	+++	80	60
	M2	-	-	+++	++	-	40	
Eye	M1	-	++	++	-	+++	60	50
	M2	-	-	++	++	-	40	
Anterior muscle	M1	-	-	+	+	-	40	40
	M2	-	-	++	++	-	40	
Mid muscle	M1	+	+	-	-	+++	60	50
	M2	-	-	+	+	-	40	
Tail muscle	M1	+	+	-	-	++	60	60
	M2	+	-	++	++	-	60	
C		30	45	90	45	60		

M1 = *M. aculeatus*, M2 = *M. armatus*, -, +, ++ and +++ represent absent, faint, moderate and deep stained bands. T1 represent the frequency (%) of esterase bands (out of total bands) present in a certain tissue; T2 stands for average frequency (%) of esterase bands in a selected tissue, C1 personates the frequency (%) of each esterase band in all selected tissues.

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