JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2013, Vol. 1, No. 4, pp:254-259
Available at www.jcrs010.com

ORIGINAL ARTICLE

TISSUE SPECIFIC ESTERASE ISOZYME VARIATION IN FOUR *Punctius* SPECIES

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ABSTRACT: Electrphoretic banding pattern of esterase isozymes was studied on 7.5% polyacrylamide gels from ten different tissues of four *Punctius* species (*P. sarana, P. sophore, P. conchonius* and *P. ticto*). All the studied species showed five esterase bands except *P. ticto* that lacked Est-5. Maximum number of bands was observed in heart (80%), liver (75%), pelvic muscle (73.75%), eye (70%) and brain (68.75%) of four mentioned species whereas, minimum in anterior muscle (47.5%). Same tissue of different species showed both similarity and dissimilarity in terms of allelic expression. As for example, four esterase bands were found in the liver of *P. conchonius* (Est-1, Est-3, Est-4 and Est-5) and *P. ticto* (Est-1, Est-2, Est-3 and Est-4) while two and three bands in *P. sophore* (Est-3 and Est-4) and *P. sarana* (Est-2, Est-3 and Est-4) respectively. On the other hand, allele of certain locus also showed tissue and species specific expression with varied type of intensity. For example, Est-4 was common in the liver of all the species and its intensity varied from faint to deep whereas, deep stained Est-5 was confined to the same tissue of *P. conchonius* only.

KEYWORDS: Electrophoresis, Esterase Isozymes, Tissue Specificity, *Punctius* spp.

INTRODUCTION

Fish is the main source of animal protein in the diet of the people of Bangladesh because more than 80% of the animal protein in our diet comes from fish alone (Ruby *et al.*, 1978). Commonly observed four species of *Puntius* (*P. sarana, P. sophore, P. conchonius* and *P. ticto*) are omnivorous fresh water fish, having a lifespan of up to 5 years and distributed throughout the Bangladesh. *P. sarana* is moderate in size, whereas *P. sophore* is comparatively smaller. The male of *P. conchonius* has a brighter pinkish color and the female is slightly plumper. *Puntius ticto* with two black spots; one just before the pectoral fin and one near the tail, is of commercial importance in the fish keeping industry and is used to create hybrid variants of tiger barbs and other barbs. They are available in the fish market throughout the year having a fewer market prices. The fish is edible with good taste and rich in protein that could solve our malnutrition problem to some extent.

Isozymes are enzymes that differ in amino acid sequences but catalyze the same chemical reaction and the existence of which permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage. They are the product of different genes and thus represent different loci. Isozymes that occur in numerous iso-forms (based on size or shape or net charge) can be separated by electrophoresis allowing rapid and inexpensive analysis of a large number of isolates. The lipid-hydrolyzing esterases split into an acid and an alcohol in a chemical reaction with water involving the hydrolysis of ester. A wide range of different esterases exists that differ in their substrate specificity, their protein structure and their biological function. Mendelian inheritance studies on these esterase loci showed that each of the bands corresponds to one allele (Stordeur, 1976). Correlation has been made in several fish species between the presence of this enzyme with fat digestion and lipid absorption (Baglole *et al.*, 1998). Since each band pattern is characteristic of one species and is practically unaltered as a result of ice storage or frozen storage of the fish, it is possible to identify any fresh and frozen fish by comparing its banding pattern. Hence, an attempt was taken to investigate the tissue specific esterase isozyme variation in the studied species.

MATERIALS AND METHODS

The present study on esterase loci from different tissues of *Punctius* species was carried out in Genetics and Molecular Biology Laboratory, Department of Zoology, University of Dhaka, Bangladesh. The adult fish samples were collected from Titas River, Brahmanbaria and were transported to the laboratory with ice cool packs. The specimens were then dissected to

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collect the desired amount (~0.016 g) of following tissues: Pelvic_, Anterior_ and Tail_ muscle, Eye, Brain, Liver, Stomach, Heart, Fore_ and Mid_ gut of intestine. 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 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 (figure and table 1), the result of which were as follows:

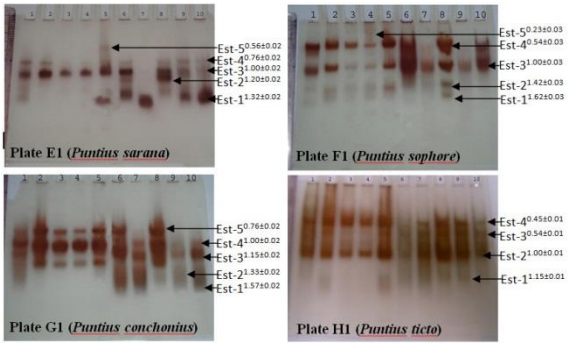

Figure 1: Esterase isozyme banding pattern in different tissues of four Puntius species stained with α and β naphthyl acetates. Arrows indicate the location and number of different esterases (relative mobility in superscript). Lane denote:1-Pelvic muscle, 2-Eye, 3-Anterior muscle, 4-Tail muscle, 5-Brain, 6-Liver, 7-Stomach, 8-Heart, 9-Fore gut of intestine, 10-Mid gut of intestine.

3.1. Pelvic Muscle

Four esterase bands were found in *P. sarana* (Est-1, Est-2, Est-4 and Est-5) and in *P. conchonius* (Est-2, Est-3, Est-4 and Est-5) with differential allelic expression. On the other hand, three (Est-2, Est-3 and Est-4) and two (Est-2 and Est-4) esterase bands were found in *P. sophore* and *P. ticto* respectively. Est-1, Est-2 and Est-5 of *P. sarana* were faintly stained but Est-4 was medium faintly stained. Est-2, Est-3 and Est-4 of *P. sophore* were faintly, medium deep and deeply stained in order. Est-2 and Est-5 were medium stained. Est-3 was medium deep but Est-4 was deeply stained in *P. conchonius.* Est-2 and Est-4 of *P. ticto* were deeply and medium deep stained accordingly. Two esterase bands were found in the same tissue of both *Clarias batrachus* and *C. gariepinus* (Rashid and Rahman, 2013) while single band was detected both in *Notopterus chitala* and *N. notopterus* (Begum *et al.,* 2012).

3.2. Eye

Two esterase bands (Est-4 and Est-5) in the eye of both *P. sarana* and *P. conchonius*, whereas three (Est-1, Est-2 and Est-4) and four bands (Est-1, Est-2, Est-3and Est-4) were detected in *P. ticto* and *P. sophore* accordingly. Est-4 that was medium to deep stained was found to be common in all the studied species while Est-3 only in *P. sophore.* Est-5 was faintly stained in *P. sarana* while deeply stained in *P. conchonius.* Est-1 and Est-2 were faintly stained in *P. sophore* while faint to medium stained in *P. ticto.* One and two bands were found in *N. chitala* and *N. notopterus* (Begum *et al.,* 2012) respectively. One and two bands were also found in the separate study on *Heteropneustes fossilis* (Begum *et al.,* 2011) and *Oreochromis niloticus* (Shahjahan *et al.,* 2008) accordingly.

3.3. Anterior Muscle

Three esterase bands were found both in *P. conchonius* (Est-2, Est-4 and Est-5) and *P. sophore* (Est-2, Est-3 and Est-4) where two bands (Est-2 and Est-4) were common for both species. Only one (Est-5) and two esterase bands (Est-2 and Est-4) were found in *P. sarana* and *P. ticto* respectively. Faintly stained Est-3 was unique to *P. sophore.* Staining intensity of Est-2 and Est-5 varied faint to medium, while Est-4 varied medium to deep. Single band was also observed in *N. notopterus* and *N. chitala* (Begum *et al.,* 2012), whereas two and four bands in *C. batrachus* and *C. gariepinus* accordingly (Rashid and Rahman, 2013).

3.4. Anal Muscle

One (Est-5), Two (Est-2 and Est-4), three (Est-2, Est-4 and Est-5) and four (Est-2, Est-3, Est-4 and Est-5) esterase bands were detected in *P. sarana, P. ticto, P. conchonius* and *P. sophore* in order. Est-2 and Est-4 were common in all the species except *P. sarana* while faintly stained Est-3 was unique to *P. sophore.* Est-2 and Est-5 was faint to medium stained while Est-4 was medium to deep stained. Only one esterase band was found

in *N. notopterus* and *N. chitala* (Begum *et al.*, 2012) while, two and three bands were observed in *C. batrachus* and *C. gariepinus* in order (Rashid and Rahman, 2013).

3.5. Brain

Three esterase bands were found in the brain of *P. sophore* (Est-2, Est-3 and Est-4), *P. conchonius* (Est-2, Est-4 and Est-5) and *P. ticto* (Est-1, Est-2 and Est-4) while only two in *P. sarana* (Est-1 and Est-4). Medium to deep stained Est-4 was common in all the species whereas medium stained Est-5 was confined to *P. conchonius.* Est-1 was faintly stained but Est-2 and Est-3 were medium to deep stained. Three bands were observed in the brain of *N. notopterus* and *N. chitala* (Begum *et al.*, 2012) while three and two bands in *H. fossilis* (Begum *et al.*, 2011) and *O. niloticus* (Shahjahan *et al.*, 2008) accordingly.

3.6. Liver

Four esterase bands were found in the liver of *P. conchonius* (Est-1, Est-3, Est-4 and Est-5) and *P. ticto* (Est-1, Est-2, Est-3 and Est-4) while two and three bands in *P. sophore* (Est-3 and Est-4) and *P. sarana* (Est-2, Est-3 and Est-4) respectively. Est-4 was common in all the species and its intensity varied from faint to deep whereas deep stained Est-5 was confined to *P. conchonius.* Est-1 was faint to medium stained, Est-2 was medium to deep stained and Est-3 was faint to deep stained. Four esterase bands were observed in the same tissue of *N. chitala* while only two bands in *N. notopterus* (Begum *et al.*, 2012). 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).

3.7. Stomach

One (Est-1), Two (Est-3 and Est-4), three (Est-1, Est-3 and Est-4) and four (Est-1, Est-2, Est-3 and Est-4) esterase bands were detected in *P. sarana, P. sophore, P. conchonius* and *P. ticto* in order. No band was common in all four species while deep stained Est-2 was confined to *P. ticto*. Est-3 was medium stained whereas staining intensity of Es-1 and Est-4 varied from faint to deep stained. 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.*, 2012).

3.8. Heart

Four esterase bands were observed in the heart of *P. sarana* (Est-2, Est-3, Est-4 and Est-5), *P. sophore* (Est-1, Est-2, Est-3 and Est-4) and *P. ticto* (Est-1, Est-2, Est-3 and Est-4) while three bands in *P. conchonius* (Est-2, Est-4 and Est-5). Faint to deep stained Est-2 and deep stained Est-4 were common to all species while faintly stained Est-1 and deep stained Est-5 were confined to *P. sophore* and *P. conchonius* respectively. Faint to deep stained Est-3 was common except *P. conchonius.* Two esterase bands were observed in *N. notopterus* and *N. chitala* (Begum *et al.*, 2012) whereas four two bands in *C. gariepinus* and *C. batrachus* (Rashid and Rahman, 2013) accordingly. On the other hand, four and three esterase bands were observed in *H. fossilis* (Begum *et al.*, 2011) and in *O. niloticus* (Shahjahan *et al.*, 2008) in order.

3.9. Fore Gut

One (Est-3), two (Est-1and Est-3), three (Est-2, Est-4 and Est-5) and four (Est-1, Est-2, Est-3 and Est-4) esterase bands were found in fore gut of *P. sophore, P. conchonius, P. sarana* and *P. ticto* accordingly. No band was common to all but faintly stained Est-5 was confined to *P. sarana.* Est-4 was faintly stained in *P. sarana* but medium deep stained in *P. ticto.* Est-3 and Est-2 were medium to deep stained while Est-1 was faint to medium stained. 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.*, 2012).

3.10. Mid Gut

Three esterase bands were found in *P. sarana* (Est-2, Est-4 and Est-5) and in *P. conchonius* (Est-1, Est-3 and Est-4) while two and four bands were found in *P. sophore* (Est-3 and Est-4) and in *P. ticto* (Est-1, Est-2, Est-3 and Est-4) respectively. Faint to medium stained Est-4 was common in all the species whereas faintly stained Est-5 was confined to *P. sarana.* Est-3 was medium to deep stained, Est-2 was deep stained and Est-1 was faint to medium stained. 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 bands were detected in *N. notopterus* and *N. chitala* (Begum *et al.*, 2012) respectively.

Except *P. ticto,* other three species showed five esterase bands. Number of bands may or may not vary within the species of same genus. As for example six and four esterase bands were observed in *C. batrachus* and *C. gariepinus* accordingly (Rashid and Rahman, 2013) while,

four bands were found both in *N. notopterus* and *N. chitala* (Begum *et al.,* 2012). Five esterase bands were also observed from the different tissues of *O. niloticus* (Shahjahan *et al.,* 2008) and of *Pangasius hypophthalmus* (Begum *et al.,* 2008) but the banding pattern varied from tissue to tissue. Staining intensity might also be a good parameter but in present study we have taken less attention on it as it need further experimentation.

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) but it needs further experimentation i.e., loading the same tissue of all species in same gel. However, current results seem to be approximate for this purpose as the gel concentrations, running time, voltage etc. were same for all the gels.

A variant type of allelic expression of these isozymes was observed from different tissues and none of the tissue showed all five bands indicating a clear picture of localization of these allelic expressions. While maximum tissues showed four esterase bands, some of the tissues showed one or two bands in certain species (Table 1). The location and function of the various esterase forms could vary from tissue to tissue and depend on the physiological demands of each system (Witzemann and Bousted, 1981). Strong enzymatic action was seen in heart (80%), liver (75%), pelvic muscle (73.75%), eye (70%) and brain (68.75%) of four mentioned species whereas, weak in anterior muscle (47.5%). Specific allele in specific tissues showed higher esterase activity due to biological need of that tissue specific function (Rashid and Rahman, 2013). However, many researchers observed frequently the high activity of esterase in the brain of different species (Brestkin *et al.,* 1975). 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*.

Est-4 was prominent band (87.5%) among the selected tissues of all four species, while Est-5 showed the lowest frequency (22.5%) 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 fish species, in toxicological study and to develop molecular markers but it needs further intensive study.

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Table 1: Electrophoretic banding pattern showing the intensity variation of esterases in different tissue of Puntius species (Scored from alpha and beta naphthyl acetates stained gels)

Tissues↓	**Est-1**				**Est-2**				**Est-3**				**Est-4**				**Est-5**				**T1**	**T2**	**T3**	**T4**	**T**
Species→	Psr	Psp	Pch	Ptc	Psr	Psp	Pch	Ptc	Psr	Psp	Pch	Ptc	Psr	Psp	Pch	Ptc	Psr	Psp	Pch	Ptc	Psr	Psp	Pch	Ptc	Avg
Pelvic muscle	F	-	-	-	F	F	M	D	M	M	M	M	M	D	D	D	-	-	M	-	80	60	80	75	73.75
Eye muscle	-	F	-	F	-	F	M	M	D	M	-	M	F	D	D	D	-	-	D	-	40	80	60	100	70
Anterior muscle	-	-	-	-	-	F	F	M	M	F	-	-	-	M	D	D	-	-	M	-	20	60	60	50	47.5
Tail muscle	-	-	-	-	-	F	F	M	M	F	-	-	-	M	D	M	-	F	M	-	20	80	60	50	52.5
Brain	F	-	-	M	-	M	M	D	D	M	-	-	F	D	D	D	F	-	M	-	80	60	60	75	68.75
Liver	D	-	M	F	F	-	-	M	D	D	D	M	F	D	M	F	-	-	D	-	80	40	80	100	75
Stomach	D	-	M	F	-	-	-	D		M	M	M	-	F	M	F	-	-	-	-	20	40	60	100	55
Heart	F	F	-	F	D	M	D	D	D	D	-	M	F	D	D	M	-	-	D	-	80	80	60	100	80
Foregut	M	-	F	M	-	-	-	D	M	M	M	M	F	-	-	M	-	-	-	-	60	20	40	100	55
Mid gut	D	-	M	F	-	-	-	M	F	D	D	M	F	M	M	F	-	-	-	-	60	40	60	100	65
C1	70	20	40	70	30	60	60	100	90	100	50	70	70	90	90	100	10	10	70	0	**54**	**56**	**62**	**85**	64.25
C2	43.33				62.5				77.5				87.5				22.5				**64.25**				

Psr = Puntius sarana, Psp = Puntius sophore, Pch = Puntius conchonius, Ptc = Puntius ticto, D = Deep Stained, M = Medium Stained, F = Faint Stained, - = Absent, T1, T2, T3 and T4 represent the frequency (%) of esterase bands (out of total bands) present in a certain tissue of corresponding species; T stands for average frequency (%) of esterase bands in a selected tissue, C1 personates the frequency (%) of each esterase band in all selected tissues of corresponding species, C2 referred to the average frequency (%) of each esterase band in all selected tissues of all four species.