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ANALYSIS OF ESTERASE ISOZYME BANDING IN SOME TISSUES OF NILE TILAPIA AND GENETICALLY IMPROVED FARMED TILAPIA OF *OREOCHROMIS NILOTICUS* L.

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Abstract: Esterase isozyme variability was analysed in eight different tissues to distinguish between, Nile Tilapia (NT) and Genetically Improved Farmed Tilapia (GIFT), of the species *Oreochromis niloticus* L. The tissues were anterior, dorsal, pectoral, buccal, anal, tail, gill and heart muscles. Polyacrylamide gel electrophoresis (7.5% PAGE) was employed for this purpose and the substrate used was α napthyl acetate. Five esterase bands, namely Est-$1^{1.65}$, Est-$2^{1.32}$, Est-$3^{1.00}$, Est-$4^{0.73}$ and Est-$5^{0.38}$ were found to be common for both GIFT and NT in the different observed tissues. Est-$6^{0.25}$ was observed only in GIFT as an extra band. Esterase had tissue-specific and species-specific expression. Est-1 band was detected in anterior and dorsal muscles; Est-2 in anterior, dorsal, pectoral, gill and heart muscles; Est-3 in all tissues; Est-4 was observed in all tissues of NT except in dorsal muscle; in GIFT samples it was absent in anterior, dorsal, gill and heart tissues. Est-5 occurred in anal, dorsal, gill, heart and pectoral muscles but not in the anterior, buccal and tail samples. Esterase-6 was identified only in GIFT with a variation in intensity ranged from faint and medium to deep in the dorsal, anal and heart tissues. This band restricted only in GIFT could be used as an identification marker to distinguish between NT and GIFT.

mvi-ms‡¶c: *Oreochromis niloticus*-Gi AšMZ bvBj †Ujwcqv (NT) Ges ‡R‡bwUK¨wj Bgc"fW dvg †Ujwcqv (GIFT) Gi g‡a¨ c_K Kivi Rb¨ 8wU wUmi G÷v‡iR AvB‡mvRvB‡gi wfbZv ch‡e¶Y Kiv n‡q‡Q| wUm¸‡jv nj m¤§L, cô‡`kxq, e¶:¯jxq, ev°vj, c"Q‡`kxq, †jR, djKv Ges ü`‡ckx| Avjdv b¨c_vBj Gwm‡UU mve÷¨vU wnmv‡e e¨envi K‡i cwjGwµjvgvBW †Rj B‡j‡±v‡dv‡iwmm (7.5% PAGE) c×wZ‡Z ch‡¶b Kiv nq| Dfq †Ujwcqvi (GIFT Ges NT) wUm‡Z 5wU G÷v‡iR e¨Û h_vµ‡g Est-$1^{1.65}$, Est-$2^{1.32}$, Est-$3^{1.00}$, Est-$4^{0.73}$ Ges Est-$5^{0.38}$ cvIqv wM‡q‡Q| GKwU AwZwi³ e¨Û, Est-$6^{0.25}$, ‡Kejgv‡Î GIFT G cvIqv wM‡q‡Q| G÷v‡iR AvB‡mvRvBg wUm wfbK ewn:cKvk nq| NT Gi m¤§L I cô‡`kxq gvsm †ckx‡Z Est-1 e¨Û cvIqv wM‡q‡Q; m¤§L, cô‡`kxq, e¶:¯jxq, djKv Ges ü`‡ckx†Z Est-2; mKj gvsm †ckx‡Z Est-3 cvIqv wM‡q‡Q| NT-Gi m¤§L gvsm †ckx e¨wZZ D‡jwLZ mKj gvsm †ckx‡Z Ges GIFT-Gi m¤§L, cô‡`kxq, djKv Ges ü`‡ckx‡Z Est-4 ch‡¶b Kiv n‡q‡Q | Est-5 cô‡`kxq, e¶:¯jxq, c"Q‡`kxq, djKv Ges ü`‡ckx†Z Dcw¯Z wQj| Est-6 †Kejgv‡Î GIFT- Gi m¤§L, cô‡`kxq, c"Q‡`kxq Ges ü`‡ckx†Z wPwýZ Kiv wM‡q‡Q| †h‡nZ Est-6 †Kejgv‡Î GIFT-Gi ‡ckx¸‡jv†Z Dcw¯Z wQj ZvB GwU NT Ges GIFT-Gi g‡a¨ c_K ci Rb¨ mbv³Kib wPý wn‡m‡e e¨eüZ n‡Z cv‡i|

Key words: Esterase, GIFT, identification marker, *Oreochromis niloticu]*

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INTRODUCTION

Tilapia is the common name applied to three genera of fish under the Family Cichlidae: *Oreochromis*, *Sarotherodon*, and *Tilapia*. The Nile Tilapia (NT) *Oreochromis niloticus* L. is the most abundant species in freshwater tilapia culture. NT was first introduced into Bangladesh by the United Nations International Children Emergency Fund (UNICEF) in 1974, and later by the Bangladesh Fisheries Research Institute (BFRI) from Thailand (Gupta *et al.* 1992). *Oreochromis* spp. ingest a wide range of food, such as benthic algae, phytoplankton, macrophytes, zooplankton, fish eggs, fish larvae, and detritus (Alceste and Jory 2000). The fish is preferred by farmers because of its desirable features such as faster growth rate compared to any other short cycled fish species including other commonly used tilapia strains, high yield, tasty flesh and ease of reproduction. Moreover, this species is a hardy fish, good converter of organic wastes into quality protein and resistant to disease (Stickney *et al.* 1979, Balarin and Haler 1982, Pullin and Lowe-McConnel1982). However, it is least cold tolerant and prefers tropical to subtropical climates. *Oreochromis* spp. exhibit maximum growth rates at temperatures between 25 and 30°C (Meyer 2002), making them more likely to become established and invasive in tropical climates. However, both tolerances to water temperature and salinity vary greatly between tilapia species.

The Genetically Improved Farmed Tilapia or GIFT (Eknath *et al.* 1993) was introduced into Bangladesh in July 1994 from the Philippines. The strain was developed by the International Center for Living Aquatic Resources Management (ICLARM) through several generations of selection from a base population involving eight different strains of NT (Eknath *et al.* 1993, Gupta *et al.* 2004). This strain was the result of five generations of combined selection for growth on a genetically variable synthetic base population derived from eight separate strain accessions. The GIFT selection programme was reported to have accumulated genetic gain of 85% in relation to the base population (Eknath and Acosta 1998) and was established as a good strain for aquaculture and widely distributed in Asia (Gupta and Acosta 2004). Uraiwan (1988) confirmed that a genetic relationship exists between growth rate, age and size at maturity in tilapia. This observation led to a suggestion that selection can be made more efficient by combining selection for body weight at a particular age with selection for increasing fish growth.

Development of the tilapia trade and marketing coupled with the aquaculture industry is becoming more intensive as the fisheries industry comes to rely more and more on fish harvested from farms than natural fishes. Tilapia makes up about 3.5% of the total amount of global aquaculture production

(Meyer 2002). Tilapia are well adapted to artificial culture environments, gain weight quickly at optimum conditions and reproduce in the farm without special management or infrastructure (Meyer 2002). It consumes aquatic foods and other varieties of supplemental feeds.

Esterases are multiple forms of hydrolytic enzymes that split ester into acid and alcohol, often have different isoelectric points and therefore can be separated by electrophoresis (Nelson *et al.* 1993). Esterase pattern study would be helpful to determine the toxic or agrochemical resistant capacity of fishes. It was thought that using the electrophoresis patterns of esterase might solve the problems associated.

Choline esterase consists of acetylcholine esterase found in the blood and pseudocholine esterase found in the blood plasma of the liver. These two composites can quickly transform the acetylcholine into acetic acid and choline. Isozymes have been amongst the most widely used molecular markers for the purpose of population genetic studies that revealed the effects of genetic variation within and between populations (Agnese *et al.* 1997, Lima-Catelani *et al.* 2004).

OBJECTIVE

The objective of the present study was to observe the tissue specific expression of esterase and to detect identification markers for NT and GIFT.

MATERIAL AND METHODS

Sample collection: The fingerlings of NT were collected from Shuari Ghat, Dhaka in June 2008, and GIFT samples were collected from Bangladesh Fisheries Research Institute (BFRI), Mymensingh in April 2008. These were brought to the Genetics and Molecular Biology laboratory, Department of Zoology, Dhaka University, and stored in an ultra freeze.

Sample preparation: Approximately 0.0156-0.0162 g tissues were taken in an eppendorf tube and squashed with 40 µl of TBE solution (1X Tris Borate at pH 8.9). Then 10 µl bromophenol blue were added in each tube and for the proper homogenization the samples were centrifuged at 12500 rpm for 15 min. Eight tissues, namely anterior, dorsal, pectoral, buccal, gill, heart, anal and tail were taken from both NT and GIFT samples. The gel preparation, sample loading and electrophoresis of esterase pattern using polyacrylamide gel electrophoresis (PAGE) were done following Shahjahan *et al.* (2008).

RESULTS AND DISCUSSION

A total of five bands, namely Est-1, Est-2, Est-3, Est-4 and Est-5 were observed in both NT and GIFT samples. An extra Est-6 band was found in the anal, dorsal and heart muscles of GIFT. The relative mobility of these bands were 1.65 ± 0.03, 1.32 ± 0.03, 1.0 ± 0.03, 0.73 ± 0.03, 0.38 ± 0.03 and 0.25 ± 0.03, respectively. Est-1 was found in all samples of anterior and dorsal muscles in both NT and GIFT, and was always faintly stained. It was absent in the rest of the tissues. Est-2 band was found in anterior, dorsal, pectoral, gill, and heart in both NT and GIFT samples. The intensity varied from faint to deep. Est-3 band was found in all the tissues and was taken as standard for the measurement of relative mobility and intensity variation of bands. In most of the cases the band was medium to deep stained. However, in some tissues the intensity was faint. Est-4 band was found in all tissues except dorsal muscle. It was faintly stained in buccal, tail and gill tissues of GIFT samples, and was absent in anterior, gill and heart tissues of NT. Staining intensity of the Est-4 ranged from faint, medium to deep. Est-5 band was observed in dorsal, pectoral, anal, gill and heart muscles of both GIFT and NT samples and was faint to medium stained. The band was absent in anterior, buccal and tail muscles. Est-6 band was exclusively identified in anal, dorsal and heart tissues of GIFT samples only. In anal muscle the band was faintly stained except for one sample. In dorsal muscle the band was faintly stained in all samples. However, intensity variation of this band was prominent and varied from medium to deep in heart muscle.

Esterase bands in NT and GIFT samples were conspicuous both in their number and intensity variation. Altogether five esterase bands were observed in NT and six bands in GIFT. Variation observed in eight tissues as stated above were described for comparison of the differences in the banding pattern. The esterase banding pattern appeared to be more or less similar in both NT and GIFT However, Est-4 was present in anterior, gill and heart tissues of NT but was absent in the same tissues of GIFT. Again, the band Est-6 which remarked in GIFT was absent in NT; this was the unique finding of this experiment. The band was exclusively present in anal, dorsal and heart tissues and in most cases the intensity of Est-6 was deep particularly in the heart tissue.

NT appears to have isoyzme variation (Taniguchi *et al.* 1985, Abdelhamid 1988, Rognon *et al.* 1996) and the current research confirms this trend. However, increased genetic variation in some NT populations is a result of interspecific introgression (Taniguchi *et al.* 1985, Macaranas *et al.* 1986, Abdelhamid 1988, Rognon *et al.* 1996, Agnese *et al.* 1997). The development of the GIFT strain of *O. niloticus* was hailed as a significant development in the history of genetic improvement of tropical fin fish. The culture performance of

the GIFT strain and the existing 'best' local *O. niloticus* strain (non-GIFT) was evaluated in different farming systems and agro-ecological zones (Mayer 2002). Ponzoni *et al.* (2005) indicated that despite having undergone several generations of selection, the GIFT population still has additive genetic variance to enable further improvement.

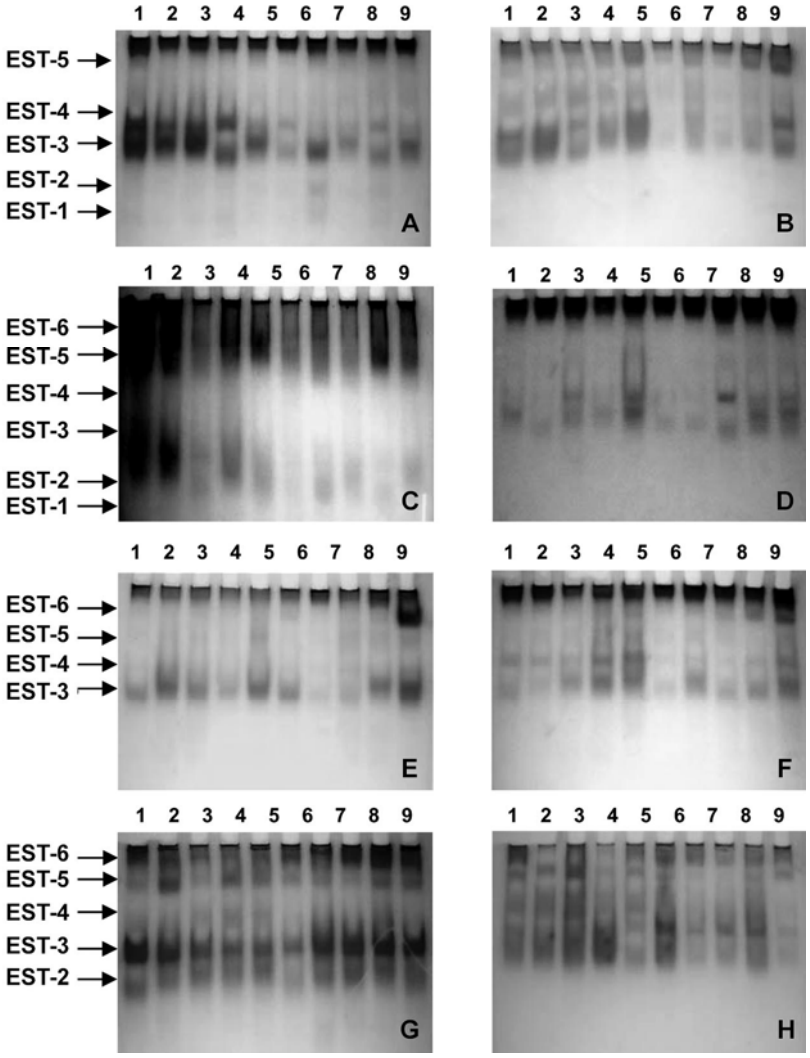

Fig. 1. Comparison of esterase isozyme banding patterns among different tissues of NT (lanes 1-5) and GIFT (lanes 6-10), *Oreochromis niloticus*, stained by α naphthyl acetate using 7.5% PAGE. Plate A= anterior muscle; Plate B= pectoral muscle; Plate C= dorsal muscle; Plate D= buccal muscle; Plate E= anal muscle; Plate F= tail muscle; Plate G= gill and Plate H= heart.

Table 1. Electrophoretic banding patterns showing the intensity variation of esterase isozymes in different tissues of the Nile tilapia (NT) and Genetically Improved Farmed Tilapia (GIFT)

Tissues	Est- $1^{1.65}$		Est- $2^{1.32}$		Est- 3^{1}		Est- $4^{0.73}$		Est- $5^{0.38}$		Est- $6^{0.25}$	
Anterior	+*	+*	+	+	+++	++	+++	-	-	-	-	-
Dorsal	+	+	+	++	++	+	-	-	+	++	-	+
Pectoral	-	-	++	++	+++	+	++	+	++	++	-	-
Buccal	-	-	-	-	+	++	+	++	-	-	-	-
Anal	-	-	-	-	++	+++	++	+	+	+	-	+
Tail	-	-	-	-	+	++	+	+	-	-	-	-
Gill	-	-	++	++	+++	+++	+	-	++	++	-	-
Heart	-	-	++	+	++	++	++	-	++	+	-	+++

*Esterase bands in NT followed by GIFT. Superscript numbers indicate the relative mobility of esterase bands. '+', '++' and '+++' marks denote faint, medium and deeply stained, respectively.

Tissue-specific esterases of the xiphophorine fishes *Platypoecilus maculatus* (platyfish), *Xiphophorus helleri* (swordtail), and their F_1 hybrid were analyzed using disc electrophoresis (Ahuja *et al.* 1977). Seven esterase zones, resolved into a maximum of nine bands, exist in these fishes, and these have been classified by employing specific inhibitors. Five of the seven zones, Est-1, Est-2, Est-5, Est-6, and Est-7, appeared to be carboxylesterases; while the two remaining zones, Est-3 and Est-4, were classified as cholinesterases. In the liver of the platyfish, all seven esterase zones were detected, while the liver of the swordtail exhibited only five esterase zones. Est-1 and Est-3 were lacking in the liver tissue of the swordtail (Ahuja *et al.* 1977). These findings are in agreement with the present results.

CONCLUSION

An extra band present in GIFT might be enrolled as an important factor for genetic improvement. It could be inferred that this extra band suggests GIFT as a genetically modified strain as compared with the NT.

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