Dhaka Univ. J. Biol. Sci. **14**(2) 193-196, 2005 (July) **– Short communication**

ESTERASE ISOZYMES PATTERN IN *PANGASIUS PANGASIUS* AND *P. SUTCHI*

MOHAMMAD AL-AMIN , GULROO BEGUM SUFI[1] AND REZA M. SHAHJAHAN*

Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh

Key words : Fish, *Pangasius*, Esterase, Isozyme pattern

The electrophoretic banding pattern of esterase isozymes was examined in two fish species of Bangladesh. Samples were taken from both the flesh and intestine of *Pangasius sutchi* (Thai pangus) and *Pangasius pangasius* (Native pangus) for investigation. A maximum number of different esterase bands observed in the genus *Pangasius* were ten of which, eight different bands were observed in *P. sutchi* distributed in six loci (*viz.* Est.-2, Est.-3, Est.-5, Est.-6, Est.-7, Est.-8) and seven bands were found in *P. pangasius* distributed in seven loci (*viz.* Est.-2, Est.-3, Est.-4, Est.-5, Est.-6, Est.-7, Est.-8). Among these, Est.-5 was found to be polymorphic in *P. sutchi.* These isozymes showed a species specific, tissue specific and intensity variation in their expression. The electrophoretic patterns of esterase isozymes appear to be genetically controlled and are unaffected by physiological or environmental factors.(1) Electrophoretic pattern of isozymes in fish is species specific which could be used for the identification of fish species.(2,4) During the last decade, large amount of genotype and allele frequency data has been obtained from a number of fish species primarily through the means of protein electrophoresis which enabled identification of many complex species.(3,4) In this study, we observed variation in the pattern and intensity of esterase bands between *P. pangasius* and *P. sutchi. P. pangasius* is known to be more delicious and *P. sutchi* is first growing. By using the electrophoretic pattern of esterase isozymes we could possibly select for the best hybrid pungas that would be delicious, fast growing and could be cultured in the pond.(5)

Pangasius pangasius (Native pungas) and *Pangasius sutchi* (Thai pungas) were investigated to study flesh and intestine. Vertical polyacrylamide gel electrophoresis was used to verify the genotype of esterase isozymes in flesh and intestine following the method of Hames(6) (with some modifications). Electrophoresis was carried out using a Tris-Borate-EDTA buffer, pH 8.9. α and β-napthyl acetate was used as an esterase substrate.

*Author for correspondence. [1]Department of Zoology, University of Dhaka, Dhaka-l000, Bangladesh.

Substrate buffer pH 6.4 was made by mixing 50 ml of 0.2 M sodiumphosphate (monobasic) to 10 ml of 0.2 M sodium phosphate (dibasic) making final volume to 100 ml with distilled water. 0.042 g α-napthyl acetate was dissolved in 1 ml acetone and 1 ml distilled water at 45°C. 0.042 g β-napthyl acetate was dissolved in 2 ml acetone at 70°C. Finally, 2 ml α-napthyl acetate solution and 2 ml β-napthyl acetate solution were added to 100 ml of staining buffer. This solution was known as substrate mixture. Bands were scored from the stained gel. Relative mobilities were estimated from the commonly found band (Est-$5^{1.0}$). The esterase isozyme loci were assigned to an increase number based on the increasing mobility from the origin following Trebatoski and Craig.[7]

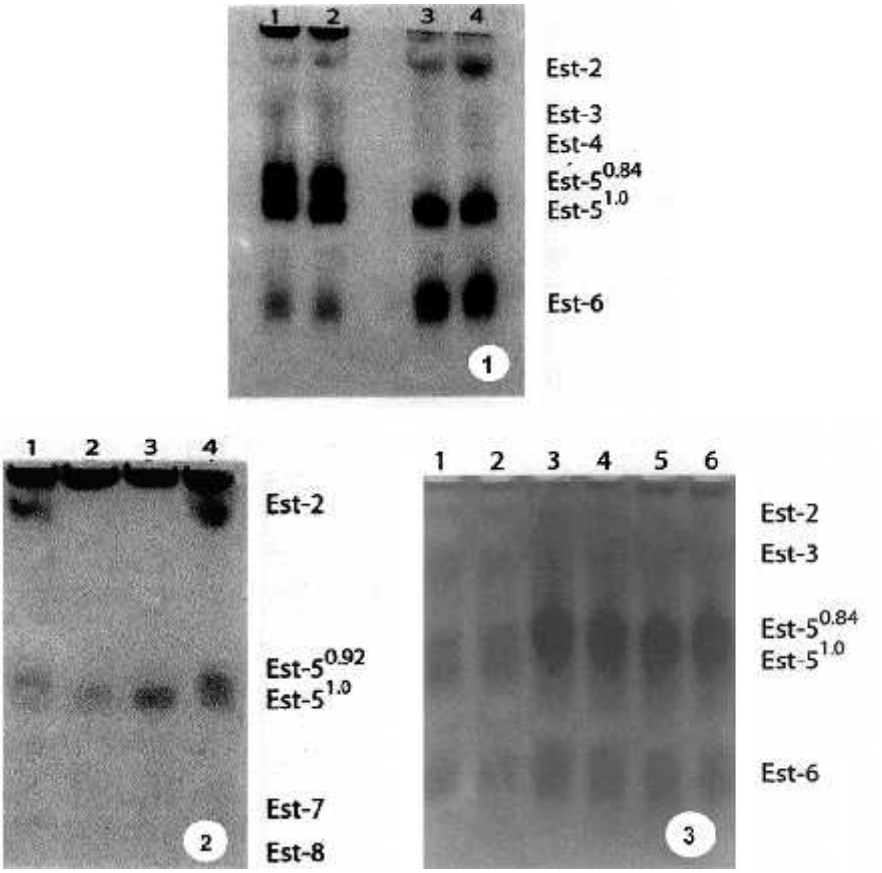
Table 1. The intensity variation of esterase isosymes bands in different tissues of *P. sutchi* and *P. pangasius*.

Relative mobility	Locus	*P. sutchi*		*P. pangasius*	
		Intestine	Flesh	Intestine	Flesh
0.18 ± 2	Est.-2	MS	DS	MS	-
0.44 ± 2	Est.-3	MDS	-	MDS	-
0.63 ± 2	Est.-4	-	-	FS	-
0.84 ± 2		DS	-	-	-
0.92 ± 2	Est.-5	-	MDS	-	-
1.0 ± 2		DS	MDS	DS	MDS
1.43 ± 2	Est.-6	MDS	-	DS	-
1.5 2 ± 2	Est.-7	-	FS	-	FS
1.75 ± 2	Est.-8	-	FS	-	FS

- = Absent; DS = Deep stained; MDS = Medium deep stained; MS = Medium stained; FS = Faint stained.

Variation was observed in the pattern and intensity of the esterase isozymes band between *P. sutchi* and *P. pangasius* as shown in Figs. l, 2 and 3. Intensity of esterase bands differed from tissue to tissue and species to species.[8] In Thai pungas, Est.-2 was stained deep in flesh (tail) but stained medium in intestine (hind parts) (Figs. 1 and 2). Est.-$5^{0.84}$ and Est.-$5^{1.0}$ was more intense in the middle and hind part of intestine of same species (Fig. 3). Species specific intensity was observed in the Est.-6 and Est.-$5^{0.84}$. In intestine, Est.-6 was stained medium-deep in *P. sutchi* and stained deep in *P. pangasius* (Fig. 1). The intensity variation of the bands is summarized in Table 1. Est.-2 and Est.-5 only were found in flesh of Thai pangas but it was not found in that of native pangas. Again, Est. $5^{0.84}$ was observed only in the intestine of Thai pungas. On the other hand, Est.-4 was only observed in the native pungas. Est.-5 was observed as a polymorphic locus in *P. sutchi* having three alleles. Two polymorphic loci were found in two species and four sub-species of the fish of the genus *Xiphophorus* (Poeciliidae) having eight alleles in the Est-2 and five alleles in the Est-3.[9]

Isozymes analysis by electrophoresis has a great significance in the field of fish genetics and fish technology particularly in taxonomy for identification of species and in breeding technology.[2,5] In the present investigation, ten esterase bands were observed in flesh and intestine of *P. pangasius* and *P. sutchi.* All the ten bands were



Figs. 1 - 3: 1. Comparison of esterase isozymes loci in the intestine (posterior part) between *P. sutchi* (1, 2) and *P. pangasius* (3, 4). 2. Comparison of esterase isozymes loci in the flesh (tail region) between *P. sutchi* (1, 4) and *P. pangasius* (2, 3). 3. Comparison of esterase isozymes banding pattern among the fore-intestine (1, 2) mid-intestine (3, 4) and hind-intestine (5, 6) of *P. sutchi.*

not observed in the same tissue and in the same species. Maximum five different bands were found in the intestine of both species. Eight esterase bands were reported to find in *Oreochromis aureus* in brain, eye, heart, muscle and liver after electrophoretic analysis.[10] In this study *P. pangasius, P. sutchi* and their hybrid from the natural source could be accurately identified by using the esterase pattern as a biochemical marker. The hybrid larvae of *Penaeus monodon* and *Penaeus esculentus,* was identified by using such biochemical rnarker.[5] We need to continue

further such research on the above mentioned fish species and others to obtain concrete data that could be used for identification of fish species, selection of the best hybrid and above all teclmology - based improvement of fish farms.

References

1. Turner BJ 1973.Genetic divergence of Death Valley pupfish populations. Species - specific esterases.Comp. Biochem. Physiol. B. **46**(1): 57-70.
2. Macranas JM, Eknath AE, Augastin LQ, Velasco RR, Ablan MaCA and Pullin RSV 1993. Genetic improvement of farmed talapia : documentation and genetic characterization of strains. Aquaculture **111**: 296.
3. Allendorf FW, Ryman N and Utter FM 1987. Genetics and fishery management past, present and future. *In:* Population genetics and fishery management. University of Washington Press Seattle. Aquaculture **129**: 215-219.
4. Rymen N 1983. Patterns of distribution of biochemical genetic variation in salmonids: differences between species. Aquaculture **33**: 1-21
5. Bezic JAH, Kenway M, Ballment E, Frusher S, Trott CC 1994. Interspecific hybridization of the tiger prawn *Penaeus monodon* and *Penaeus esculentus.* Aquaculture **133**: 103-111.
6. Hames BD 1986. An introduction to polyacrylamide gel electrophoresis. *In:* Gel Electrophoresis of proteins (D. Rickwood and B.D. Hames Eds.), pp. 1-45. RIL Press Limited. Oxford. Washington, D.C.
7. Trebatoski AM and Craig GB 1969. Genetics of and esterase in *Aedes aegypti.* Biochemical Genetics. **3**: 383-392.
8. Debnath JC 1978. Electrophoretic and biochemical studies of proteins and isozymes of non-specific esterase, lactate and malate dehydrogenares in the three species of freshwater fishes of Bosnia and Hercegovina. Ph.D. thesis, University Medical Centre, Sarajevo. Bosnia-Herzegovina.
9. Donald CM and Michael JS 1979. Polymorphism, linkage and mapping of four enzyme loci in the fish Genus *Xiphophorus* (poeciliidae): Genetics **93**: 947-960.
10. Hongtuo Fu Zin Dequan and Wu Tingting 1993. Isozyme of *Oreochromis aureus.* Aquaculture **111**: 326.

(Manuscript received on 30 July, 2003; revised on 16 May, 2004)