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## Tissue Specific Esterase Isozyme Variation in Ocellated Pufferfish, *Tetraodon cutcutia*

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**ABSTRACT**

Variation in the expression of esterase isozymes was studied from twenty different tissues of *Tetraodon cutcutia* on 7.5% polyacrylamide gel electrophoresis (PAGE) stained with  $\alpha$  and  $\beta$  naphthyl acetates. A variant type of allelic expressions was observed in different tissues where altogether, five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were detected. Est-4 showed the highest frequency (95%) whereas Est-3 was found to be lowest (30%). Localization of esterase isozymes were also observed in different tissues that showed tissue specific isozyme variation. Tissues that were related to digestive system (liver, intestine) showed higher allelic variation (80%). All the bands were present only in eye tissue. One the other hand, only one band (Est-4) was present in anterior\_ and mid\_ muscle. Est-3 was absent in digestive, nerve and muscle tissues but was present in skin, fin, eye and adipose tissues.

**Keywords:** PAGE, Esterase isozyme, Tissue specificity, *Tetraodon cutcutia*

**1. Introduction**

Esterases are the lipid-hydrolyzing enzymes which have a great significance in the field of genetics and toxicology<sup>[1]</sup>. 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. These enzymes occur in numerous iso-forms expressed by distinct gene loci that generally have a high degree of genetic variability. Mendelian inheritance studies on these esterase loci showed that each of the bands corresponds to one allele<sup>[2]</sup>. Isozymes that have large variations in size or shape or that differ in net charge can be separated by electrophoresis allowing rapid and inexpensive analysis of a large number of isolates. The level of esterases are highly variable depending on the life stage, sex, tissue, hormones, strain, food, environmental conditions and numerous other factors<sup>[3]</sup>. Correlation has been made in several fish species between the presence of this enzyme with fat digestion and lipid absorption<sup>[4]</sup>.

Puffer fish (Tetraodontiformes: Tetraodontidae) is a balloon shaped fish that is found both in freshwater and marine environments having a wide distribution, from Africa to Southeast Asia. The skin and visceral organs of this fish carry acute toxic substances viz. tetrodotoxin and saxitoxin. However, it should be noted that all puffers are not poisonous and its neurotoxin is not necessarily as toxic to other animals as it is to humans. It has no important commercial value, may occasionally be eaten by some people, but is a very popular aquarium fish. Hence, an attempt was taken to investigate the tissue specific esterase isozyme variation in the studied species.

**2. Materials and Methods**

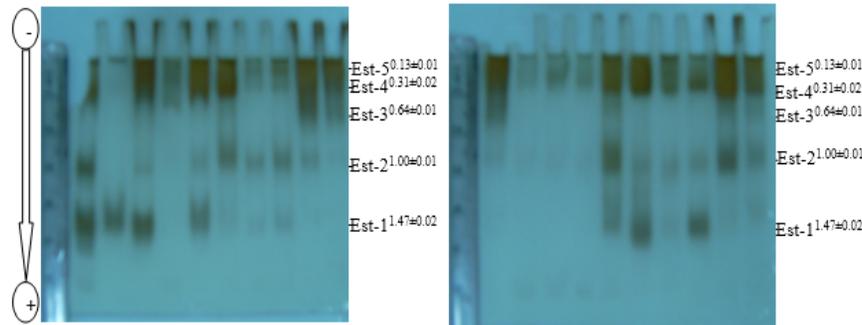
The present study on esterase loci from different tissues of *Tetraodon cutcutia* was carried out in Genetics and Molecular Biology Laboratory, Department of Zoology, University of Dhaka, Bangladesh. The adult fish samples were caught from the ponds of Gopalganj and were transported to the laboratory with ice cool packs. The specimen was then dissected to collect the following tissues: Liver, Stomach, Intestine, Brain, Eye, Kidney, Ovary, Heart, Gill, Buccal\_, Lip\_, Anterior\_, Mid\_ and Posterior\_ muscle, Lip\_, Anterior\_, Mid\_ and posterior\_skin, Adipose tissue and Tail fin.

Each tissue was squashed in TBE buffer (40  $\mu$ l) and aliquots from each sample (15  $\mu$ l) 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.*<sup>[5]</sup> and the electrophoretic bands of esterase isozymes resulting from stained gel with  $\alpha$  and  $\beta$  naphthyl acetates were assigned to increasing numbers based on decreasing mobility following Richardson<sup>[6]</sup>. 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.

### 3. 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:



**Fig 1:** Esterase isozyme banding pattern in different tissues of *Tetraodon cutcutia* stained with both  $\alpha$  and  $\beta$  naphthyl acetates by using 7.5% polyacrylamide gel electrophoresis (PAGE). Lane indicates 1- Liver, 2- Stomach, 3- Intestine, 4- Buccal muscle, 5- Lip muscle, 6- Lip skin, 7- Gill, 8- Brain, 9- Anterior skin and 10- Mid skin, 11- Posterior skin, 12- Anterior muscle, 13- Mid muscle, 14- Posterior muscle, 15- Eye, 16- Kidney, 17- Ovary, 18- Heart, 19- Adipose tissue and 20- Tail fin. “ $\rightarrow$ ” mark represents esterase band with number (allele) and relative mobility (in superscript)

**Table 1:** Electrophoretic banding pattern showing the staining intensities of esterase isozymes from different tissues of *Tetraodon cutcutia* collected from Gopalgonj (scored from both  $\alpha$  and  $\beta$  naphthyl acetates stained gels).

Sl.	Locus $\rightarrow$ Tissues	Est-1 1.47 $\pm$ 0.02	Est-2 1.00 $\pm$ 0.01	Est-3 0.64 $\pm$ 0.01	Est-4 0.31 $\pm$ 0.02	Est-5 0.13 $\pm$ 0.01	T1
1	Liver	D	D	-	D	D	80
2	Stomach	M	-	-	-	-	20
3	Intestine	D	M	-	D	D	80
4	Buccal muscle	-	-	-	M	M	40
5	Lip muscle	M	M	-	D	D	80
6	Lip skin	F	D	-	D	D	80
7	Gill	F	M	-	F	M	80
8	Brain	F	M	-	M	M	80
9	Anterior skin	-	F	D	D	D	80
10	Mid skin	-	-	M	D	D	60
11	Posterior skin	-	-	M	D	D	60
12	Anterior muscle	-	-	-	F	-	20
13	Mid muscle	-	-	-	M	-	20
14	Posterior muscle	-	-	-	M	M	40
15	Eye	M	D	F	D	D	100
16	Kidney	D	M	-	D	D	80
17	Ovary	F	F	-	D	D	80
18	Heart	D	M	-	M	M	80
19	Adipose tissue	-	D	F	D	D	80
20	Tail fin	-	M	F	D	D	80
	C1	55	65	30	95	85	66

(-, F, M and D denote absent, faint, medium and deep stained bands; T1 represents the frequency (%) of esterase bands (out of five bands) present in a certain tissue of above mentioned species; C1 personates the frequency (%) of each esterase band in all selected tissues)

#### 3.1. Liver

Altogether, four esterase bands (Est-1, Est-2, Est-4 and Est-5) were found in liver, all of which were deep stained indicating higher esterase activity. Four esterase bands were also observed in the same tissue of *Oreochromis niloticus*<sup>[5]</sup>, *Heteropneustes fossilis*<sup>[7]</sup>, *Notopterus chitala*<sup>[8]</sup> and of *Clarias gariepinus*<sup>[9]</sup>. However, five and two bands were observed in *C. batrachus*<sup>[9]</sup> and in *N.*

*notopterus*<sup>[8]</sup> respectively.

#### 3.2. Stomach

Only one esterase band (Est-1) was found in stomach which was medium stained. Five, four, three and two bands were observed in *C. batrachus*<sup>[9]</sup>, *O. niloticus*<sup>[5]</sup>, *C. gariepinus*<sup>[9]</sup> & *H. fossilis*<sup>[7]</sup> and in *N. notopterus* & *N. chitala*<sup>[8]</sup> in order.

### 3.3. Intestine

As like liver tissue, intestine also showed four esterase bands (Est-1, Est-2, Est-4 and Est-5), all of which were deep stained except Est-2, which was medium stained. Three esterase bands were also observed in the same tissue of *H. fossilis* <sup>[7]</sup>.

### 3.4. Gill

Total four esterase bands (Est-1, Est-2, Est-4 and Est-5) were found in gill tissue, of which Est-1 and Est-4 were faint stained and rest of the bands were medium stained. Two bands were found both in the gill of *C. batrachus* and *C. gariepinus* <sup>[9]</sup> whereas only one band in *H. fossilis* <sup>[7]</sup>.

### 3.5. Brain

Altogether, four esterase bands (Est-1, Est-2, Est-4 and Est-5) were found in brain, of which Est-1 was faint stained and rest of all were medium stained. Three bands were observed in *H. fossilis* <sup>[7]</sup> and *N. chitala* <sup>[8]</sup> and two bands in *N. notopterus* <sup>[8]</sup> and *O. niloticus* <sup>[5]</sup>.

### 3.6. Eye

All the bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were present only in eye tissue, of which Est-3 was faint stained, Est-1 was medium stained and rest of all were deep stained. Two bands were found in *N. notopterus* <sup>[8]</sup> and *O. niloticus* <sup>[5]</sup> whereas only one band in *H. fossilis* <sup>[7]</sup> and *N. chitala* <sup>[8]</sup>.

### 3.7. Kidney

As like liver, four esterase bands (Est-1, Est-2, Est-4 and Est-5) were also found in kidney, of which Est-1 was faint stained, Est-2 was medium stained and rest of two were deep stained. Three bands were observed in *C. batrachus* and *C. gariepinus* <sup>[9]</sup> and in *H. fossilis* <sup>[7]</sup>.

### 3.8. Ovary

Altogether four esterase bands (Est-1, Est-2, Est-4 and Est-5) were found in ovary, of which Est-1 & Est-2 were faint stained and rest of two were deep stained. Higher esterase activity in ovary might be due to embryonic activity as like *Lepomis cyanellus* and *Erimoizon sucetta* <sup>[10]</sup>.

### 3.9. Heart

Total four esterase bands (Est-1, Est-2, Est-4 and Est-5) was found in heart, of which Est-1 was deep stained and rest of all were medium stained. Four, three and two bands were observed in *C. gariepinus* <sup>[9]</sup> & *H. fossilis* <sup>[7]</sup>, *O. niloticus* <sup>[5]</sup> and in *N. notopterus* & *N. chitala* <sup>[8]</sup> in order.

### 3.10. Lip skin

Expression of esterase isozymes in lip skin was more or less same as liver tissue (Est-1, Est-2, Est-4 and Est-5) but Est-1 was faintly stained.

### 3.11. Anterior skin

Four esterase bands (Est-2, Est-3, Est-4 and Est-5) were also found in anterior skin of which Est-2 was faint stained and rest of all were medium stained. It was noticed that Est-1 was expressed in the lip skin, but was absent in the other part of the studied skin (anterior, mid\_ and posterior\_skin).

### 3.12. Mid skin

Only three esterase bands (Est-3, Est-4 and Est-5) were found in mid skin of which Est-3 was medium stained and rest of two were deep stained.

### 3.13. Posterior skin

As like mid skin, posterior skin also showed three esterase bands (Est-3, Est-4 and Est-5), the staining intensity of which were more or less same as mid skin. Both mid\_ and posterior\_skin lack Est-1 as well as Est-2 showing a differential expression from anterior to posterior part of the body.

### 3.14. Buccal muscle

Two medium stained esterase bands namely Est-4 and Est-5 were present in buccal muscle. One and two bands were observed in *C. batrachus* and *C. gariepinus* accordingly <sup>[9]</sup>.

### 3.15. Lip muscle

Same as liver and intestine, lip muscle also expressed four esterase bands (Est-1, Est-2, Est-4 and Est-5) but their staining intensity varied as Est-1 and Est-2 were medium stained.

### 3.16. Anterior muscle

Only one esterase band (Est-4) was found in anterior muscle which was faint stained. Single band was also observed in *N. notopterus* & *N. chitala* <sup>[8]</sup>, whereas two and four bands in *C. batrachus* and *C. gariepinus* accordingly <sup>[9]</sup>.

### 3.17. Mid muscle

As like anterior muscle, mid muscle also showed only one esterase band (Est-4) but it was medium stained. Four esterase bands were observed in the same tissue of both *C. batrachus* and *C. gariepinus* <sup>[9]</sup>.

### 3.18. Posterior muscle

Unlike anterior and mid muscle, two esterase bands namely Est-4 and Est-5 were found in posterior muscle both of which were medium stained. One, two and three bands were observed in *N. notopterus* & *N. chitala* <sup>[8]</sup>, *C. batrachus* and *C. gariepinus* in order <sup>[9]</sup>. Slightly greater activity of this enzyme was observed from anterior to posterior muscle.

### 3.19. Adipose tissue

Altogether four esterase bands (Est-2, Est-3, Est-4 and Est-5) were found in adipose tissue of which Est-3 was faint stained and rest of all were deep stained. 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 <sup>[4]</sup>.

### 3.20. Tail fin

Tail fin showed four esterase bands (Est-2, Est-3, Est-4 and Est-5) of which Est-3 was faint stained, Est-2 was medium stained and rest of two were deep stained. Two and four esterase bands were observed in the larvae and adult of *H. fossilis* <sup>[7]</sup>.

Altogether, five esterase bands were observed from the different tissues of *Tetraodon cutcutia* that could be specific for this species. Eight, seven and six esterase bands were observed in different tissues of *O. aureus* <sup>[11]</sup>, *Megalobrama mblycephala* <sup>[12]</sup> and of

*Ictalurus punctatus* <sup>[13]</sup> respectively. However, 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 *Clarias batrachus* and *C. gariepinus* accordingly <sup>[9]</sup> while, four bands were found both in *N. notopterus* & *N. chitala* <sup>[8]</sup>. Five esterase bands were also observed from the different tissues of *O. niloticus* <sup>[5]</sup> and of *Pangasius hypophthalmus* <sup>[14]</sup> but the banding pattern varied in different tissues.

A variant type of allelic expression of these isozymes was observed from different tissues. As for example, all the bands were present only in eye tissue while maximum tissues showed four esterase bands. One the other hand only one band (Est-4) was present in anterior muscle and mid muscle. Localization of esterase isozymes was clearly visible in different tissues in aspect of allelic expression and intensity variation (Figure-1).

Strong enzymatic action was seen in liver and intestine but weak in stomach. Hirj and Courtney <sup>[15]</sup> 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 fluviatilis*. The differences might be due to differential feeding habit with the presence of digestive esterases.

In brain, four alleles were to be expressed indicating higher enzymatic action. Many researchers observed frequently the high activity of esterase in the brain of different species <sup>[16]</sup>. Specific allele in specific tissues showed higher esterase activity due to biological need of that tissue specific function <sup>[9]</sup>. It is also mentionable that an organism may develop resistance to insecticides by producing large amount of specific esterases which either breakdown the insecticide molecules or bind them so tightly that they cannot function <sup>[7]</sup>.

It is important to note that certain bands were absent in one tissue, while the other bands were present in other tissues. As for example, Est-3 was absent in digestive, nerve and muscle tissues but was present in skin, fin, eye and adipose tissue. The location and function of the various esterase forms could vary from tissue to tissue and depend on the physiological demands of each system <sup>[17]</sup>. Staining intensity might also be a good parameter but in present study we have taken less attention on it as it need further experimentation.

Est-4 was prominent band (95%) among the selected tissues, while Est-3 showed lowest frequency (30%) which indicated that each allele might have underlying mechanisms regulating the esterase related processes <sup>[18]</sup>. Certain band was also common in all the studied tissues of *H. fossilis* <sup>[7]</sup>, *O. niloticus* <sup>[5]</sup> and of *P. hypophthalmus* <sup>[14]</sup>.

#### 4. Conclusion

Expression of tissue specific esterase isozymes showed differential banding pattern that could be used in toxicological study and also could be used for the development of molecular markers.

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