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Tissue and Substrate Specific Esterase Isozyme Banding Pattern in *Macrobrachium rosenbergii*

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ABSTRACT
Tissue and substrate specificity of esterase isozymes was studied from five different tissues of *Macrobrachium rosenbergii* on polyacrylamide gels (7.5 %) stained with α or β or both α and β naphthyl acetates together. Altogether, five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were observed that showed both tissue and substrate specific expression. Maximum, four esterase bands were found in eye (Est-1, Est-3, Est-4 and Est-5) and tail muscle (Est-1, Est-2, Est-4 and Est-5) while other tissues showed three bands stained with both α and β naphthyl acetates together. Est-5 was highly expressed (100%) in all the tissues as well as in all three substrates (α or β or α-β naphthyl acetates together). Most of the bands were found to be α-β specific (68 %) while few bands were α (28 %) or β (36%) specific. Both Est-1 and Est-4 were completely absent in α as well as in β stained gels.

Keywords: Electrophoresis, Esterase isozyme, Tissue and substrate specificity, *Macrobrachium rosenbergii.*

1. Introduction
The freshwater prawn farming has already become in the last years a major contributor to global aquaculture, in terms of quantity and value [1] and plays an important role in the national economy of Bangladesh. Because of favorable resources and agro-climatic conditions, Bangladesh is considered one of the most suitable countries in the world for giant fresh water prawn farming [2]. The commercial farming of *Macrobrachium rosenbergii* has not flourished in our country because of the lack of adequate knowledge about its biology and culture techniques and little attempt has been made to culture this species scientifically. A substantial body of anecdotal evidence suggests that pesticide poisonings and ecological damage have become commonplace in Bangladesh [3, 4]. In addition, pesticides that do accumulate biologically in foods through different tropic level which are then harmful to humans when ingested. As a result, consumer health still is not protected from potentially dangerous chemicals used to control the pest organisms. Esterases are important for insecticide breakdown and its isozymes have been amongst the most widely used molecular markers for this purpose [5]. They are lipid-hydrolyzing enzymes having a wide significance in the field of genetics and toxicology and may be used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture [6]. A wide range of different esterases exists that differ in their substrate specificity, their protein structure and their biological function. Isozymes are the variants of same enzyme. Whilst isozymes may be almost identical in function, they may differ in other ways. In particular, amino acid substitutions that change the electric charge of the enzyme are simple to identify by gel electrophoresis [7], and this forms the basis for the use of isozymes as molecular markers. They are still amongst the quickest and cheapest marker systems to develop, and remain an excellent choice for projects that only need to identify low levels of genetic variation. The aims of the present study were to investigate the esterase isozyme variability in terms of tissue and substrate-specificity.

2. Materials and Methods
The present study on esterase loci from different tissues of *M. rosenbergii* was carried out in Genetics and Molecular Biology Laboratory, University of Dhaka, Bangladesh.

Twenty grade prawn samples (20=1kg) were collected from Kawran Bazar fish market, Dhaka (original source unknown) and were transported to the laboratory with ice cool packs to prevent degradation of esterases. The specimens were then dissected carefully maintaining ice cool temperature to collect desired amount (~0.016g) of the following tissues: Tail_, Dorsal_ and Ventral muscle, Nerve cord and Eye. Each tissue was squashed finely in TBE (1X) buffer (40 µl) with the help of electric squashier and aliquots from each sample (15 µl) were loaded on the separate gel slots (7.5 % polyacrylamide gel) 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) in TBE (1X) buffer was followed as that of Shahjahan *et al* [8] and the electrophoretic bands of esterase isozymes resulting from stained gels (α or β or both α and β naphthyl acetates together, incubated at 37 ºC for 50 min) were assigned to increasing numbers based on decreasing mobility following Richardson [9]. Bands were categorized as deep, medium and faintly stained according to their intensity. 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, intensity variation and also the substrate specificity, the results of which were as follows:

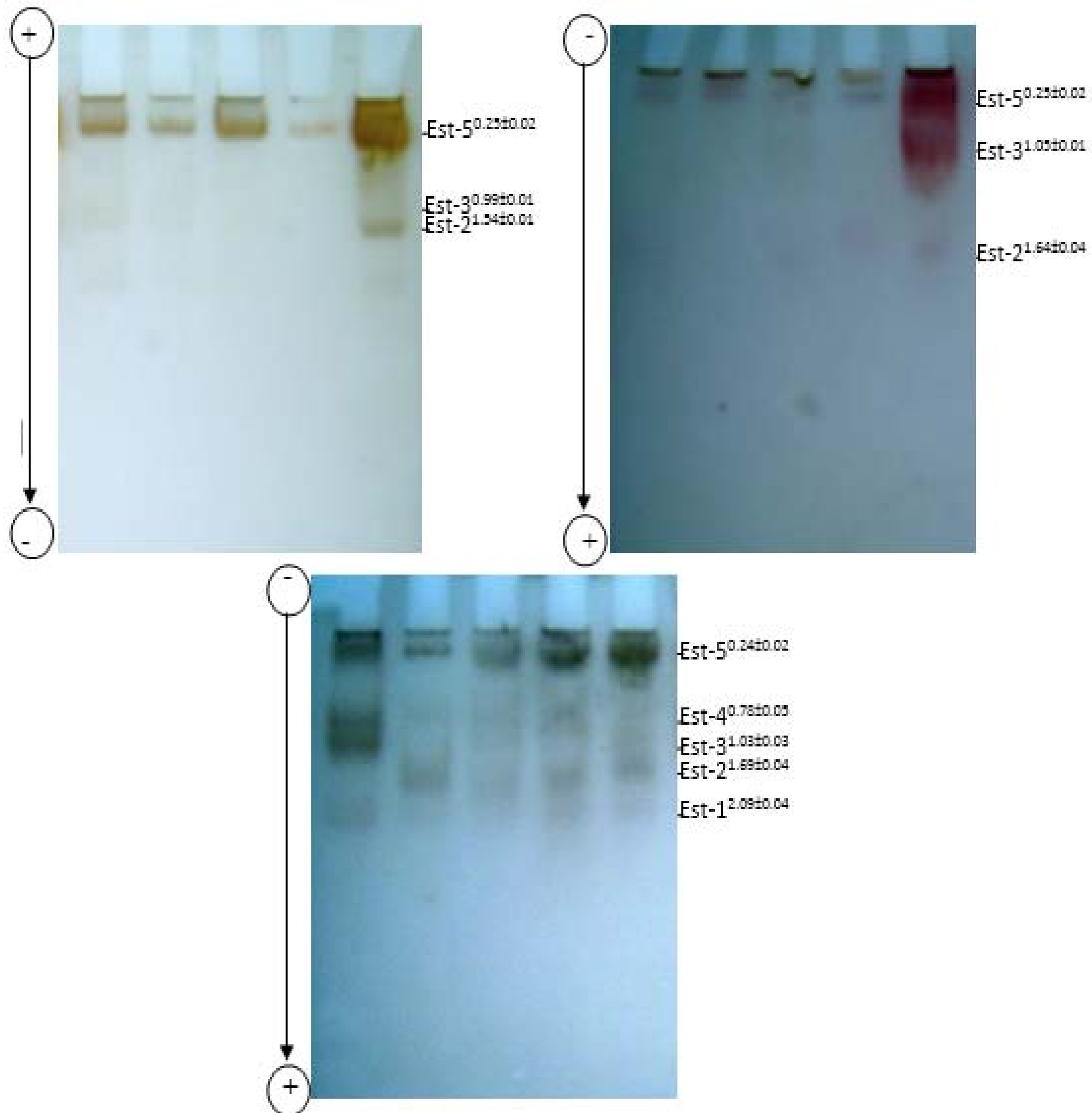

Fig 1: Representative gel plates showing the esterase isozyme banding pattern in different tissues of *M. rosenbergii* stained in α or β or both α and β naphthyl acetates together on 7.5 % polyacrylamide gels where D, V and T represent tissues of dorsal_, ventral_ and tail muscle respectively, E-eye and N-nerve cord.

Table 1: Tissue specific esterase isozyme banding pattern of *M. rosenbergii* showing the absence or presence of bands with intensity variation (scored from α or β or both α and β naphthyl acetates stained gels).

Alleles→	**Est-1**			**Est-2**			**Est-3**			**Est-4**			**Est-5**			**T1**			**T2**
Substrate→	**α**	**β**	**αβ**	**α**	**β**	**αβ**	**α**	**β**	**αβ**	**α**	**β**	**αβ**	**α**	**β**	**αβ**	**α**	**β**	**αβ**	
D-muscle	-	-	+	-	-	++	+	-	-	-	-	-	+++	++	+++	40	20	60	40
T-muscle	-	-	+	-	-	++	-	-	-	-	-	++	+++	++	+++	20	20	80	40
Nerve cord	-	-	+	-	+	++	-	-	-	-	-	-	+++	++	+++	20	40	60	40
V-muscle	-	-	-	-	+	+	-	-	-	-	-	+	++	++	+++	20	40	60	40
Eye	-	-	+	++	+	-	-	+++	+++	-	-	+++	+++	+++	+++	40	60	80	60
C1	0	0	80	20	60	80	20	20	20	0	0	60	100	100	100	28	36	68	44
C2	26.67			53.33			20.00			20.00			100.00			44.00			

(-, +, ++ and +++ denote absent, faint, medium and deep stained bands; T1 represents the frequency (%) of esterase bands (out of five bands) present in a certain tissue stained with specific substrate; T2 stands for the frequency (%) of total bands in a certain tissue; C1 personates the frequency (%) of each esterase band in each substrate; C2 represents the average frequency (%) of each esterase band stained with all three substrate)

3.1 Dorsal muscle (anterior)

Two (Est-3 and Est-5), one (Est-5) and three (Est-1, Est-2 and Est-5) esterase bands were found from α, β and both α-β stained gels accordingly in anterior dorsal muscle. Est-4 was totally unexpressed in all three substrates (α, β and both α-β together) in this tissue. In α stained gel, Est-5 was deep stained while Est-3 was faintly stained. Est-5 was medium stained in β stained gel. Est-1, Est-2 and Est-5 were faint, medium and deep stained respectively when stained with both α and β napthyl acetates together. Single esterase band was observed in the same tissue of *Penaeus monodon* from both α-β stained gels [10].

3.2 Ventral muscle (anterior):

One (Est-5), two (Est-2 and Est-5) and three (Est-2, Est-4 and Est-5) esterase bands were observed from α, β and both α-β stained gels respectively in anterior ventral muscle. Est-5 was moderate to deep stained in all three cases while, Est-2 and Est-4 were faintly stained. Two esterase bands were found in the same tissue of *P. monodon* from both α-β stained gels [10].

3.3 Tail muscle (base of telson)

Only one moderate to deep stained esterase band (Est-5) was observed in tail muscle when stained with α or β napthyl acetate alone. However, four esterase bands (Est-1, Est-2, Est-4 and Est-5) were observed when stained with both α and β napthyl acetates. Here, Est-1 was faintly stained, Est-2 and Est-4 were medium stained and Est-5 was deeply stained. Only one esterase band was found in the same tissue of *P. monodon* from both α-β stained gels [10].

3.4. Nerve cord (ventral):

One (Est-5), two (Est-2 and Est-5) and three (Est-1, Est-2 and Est-5) esterase bands were observed from α, β and both α-β stained gels in order in the squashed tissue of ventral nerve cord. Moderate to deep stained Est-5 was common in all three cases while faintly stained Est-1 was both α and β specific in this tissue. Est-2 was faint and moderately stained in β specific and in α-β specific gels respectively. One, two, three and four esterase bands were found in the same tissue of *M. lamarrei, M. malcolmsonii, M.* rosenbergii [11] and *P. monodon* [10] in order from both α-β stained gels.

3.5 Eye (black portion):

Two (Est-2 and Est-5), three (Est-2, Est-3 and Est-5) and four (Est-1, Est-3, Est-4 and Est-5) esterase bands were observed from α, β and both α-β stained gels accordingly in eye. Est-5 was deep stained in all three gels while Est-2 was medium and faintly stained in α and β specific gels. Est-3 was deep stained both in β and α-β specific gels. Est-1 and Est-4 were unique to α-β specific gel where Est-1 was stained faintly and Est-4 deeply. Two, three, four and five esterase bands were found in the same tissue of *M. malcolmsonii, M. rosenbergii, M. lamarrei* [11] and *P. monodon* [10] respectively from both α-β stained gels. Differences in the number of bands with previous study might be due to differential expression pattern of these alleles in population. Variation in the number of bands was also observed in *Culex pipiens* where 0-, 1-, 2- and 3-banded individuals were found to be frequent [12]. Moreover, the level of esterases were found to be highly variable depending on the life stage, sex, tissue, hormones, strain, food, environmental conditions and numerous other factors [13].

Maximum, five esterase bands were found from the different tissues of studied species that slightly differ from the result of earlier study where four esterase bands were detected [11]. These differences might be due to differential tissue selection and also to sample collection sites (unknown). However, two and four esterase bands were observed in the previous study on *M. malcolmsonii* and *M. lamarrei* respectively [11]. Number of esterase bands may vary from species to species. As for example, three, four, five, six, seven and eight esterase bands were found in *Poecilia* reticulata [14], *Heteropneustes* fossilis [15], *Oreochromis niloticus* [8], *Ictalurus punctatus* [16], *Megalobrama amblycephala* [17] and in *O. aureus* [18] respectively.

Maximum, four esterase bands were found in eye and tail muscle while, other tissues showed three bands. It is important to note that certain band was absent in one tissue, while it was present in other tissues and vice versa. As for example, Est-3 was present in eye while absent in all other tissues except α stained dorsal muscle where it was faintly stained. The location and function of the various esterase forms could vary from tissue to tissue and depend on the physiological demands of each system [19]. Staining intensity might also be a good parameter but in present study we have taken less attention on it as it need further experimentation viz. total protein estimation and equalization before loading into gel slots.
Altogether, four esterase bands (Est-1, Est-2, Est-4 and Est-5) were found from the above mentioned three muscles of the studied species when stained with both α and β naphthyl acetates together (Table 1). However, one, two and three esterase bands were observed in a previous study on *M. lamarrei, M. malcolmsonii* and *M. rosenbergii*[11]. Greater number of bands (four) in current study might be due to higher number of tissue selection.
Est-5 was prominent band (100 %) among the selected tissues, while Est-3 showed lowest frequency (20 %) which indicated that each allele might have underlying mechanisms regulating the esterase related processes [20]. Previous study on esterases suggested that the band with highest molecular weight was common in all selected tissues of *M. rosenbergii, M. lamarrei* and *M. malcolmsonii* [11]; the cause of such tendency was unknown. Certain band was also common in all the studied tissues of *H. fossilis* [15], *O. niloticus* [8] and of *Pangasius hypophthalmus* [21].
Most of bands were found to be both α-β specific (68 %) followed by β specific (36 %) and α specific bands (28 %) (Table-1). Both α-β specific esterases were also found abundantly in *O. niloticus* (Shahjahan *et al*., 2008), *P. reticulata*[14] and *Gambusia affinis* [22].
Relatively higher esterase bands were observed from anterior to posterior part of the body when stained with both α and β naphthyl acetates together (Table 1) but their average expressions with all three staining groups were same (40 %). Hirj and Courtney [23] 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*.

4. Conclusion
Expression of tissue specific esterase isozymes showed differential banding pattern that could be used in toxicological study but it needs further intensive study to investigate the toxic potency. Present studies may also be used for the development of molecular markers.

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