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Research Article



Lactate dehydrogenase isozymes and their genetic variation in *Poecilia Latipinna* (Lesueur, 1821) and *Poecilia Sphenops* (Valenciennes, 1846)

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Abstract

The *Poecilia latipinna* and *Poecilia sphenops* are a very popular aquarium fishes belonging to the family Poeciliidae. The common name is molly. The taxonomic status of these fish is very controversial, with a paucity of molecular research on their population genetic structure and species identification. Information on molecular genetic markers, especially isoenzymes, in search of a better understanding of the population genetic structure and correct identification of fish species has been receiving more attention when elaborating and implementing commercial fishery management programs. In the present study, isozyme profiling of lactate dehydrogenase (LDH) was carried out, to analyse the biochemical genetic structure and variability between two species. The isozyme banding pattern showed variation among *P.latipinna* and *P.sphenops*. Location specific markers were obtained for *P.sphenops* from Kolathur and *P.latipinna* from Perungalathur.

Keywords: *P.latipinna* , *P.sphenops*, Isozyme, LDH

Introduction

Population genetics is a study of the causes and effects of genetic variation within and between populations, and in the past, isozymes have been amongst the most widely used molecular markers for this purpose. Although they have now been largely superseded by more informative DNA-based approaches such as direct DNA sequencing, single nucleotide polymorphisms and microsatellites, they are still amongst the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.

Lactate dehydrogenase (LDH: 3.1.1.27) enzyme in fishes have always been the subject of much attention (Market et al., 1975, whitt, 1983; Almedia and Luis Val 1993). But, scarce reports are available from

India dealing either with the expression of LDH genes /presence of loci or their quantitative changes in freshwater teleosts (Padhi and khuda-Bukhsh, 1989). Lactate dehydrogenase is one of the chief enzyme of carbohydrate metabolism which catalyses the oxidation of lactate and reduction of pyruvate during anaerobic glycolysis.

It is evident that the LDH isoenzymes have also been utilized as an indispensable tool in systematic and phylogenetic studies due to either the quantitative or qualitative differences or their differential expression (Avisé, 1974). Therefore, the significance of these important characters in evolutionary protocols cannot be ignored. The isoenzymatic patterns of six enzymes were studied in *Symphysodon aequifasciatus*, *S.discus* and *S.Tarzo* (Silva, 2008). The presence or absence of

LDH isoenzymes or genes and their quantitative differences have been determined in the air breathing teleosts, *Channapunctata*, *C.gachua* and *C.striatus* (Ahmad,2008) which reveals great variation in the quantitative as well as qualitative presence of isoenzymes. The tissue specific LDH isozyme pattern were examined in three species of the economically important tilapiine fishes, *Oreochromisniloticus*, *O.aureus* and *Tilapia zillii* (Sherif *et al.*, 2008).

The *Poecilia latipinna* and *Poecilia sphenops* are a very popular aquarium fishes belonging to the family Poeciliidae. The common name is molly. The taxonomic status of these fish is very controversial, with a paucity of molecular research on their population genetic structure and species identification. Information on molecular genetic markers, especially isoenzymes, in search of a better understanding of the population genetic structure and correct identification of fish species has been receiving more attention when elaborating and implementing commercial fishery management programs. In the present study, isozyme profiling of lactose dehydrogenase (LDH) was carried out, to analyse the biochemical genetic structure and variability between two species.

Materials and Methods

Preparation of Tissue Extract and Isozyme Analysis

Approximately 1g tissue (muscle) of *P.latipinna* and *P.sphenops* were dissected out into microcentrifuge tubes. 1-2ml of chilled homogenizing buffer (0.1 M Tris-HCl p^H 7.0 1mM EDTA and 7mM 2-mercaptoethanol) were added and the mixture homogenized thoroughly with a chilled steel pestle. The mixture was centrifuged at 4°C at 10,000 g for 20 min, and the clear supernatant was kept in storage tubes. Protein concentration was determined by Lowry's method. Polyacrylamide gel electrophoresis was performed in a vertical slab apparatus of discontinuous buffer system with 5.5% separating gel and 4% stacking gel.

The crude protein samples were diluted with 40% sucrose solution, in the ratio of 1:1, and then 30µl of the diluted samples was loaded on to 5.5% poly acrylamide gel and electrophoresed at 30volts at 16°C for 2 hours until the dye front reaches the lower end of the gel.

After electrophoresis was performed, the gel was rinsed well in distilled water and then the gel was incubated at 37°C in the dark room for about 30 minutes in the freshly prepared staining solution containing 1M Lithium lactate, Nicotinamide adenine dinucleotide (NAD)(10mg/ml), 0.1M Sodium chloride, 0.1M Magnesium chloride, 0.5M Phosphate buffer (p^H7.5), Nitro blue tetrazolium (NBT) (1mg/ml), Phenyl methyl sulphate (PMS) (1mg/ml).

After staining for LDH, the gel was washed in running cold water to remove the unused staining reagents and then placed in 7% acetic acid.

Appropriate amount of protein was loaded onto 5% acrylamide gel and electrophoresed at 200V for 1.5 hr for (80 mm long gel). The gel was stained in staining buffer containing substrate (Lactate dehydrogenase), cofactor (NAD₊ or NADP₊), nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. LDHD dendrogram for LDH-isozyme were constructed based on SPSS (version 16, 2007) software tool.

Results and Discussion

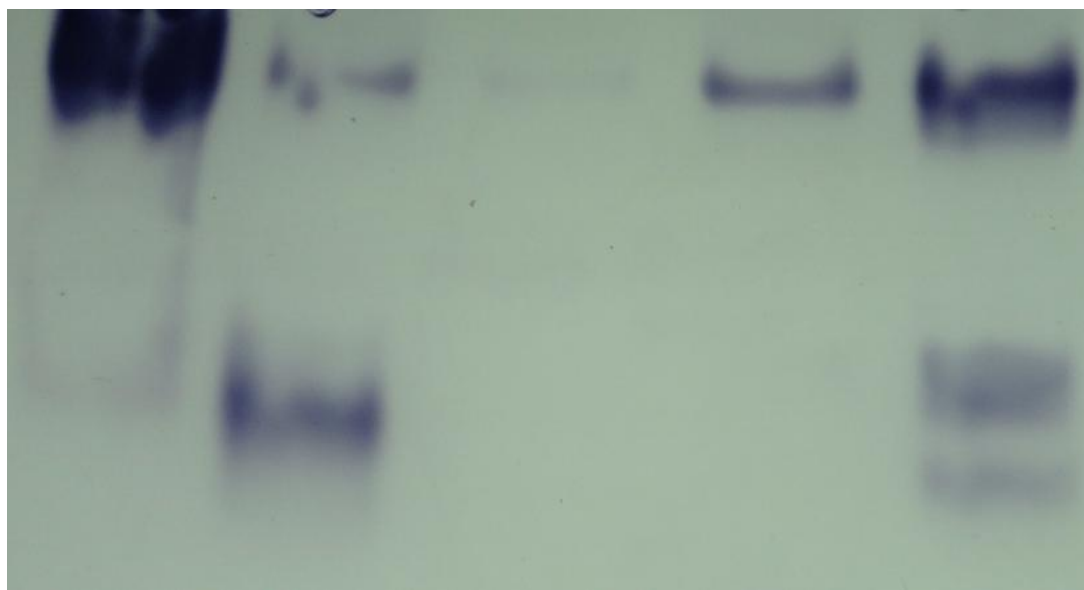
In taxonomic and systematic studies, isozymes help in distinguishing species and hypothesize their relationships by genetically based differences and similarities among population (Leary and Booke, 1990). The general principle of this is that the more closely the species are related the more likely they have similar protein enzymes (Kitto and Wilson, 1966).

In the present study, LDH enzyme mobility was optimized in a 4% PAGE and subjected to enzyme specific staining. The LDH bands were recorded as LDH 1 – LDH 4 based on their Relative Mobility in the gel. The banding pattern showed variation among *P.latipinna* and *P.sphenops* (Fig.1). Location specific markers were also obtained for *P.sphenops* from Kolathur and *P.latipinna* from Perungalathur. Four isoforms were obtained in total, of which LDH-1 was monomorphic among *P.latipinna* and *P.sphenops* (Table.1). LDH - 3 and LDH - 4 were specific to *P.latipinna*. LDH - 3 can be designated as a molecular marker for *P.latipinna*, Perungalathur. LDH - 2 A marker for *P.sphenops*, Kolathur was also obtained. Quantitative analysis of isoforms was performed using an imaging software Image J v1.46 (Table.2). The monomorphic band LDH-1 was highly expressed in *P.sphenops*, Kolathur followed by

Perungalathur location. *P.latipinna*, Perungalathur showed high expression of LDH -1 when compared to Kolathur location. Expression of LDH-3 was high in *P.latipinna*, Perungalathur than Kolathur location. The present study is comparable with the work done by Killebrew and Sweet (1988). They also concluded that

genetic differentiation between two widely separated *Clibanarius vittatus* has been minimized. In addition Mulley and Latter (1981); Richardson (1982); Lester (1983); Sunden and Davis (1991) have reported that the overall degree of divergence between populations of the *Penaeids* were found to be quite small.

Fig . 1 LDH Isoenzymes patterns of *P.sphenops* and *P. latipinna*



LANE 1 LANE 2 LANE 3 LANE 4

LANE 1: *P. sphenops* – Kolathur, LANE 2: *P. latipinna* – Kolathur,LANE 3: *P. sphenops* - Perungalathur, LANE, 4: *P. latipinna* - Perungalathur

Table. 1 Scoring for lactate dehydrogenase

LOCI	<i>P.sphenops</i> KOLATHUR	<i>P.latipinna</i> KOLATHUR	<i>P.sphenops</i> PERUNGA- LATHUR	<i>P.latipinna</i> PERUNGA - LATHUR
LDH-1	1	1	1	1
LDH-2	1	0	0	0
LDH-3	0	1	0	1
LDH-4	0	0	0	1

Table. 2 Quantitative estimation of LDH

	<i>P.sphenops</i> KOLATHUR	<i>P.latipinna</i> KOLATHUR	<i>P. phenops</i> PERUNGALATHUR	<i>P. latipinna</i> PERUNGALATHUR
LDH 1	172081.954	55345.04	75177.479	71352.437
LDH2	30316.566	-	-	-
LDH3	-	56878.42	-	65417.642
LDH4	-	-	-	90023.281

Table. 3 Agglomeration schedule

STAGE	CLUSTER COMBINED		COEFFICIENTS	STAGE CLUSTER FIRST APPEARS		NEXT STAGE
	CLUSTER 1	CLUSTER 2		CLUSTER 1	CLUSTER 2	
1	2	4	1.00	0	0	3
2	1	3	1.00	0	0	3
3	1	2	2.00	2	1	0

Dendrogram was constructed by scoring the LDH bands as 0 and 1 for its absence and presence. A statistical software SPSSv16 was used to construct dendrogram (Fig.2). Two clusters were obtained in cluster1 and cluster 2. The coefficient between the clusters was 2.00 (Table.3). Cluster 1 contained *P.latipinna* from Kolathur and Perungalathur with a

coefficient of 1.00. Cluster 2 contained *P. sphenops* from Kolathur and Perungalathur with a coefficient of 1.00. The isozyme banding pattern showed variation among *P.latipinna* and *P.sphenops*. Location specific markers were obtained for *P.sphenops* from Kolathur and *P.latipinna* from Perungalathur.

***** H I E R A R C H I C A L C L U S T E R
A N A L Y S I S *****

Dendrogram using Average Linkage (Between Groups)

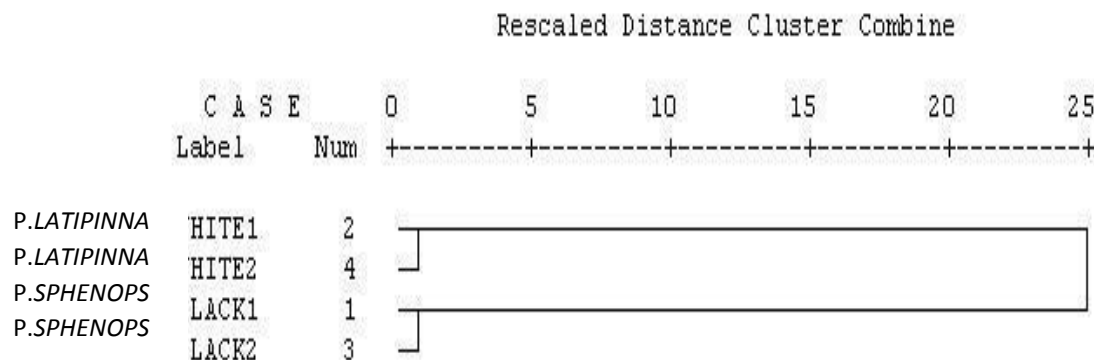


Fig. 2 Dendrogram was constructed by scoring the LDH bands

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