Tissue Specific Isoenzyme variation or polymorphism in Tangra Fish (*Mystus gulio* and *Mystus vittatus*)

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Abstracts

Studies on native polyacrylamide gel electrophoresis have been used to analyze malate dehydrogenase (MDH) and lactate dehyrogenase (LDH) isoenzymes in different tissues (liver, muscle and heart) of tangra fish-*Mystus gulio* (salt water) and M*ystus vittatus* (fresh water) in order to study the tissue specificity of these isoenzymes. LDH and MDH isoenzymes are major system found in fishes. The percentage amount of MDH and LDH in general varied significantly in different tissues. In case of LDH the percentage amount of total enzyme showed significant difference between liver and another tissues and this variation may be due to higher gene activity in liver. In case of MDH the percentage amount of the total enzyme showed significant difference between liver and heart. This variation is also due to the higher gene activity. *Mystus gulio* lives in estuary with stress conditions and express high level of LDH in heart established hypoxia tolerance. Whereas *Mystus vittatus* did not express LDH in heart as it is fresh water dweller. In *M. gulio, and M. vittatus* mitochondrial MDH is found in liver, heart and muscle. One isoenzyme system for MDH is also found in *Lepidosiren* also. This one enzyme system probably establishes the adaptive lineage between both species. Present studies also confirm the adaptive feature of *M.gulio* for estuarine habitat.

Keywords: lactate dehyrogenase; malate dehydrogenase; polymorphism; electrophoresis; tangra fish; gluconeogenesis.

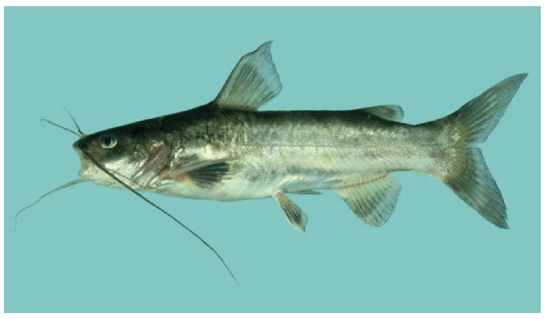
To maintain circulating glucose during unfavourable conditions gluconeogenesis is an alternative in vertebrates [1,2]. Low dietary carbohydrate influence hepatic glucose metabolism [3,4,5]. Environmental hypertonicity stimulates gluconeogenesis [6]. Lactate, pyruvate and amino acids are alternate carbohydrate sources.

Lactate dehydrogenage (LDH; EC 1.1.1.27) and malate dehydrogenage (MDH; EC 1.1.1.37) are two well studied isoenzyme system and suited for metabolic, genetic, ecological, evolutionary as well as systematic studies [7].

Two species of *Mystus* are adapted for different habitat, *Mystus gulio* are adapted in estuarine habitat and *Mystus vittatus* are in fresh water [8]. So that estuarine *Mystus gulio* is adapted for withstanding osmotic stress. In laboratory exposure to higher environmental salinity causes induction of gluconeogenesis [9]. Sum of the present study is to focus on the difference in genetic makeup of this two species with reference to the key enzyme i.e. MDH and LDH how to withstand different osmotic environment. To address strategic ecological evolution, fish may be an indispensible environmental model.

Methods and materials

Specimens were collected from fish markets of Kolkata and adjoining of Howrah and brought to the laboratory in live condition. They have different anatomical and morphological features [8].



Mystus gulio (estuarine fish)

Mystus vittatuas (fresh water fish)

Preparation of tissue extract

Specimens are autopsied and desired tissues i.e. heart, liver, and muscles were taken on ice for homogenization. Tissues were cut into small pieces and homogenised in 0.1 M Tris-HCl buffer (pH 7.5) containing Phenyl methyl sulfonyl fluoride (PMSF) and centrifuged at 16000 rpm for 15 minutes at 4 °C. Supernatant were used for electrophoresis [7].

Electrophoretic analysis and enzyme staining

Native gel electrophoresis were performed on 10% polyacrylamide gel at 4°C. LDH staining solution consisted of 60mg calcium lactate, 1mg PMS (Phenazine methosulphate), 4mg NAD (Nicotine adenine dinucleotide), 2mg MTT [3- (4,5-demethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] in 30ml 0.5M Tris-HCl and MDH specific stain consisted of 12mg MTT,15 mg NAD, 7mg Sodium carbonate(Na_2CO_3), 15mg Malic acid, 1mg of PMS in 30 ml 0.5M Tris-HCl was utilized.

Results

Electrophoretic profiles of LDH and MDH varies in three tissues of two *Mystus* species.

The expression of two LDH isoform (A4 and B4) to vivid as single band in all tisues of both species except absent in heart of *Mystus vittatus* (Fig. 1). Isoform of LDH in heart (B4) is different from liver and muscle (A4).

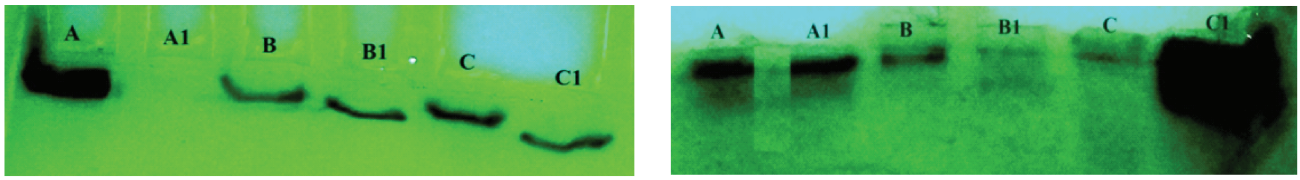


Fig. 1. Protein band structure studied in the tissue samples of Tangra fish in *LDH* isoenzymes are as follow: A-A1 (heart); B-B1 (muscle) and C-C1 (liver).

Two MDH band i.e. cytosolic MDH (MDH1) and mitochondrial MDH (MDH2) was developed after staining. Both bands in both species are also present in *Mystus vittatus,* but single band (MDH2) is found in *Mystus gulio* (Fig. 2) in liver and muscle.

Fig. 2. Protein band structure studied in the tissue samples of Tangra fish in *MDH* isoenzymes are as follow: A-A1 (heart); B-B1 (muscle) and C-C1 (liver).

Table.1

ENZYME		HEART	MUSCLE	LIVER
Lactate Dehydrogenase (LDH) (Fig.1)	***Mystus gulio***	**A**	**B**	**C**
	Mystus vittatus	**A1**	**B1**	**C1**
Malate Dehydrogenase (MDH) (Fig.2)	***Mystus gulio***	**A**	**B**	**C**
	Mystus vittatus	**A1**	**B1**	**C1**

Discussion

Estuarine organisms are adapted to withstand two types of stress; hypoxia and osmotic stress. Anaerobic respiration as well as gluconeogenesis is of adaptive strategies to overcome the crisis [10] Anaerobic glycolysis is less efficient. High loss of free energy accelerates fast reaction and is independent of substrate or product.

MDH involve in glyconeogenesis, pyruvate carboxylase utilize pyruvate in mitochondria to from oxaloacetate. Malate-aspartate shuttle works in heart and liver [11]. Both isoform of MDH, present in *M. vittatus* and may be involved in shuttle. But in *M. gulio*, shuttle is only possible in heart, may be to preserve carbohydrate intermediates. One isoenzyme system in liver and muscle also found in lepidosiren [12] as *M. gulio*.

To withstand hypoxia *M. gulio* specifically express higher LDH in heart. LDH express both in liver and muscles establish possibility of Cori cycle in both fishes, which also persist in both species. Lactate produced in skeletal muscle is probably re-oxidized in liver. The liver and skeletal muscles have same capability to catabolise lactate [13].

Both ways *M. gulio* is adapted for estuarine life, its heart is efficient for gluconeogenesis utilizing malate-aspertate shuttle as well as anaerobic respiration. Through higher LDH expression in liver and muscle are adapted for Cori cycle. Further study may reveal cross talk between the adaptive strategies.

Acknowledgement :

The present work supported by DST-FIST, Department of Zoology, Bethune College is also gratefully acknowledged.

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