LACTATE DEHYDROGENASE ISOZYMES IN TILAPIINE FISHES (CICHLIDAE): TISSUE EXPRESSION AND GENETIC VARIABILITY PATTERNS

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Abstract

The tissue specific LDH isozyme pattern is examined in three species of the economically important tilapiine fishes, *Oreochromis niloticus*, *O. aureus* and *Tilapia zillii*, using horizontal starch gel electrophoresis. Like the majority of vertebrates, tilapiine fishes exhibited the usual relative electrophoretic mobility of the A_4 and B_4-that is, B_4 carries a greater net negative charge. Isozyme of A_4 homotetramer was predominant in the skeletal muscle, while B_4 one was greatly expressed in heart muscle. Heteropolymeric isozymes of A and B composition were recognized. They were expressed in some organs and absent in others. Therefore, the present study indicated that tilapiine species could be considered 3-5 isozyme fishes. The total number of LDH isozymes of A and B composition is under genetic control of two loci, *Ldh-A* and *Ldh-B*. The LDH patterns of eye and brain displayed an additional band which was detected anodal to B_4. This anodal band is designated as LDH-C_4 isozyme. The latter isozyme is encoded in a third *Ldh* locus designated as *Ldh-C*. While the brain has heteropolymers of CA, CB and CAB polypeptide composition, the eye was the only organ that expressed the C_4 homopolymer. The present investigation showed that C gene was able to function but at lower levels in non-neural tissues. The LDH isozyme patterns of muscle, heart and eye were also compared among the three tilapiine species. The isozymatic LDH patterns in the eye of the examined species were similar except at *Ldh-C* locus. The genetically controlled restriction of the heterotetramers assembly along with the homology of the three *Ldh* loci among species was discussed.

Keywords: Lactate dehydrogenase, isozymes, tilapiine fishes, *Oreochromis*, *Tilapia*, electrophoresis.

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INTRODUCTION

Lactate dehydrogenase (LDH: L-lactate, NAD^+ oxidoreductase, EC 1.1.1.27) in higher vertebrates has been shown to exist in the catalytically active form as a tetramer of four polypeptide chains. LDH enzyme is involved in the reversible reaction of lactate oxidation and pyruvate reduction (Zietara and Skorkowski, 1991, 1993). Electrophoresis of tissue extracts, followed by the appropriate visualization procedure revealed five isozyme bands corresponding to the five possible tetrameric

combinations of two kinds of subunits (Appella and Markert, 1961). The occurrence of the five possible HM or AB tetramers in fishes is not as common as in higher vertebrates. Frequently either only the homopolymers A_4 and B_4 isozymes or these two plus A_2B_2 heteropolymer are all observed. In fact, it would appear that there is such a range of LDH isozymes in fishes that few generalizations can be made. In Petromyzontiformes, LDH is encoded by a single *Ldh-A* gene locus. In Myxiniformes, however, two loci *A* and *B* are present forming the two homotetramers A_4 and B_4, and these remained in the other vertebrates (Whitt, 1984; Baldwin and Lake, 1987; Stock and Whitt, 1992). Certain fish species, particularly flatfish, apparently contain only one isozyme of LDH in most their tissues (Markert, 1968). Electrophoresis of tissue extracts of longnose and blacknose dace (*Rhinichthys cataractae* and *R. atratulus*) and some phenotypes of the carp and the barb (Cypriniformes) revealed five isozymes corresponding to tetramers of heart and muscle type subunits in birds and mammals (Clayton and Gee, 1969; Engel *et al.*, 1973; Beck *et al.*, 1983; Frankel, 1987; Gronczewska *et al.*, 2003). In Salmoniformes, multiple genes have been described both for *Ldh-A* and *Ldh-B*. A gene duplication has produced two polypeptides, B^- from B and A^- from A (Bailey *et al.*, 1976; Kettler and Whitt, 1986). The relative electrophoretic mobility of the A_4 and B_4 isozymes was reversed from the usual in some fishes like the smallmouth and largemouth bass, and in some Serrasalmidae (Characiformes) (Whitt *et al.*, 1971; Almeida-Val *et al.*, 1991). The reverse mobility pattern does not imply on a reverse actuation of LDH enzyme on tissue glycolytic metabolism (Almeida-Val *et al.*, 1991).

An LDH isozyme unique to teleostean fishes is the LDH-C_4 isozyme. This isozyme is presumably encoded in a third *Ldh* locus (Markert and Faulhaber, 1965; Whitt *et al.*, 1971; Coppes *et al.*, 1987). The *Ldh-C* gene was expressed in many tissues in primitive teleosts while in advanced teleosts, LDH-C_4 was restricted to few tissues and it was much more specialized (Zietara and Skorkoski, 1991; Alameida-Val and Val, 1993; Farias *et al.*, 1997). The LDH-C polypeptides are synthesized almost solely in the region of the nervous system involved in vision (Goldberg, 1965; Markert and Faulhaber, 1965; Frankel, 1982). The highest level of C_4 isozyme activity is found in the neural retinal cells, particularly in the inner segment region of the photoreceptor cells (Whitt, 1970b; Whitt and Booth, 1970; Quattro *et al.*, 1993). In species belonging to the orders Gadiformes and Cypriniformes, C_4 isozyme was active in the liver, with cathodic migration (Shaklee *et al.*, 1973; Champion *et al.*, 1975; Kettler and Whitt, 1986; Rao *et al.*, 1989; Zietara and Skorkowski, 1991). Basaglia (1991) demonstrated that the hepatic electropherograms for some Sparidae species (Perciformes) unexpectedly showed evidence of the C_4 isozyme with a high anodic

rate. Rehse and Davidson (1986) suggested a close homology between C subunits of teleosts and mammals based on amino acid composition data. Markert (1994) used a chicken B-actin promoter to drive the coding sequence of a human *Ldh-C* transgene in order to examine the physiological effects of LDH isozymes containing C subunits when functioning in somatic tissues. In this study, the differential LDH patterns in different tissues of three tilapiine species are examined and the possible genetic controls of different tetramer expressions are discussed. In addition, the genetic heterogeneity among the candidate species, based on LDH patterns, is investigated.

MATERIALS AND METHODS

Chemicals and animals

Most chemicals were obtained from Sigma Chemical Co. (St Louis, MO). Fishes utilized in this study comprised *Oreochromis niloticus*, *Oreochromis aureus* and *Tilapia zillii*. These tilapiine fishes were collected from the Nile River in the region between Giza and Maser El-Kadima, Egypt. The total number of collected adult fishes was about 13 *O. niloticus*, 8 *O. aureus* and 12 *T. zillii*. The live specimens were transported to the laboratory and carefully dissected. The tissues examined were the left lobe of liver (hepatopancreas), heart muscle, ovary, testis, brain, stomach, eye, kidney and skeletal muscle. The dissected organs were stored at -20 °C until use.

Homogenization and sample preparation

Tissue samples were diluted in a ratio 1:1 with a precooled grinding solution (0.6% saline solution) except for stomach, kidney and liver where dilution was 3-folds. Homogenization was achieved using a motor-driven Teflon pestle. Homogenates were frozen and thawed twice, and then centrifuged at 14,000 rpm for 30 min in a cooling microfuge. The supernatants were isolated and recentrifuged for another 30 min under the same condition. Clear supernatant was divided into aliquots of 50 µL and kept at -20 °C.

Electrophoresis

Horizontal starch gel electrophoresis was carried out according to May (1992) to separate LDH isozymes. 11% starch in Tris (0.03M) citrate (0.005M) buffer, pH 8.5 (gel buffer) was used. The tray buffer (pH 8.1) was made up of lithium hydroxide (0.06M) and boric acid (0.3M). Run was carried out at a potential gradient of 10 V/cm for 6-7 h at 6 °C.

Staining

Visualization of LDH was carried out according to May (1992) with a slight modification. Gel slices were incubated in the dark at 37 °C for 1-2 h in a solution contained 45 ml gel buffer (pH 8.5), 5 ml substrate, 10 mg nicotinamide adenine

dinucleotide (NAD), 10 mg nitro blue tetrazolium (NBT) and 2 mg phenazine methosulphate (PMS). LDH substrate was prepared according to Shaw and Prasad (1970). After staining, the gels were fixed in a solution consisted of a mixture of methanol, H_2O and glacial acetic acid (5:4:1), and were then photographed.

RESULTS

I. Tissue distribution of the LDH isozymes of *Oreochromis niloticus*.

The tissue specific LDH isozyme pattern is displayed in Fig. 1 and Table 1. The subunit composition of LDH isozymes was designated on the basis of their tissue specificity and electrophoretic mobility according to Whitt (1970b). Like the majority of vertebrates, tilapiine fishes exhibited the usual (not reversal) relative electrophoretic mobility of the A_4 and B_4 - that is, the B_4 carries a greater net negative charge. Isozyme of B_4 homotetramer was well expressed in all tissues with their greatest activity in heart, while isozyme of A_4 homotetramer was found in varying degrees in all tissues but predominates in extracts of skeletal muscle. Heteropolymeric isozymes of mobility intermediate to the A_4 and B_4 homopolymers were recognized in this tilapiine fish. Among heteropolymers is A_2B_2 isozyme which gave the greatest stain density in ovary and brain. The remaining heteropolymers are A_3B_1 and B_3A_1. They were present in some organs and absent in others. A_3B_1 isozyme form was seen in the eye, ovary and stomach. The other heteropolymer B_3A_1 was detected in liver, eye, ovary, kidney and stomach. The LDH patterns of eye and brain are of special interest, because additional bands were detected anodal to LDH-B_4 (heart type LDH) in these organs. These anodal bands are designated as LDH-C isozymes. While the brain has heteropolymers of CA and CAB polypeptide composition, the eye was the only organ that expressed the C_4 homopolymer in addition to A_2C_2 heteropolymer. Moreover, a weak activity for A_3C_1 was scored in stomach and kidney.

II. Tissue distribution of the LDH isozymes of *Oreochromis aureus*.

The tissue specific LDH isozyme pattern is shown in Fig. 2 and Table 1. As in the case of *O. niloticus*, isozyme of B_4 homotetramer of *O. aureus* was well expressed in all tissues with their greatest activity in heart. Isozyme of A_4 homotetramer showed weak to strong stain density in all organs tested. Very strong activity for A_4 isozyme was noticed in muscle extract. Heteropolymeric isozymes that lie between A_4 and B_4 homopolymers were also recognized in this tilapiine fish. The symmetrical heteropolymers, A_2B_2 isozymes were well expressed in all organs investigated except liver which gave rather a trace of activity. The asymmetrical heteropolymer B_3A_1 isozyme gave weak staining activity in all organs except liver which has no activity for this isozyme. The other heteropolymer A_3B_1 showed a strong activity in muscle. Most

of the remaining organs showed weak to moderate activity for the latter isozyme. The anodal isozymes of C subunit composition were restricted in their expression to eye and brain. Extracts of brain contained heteropolymers of CA, CB and CAB polypeptide composition, while extracts of eye contained CAB composition in addition to C_4 homopolymer.

III. Tissue distribution of the LDH isozymes of *Tilapia zillii*.

The LDH isozyme patterns are portrayed in Fig. 3 and Table 1. The homopolymer A_4 was found in all tissues examined except liver. The most pronounced activity for A_4 isozyme was seen in muscle. The other homopolymer B_4 which occupies the anodal terminal was expressed in all tissues investigated. Expression of B_4 in heart comes on the top since a very strong activity for this isozyme was obtained. Heteropolymeric isozymes of electrophoretic mobility intermediate to the A_4 and B_4 homopolymers were also recognized in this species. The symmetrical heteropolymer A_2B_2 was expressed in varying degrees in all tissues. The asymmetrical A_3B_1 isozyme was found in all organs studied except liver and heart. The remaining heteropolymer B_3A_1 could not be detected in any of the organs analyzed in *T. zillii*. Brain extracts contained heteropolymers of CA and CAB polypeptide composition, while eye extracts contained CA and CB composition in addition to C_4 homopolymer. Heart extract contained heteropolymers of CA and CAB composition which indicated that the C subunit was synthesized in that tissue.

IV. LDH-species specificity (interspecific variation)

The LDH isozyme patterns of muscle, heart and eye were compared among the three tilapiine species (Figs. 4 and 5). Each species has its own specific LDH pattern. The electrophoretic mobilities of LDH of muscle and heart extracts from the three tilapiine species were quite similar. The A_4 (with average cathodic rate) and B_4 (with average anodic rate) isozymes of *O. niloticus* appeared to be identical in electrophoretic mobilities to those of *O. aureus* and *T. zillii*. Thus these bands were considered as monomorphic bands. The isozymatic LDH patterns in the eye of the three tilapiine species studied were also similar except for C_4 isozymes. The mobility of the latter homotetramer was different on the genus level. In the two species belonging to the genus *Oreochromis*, the mobility was the same while it differed from that of the species belonging to the other genus, *Tilapia*. The C_4 homotetramer of *O. niloticus* and *O. aureus* had a slightly faster mobility than that of *T. zillii*. Thus C_4 band was considered as polymorphic band. Concerning the heteropolymeric isozymes of CA and CB composition, they were considered in turn as polymorphic bands.

DISCUSSION

In most vertebrates, LDH is encoded by two gene loci, *Ldh-A* and *Ldh-B* which synthesize two subunits, A and B or M and H (Appella and Markert, 1961; Markert, 1968; Karlsson and Larsson, 1971; Champion *et al.*, 1975; Holt and Leibel, 1987; Haggblom *et al.*, 1988; Heinova and Blahovec, 1994; Tsoi and Li, 1994; Abdelmordy, 1999). These subunits are associated in the cytoplasm and produce five different tetramers: two homotetramers A_4 and B_4, and three heterotetramers A_3B_1, A_2B_2 and A_1B_3. These tetramers have different distribution and different kinetic and physicochemical properties (Appela and Markert, 1961; Markert, 1968; Wu *et al.*, 1993; Schulte *et al.*, 2000; Chippari-Gomes *et al.*, 2005; Ishibashi *et al.*, 2007). Markert and Faulhaber (1965) found that out of 30 species of fishes, only three which produced all the five possible HM tetramers. The present results indicated that *T. zillii* is the only tilapiine fish species which did not show the five possible HM tetramers. The five possible HM tetramers were only detected in eye, ovary and stomach of *O. niloticus* and in most tissues of *O. aureus*. Markert and Faulhaber (1965) found that the electrophoretic mobility of the LDH isozymes of 10 out of 30 fish species with reversed heart-muscle LDH mobility. The same relative mobility of LDH isozymes was observed in some Poeciliidae, Percichthyidae and Serrasalmidae (Frankel, 1982; Almeida-Val *et al.*, 1991; Xia *et al.*, 1992). The present results indicated that the electrophoretic mobility of the LDH isozymes of tilapiine fishes is quite similar to what has been described for higher vertebrates and most teleosts (Battellino and Blanco, 1970; Champion *et al.*, 1975; Holt and Leibel, 1987; Rao *et al.*, 1989; Basaglia, 1991; Heinova and Blahovec, 1994; Abdelmordy, 1999). More specifically, the homotetramer A_4 has a lower negative charge and thus lower mobility (cathodic). B_4 has an intermediate negative charge and thus faster mobility (anodic). In the three tilapiine fishes, *Ldh-A* and *Ldh-B* were all expressed together in all tissues examined. A_4 homotetramer was predominant in the skeletal muscle (anaerobic tissue), while B_4 was expressed mainly in heart muscle (aerobic tissue). This distribution reflects functional characteristics of the isozymes that are well established since it involves regulatory genes and represents preferential metabolism of each tissue.

Sensabaugh and Kaplan (1972) have reported that LDH hybridization in fish is not random as it is for LDH of higher vertebrates, while Beck *et al.* (1983) have shown that the different LDH subunits associate at random to form the observed isozymes. It has been suggested that the A and B subunits of certain teleosts do not associate in *vivo* or in *vitro* producing 2 banded LDH pattern as a result of genetically controlled restriction on their assembly (Whitt, 1970a; Frankel, 1982). According to Markert (1984) and Zawadzki *et al.* (2001), restriction in copolymerization between paralogous

Ldh-A and *Ldh-B* gene products is common in fishes, amphibians and reptiles. It was assumed that the restricted (2-4) banding pattern may be explained by the evolutionary divergence between the two *Ldh* loci after genome duplication (Markert and Faulhaber, 1965; Whitt, 1970a, b). Furthermore, several reports have suggested that the occurrence of restricted LDH patterns in the majority of advanced teleosts may reflect a strong pressure for selecting subunits with low copolymerization abilities (Morizot and Siciliano, 1979; Philipp *et al.*, 1979). Almeida-Val *et al.* (1992) have shown that the isozyme number made up from A and B subunits within the family Serrasalmidae exhibits a non-phylogenetic variation, and the existence of different isozyme number can be explained by suitable differences in electric charges of subunits A and B. So in this case, the LDH isozyme number apparently is not determined by natural selection.

The present studies on tilapiine fishes indicated that these species could be considered 3-5 isozyme fishes. These restricted 3-5 banded patterns could be explained on the basis of either one of the two following alternatives: the first is the evolutionary divergence between the two *Ldh* loci after genome duplication. This divergence could provide molecular instability for co-polymerization of one homodimer (A_2 or B_2) with two different monomers (A and B subunits) leading to absence of the two or one of the asymmetrical heterotetramers. A_3B_1 and B_3A_1 were absent in liver and heart of *T. zillii*, and in brain, muscle and heart of *O. niloticus*. In the latter species, only A_3B_1 was absent in kidney and liver. On the other hand, B_3A_1 was absent in all organs of *T. zillii* and in liver of *O. aureus*. The result is the formation of LDH patterns of less than 5 isozymes in some organs of *O. niloticus* and *O. aureus*, and in all organs of *T. zillii*. The second explanation is the gene product of B_4 homotetramer is relatively not enough favoring the occurrence of the A_3B_1 heterotetramer in case of *T. zillii*. On the contrary, absence of A_3B_1 in some organs of *O. niloticus* reflects that gene product of A_4 in these organs is relatively not enough since it involves regulatory mechanisms favoring the absence of A_3B_1. Ahmad and Hasnain (2005) reported that the contribution of heterotetramers depends on the levels of LDH homotetramers. Absence of the two asymmetrical heterotetramers A_3B_1 and B_3A_1 in heart, muscle and brain of *O. niloticus* although good expression of both A_4 and B_4 homotetramers may provide a supporting evidence to favor the first explanation in this case. The present results are not in common to what has been described by Rao *et al.* (1989). They have shown that *O. niloticus* contained only 1 or 2 LDH bands in their organs.

The members of tilapiine fishes showed a third homotetramer (LDH-C_4) that carries a strong negative charge and hence migrates rapidly to the anode during electrophoresis. The activity of the C type isozyme was found in eye and brain

extracts. This observation is consistent with most teleosts (Whitt, 1970b; Frankel, 1982; Holt and Leibel, 1987; Basaglia, 1991; Farias *et al.*, 1997). Isozyme C_4 is encoded in a third *Ldh* locus designated *Ldh-C* (Markert and Faulhaber, 1965; Markert, 1968; Whitt *et al.*, 1971; Coppes *et al.*, 1987; Farias *et al.*, 1997). The *Ldh-C* product is probably involved in the visual metabolism (Frankel, 1982). The present investigation showed that the C gene was able to function in non-neural tissues such as heart in *T. zillii*, kidney and stomach in *O. niloticus*. This observation is consistent with Whitt *et al.* (1971) and Basaglia (1991, 2002). However, the level of C polypeptide synthesis was lower in these tissues than in the eye and brain. According to Almeida-Val and Val (1993), the regulatory pattern of *Ldh-C* gene appears to be the result of a high selective pressure upon an ancient gene.

The heteropolymeric isozymes intermediate to the B_4 and C_4 isozymes in the eye of the bass fish appeared to be composed mainly of B and C subunits rather than A and C subunits (Whitt *et al.*, 1971; Philipp *et al.*, 1979). The eye of *Phallichthys amates* contains all heteropolymers of C subunit composition (CA, CB and CAB) (Frankel, 1982). The present investigation showed that eye and brain of tilappine fishes run in common with *P. amates* in that respect. This proves that there is no isolation of C and A as well as B subunit synthesis. In contrast, in birds and mammals, during the time the C gene is turned on, the A and B genes are turned off. This coordinated behavior is not applicable to the LDH isozymes in many fish tissues.

Comparison of the LDH isozyme patterns of muscle, heart and eye between the three tilapiine species demonstrates that the lactate dehydrogenase isozymes are species-specific. Nonetheless, large similarities in terms of mobility and intensity exist in the patterns of these species, particularly those belonging to the same genus (*Oreochromis*). The electrophoretic mobilities of LDH-A_4 and LDH-B_4 from the three tilapiine species were quite similar indicating the homology of *Ldh-A* and *Ldh-B* genes among tilapiine fishes. The mobility of the C_4 in the eye of the three species was different on the genus level. In the two species belonging to genus *Oreochromis* the C_4 homotetramer migrated faster than the corresponding isozyme of the genus *Tilapia*. It must therefore carry a greater net negative charge than the C_4 isozyme of *T. zillii*. This in turn will be reflected on the mobility of heteropolymeric isozymes of CA, CB and CAB composition. It may be assumed that the *Ldh-C* locus was monomorphic at a time and the polymorphism was taken place prior to the divergence of the *Oreochromis* and *Tilapia*. Thus the evolution occurred more rapidly on an ancient gene (*Ldh-C* locus) than on the relatively more recent genes (*Ldh-A* and *Ldh-B* loci).

Table 1. Distribution and rate of activity of LDH isozymes in different organs of *Oreochromis niloticus*, *O. aureus* and *Tilapia zillii*.

Organs	Species	LDH isozymes											
		A_4	A_3B_1	A_2B_2	B_3A_1	B_4	A_3C_1	$A_2B_1C_1$	A_2C_2	$A_1B_1C_2$	B_2C_2	B_1C_3	C_4
Liver	*O. niloticus*	+	-	(+)	++	++	-	-	-	-	-	-	-
	O. aureus	+	+	(+)	-	++	-	-	-	-	-	-	-
	T. zillii	(+)	-	+	-	++	-	-	-	-	-	-	-
Heart	*O. niloticus*	++ +	-	++	-	++ ++	-	-	-	-	-	-	-
	O. aureus	(+)	(+)	++ +	(+)	++ ++	-	-	-	-	-	-	-
	T. zillii	+	-	++	-	++ ++	++ +	+++	-	-	-	-	-
Brain	*O. niloticus*	++ +	-	++ +	-	++ +	-	-	++	++	-	-	-
	O. aureus	++	+	++ +	+	++ +	+	-	+	++	++	-	-
	T. zillii	++ +	++ +	++ +	-	++ +	++	-	++	++	-	-	-
Stomach	*O. niloticus*	+	+	++	+	++ +	+	-	-	-	-	-	-
	O. aureus	(+)	-	++ +	+	++ +	-	-	-	-	-	-	-
	T. zillii	++ +	++	++	-	++ +	-	-	-	-	-	-	-
Eye	*O. niloticus*	++	++	++	++	++ +	-	-	+	-	-	-	++ +
	O. aureus	++	++	++	+	++ +	-	-	-	+	-	-	++ ++
	T. zillii	++ +	++ +	++	-	++ +	-	-	+	-	+	++	++ +
Kidney	*O. niloticus*	++	-	++	+	++ +	+	-	-	-	-	-	-
	O. aureus	++	-	++	+	++ +	-	-	-	-	-	-	-
	T. zillii	++	+	++	-	++ +	-	-	-	-	-	-	-
Muscle	*O. niloticus*	++ ++	-	++	-	++ +	-	-	-	-	-	-	-
	O. aureus	++ ++	++ ++	++	+	++	-	-	-	-	-	-	-
	T. zillii	++ ++	++ ++	+	-	++	-	-	-	-	-	-	-
Ovary	*O. niloticus*	++ +	+	++ +	+	++ +	-	-	-	-	-	-	-
	O. aureus	?	?	?	?	?	?	?	?	?	?	?	?
	T. zillii	++ +	++ +	++ +	-	++ +	-	-	-	-	-	-	-
Testis	*O. niloticus*	?	?	?	?	?	?	?	?	?	?	?	?
	O. aureus	?	?	?	?	?	?	?	?	?	?	?	?
	T. zillii	++	+	+	-	++ +	-	-	-	-	-	-	-

(+) = trace of activity; + = weak activity; ++ = moderate activity; +++ = strong activity; ++++ = very strong activity; - = no activity; ? = not tested.

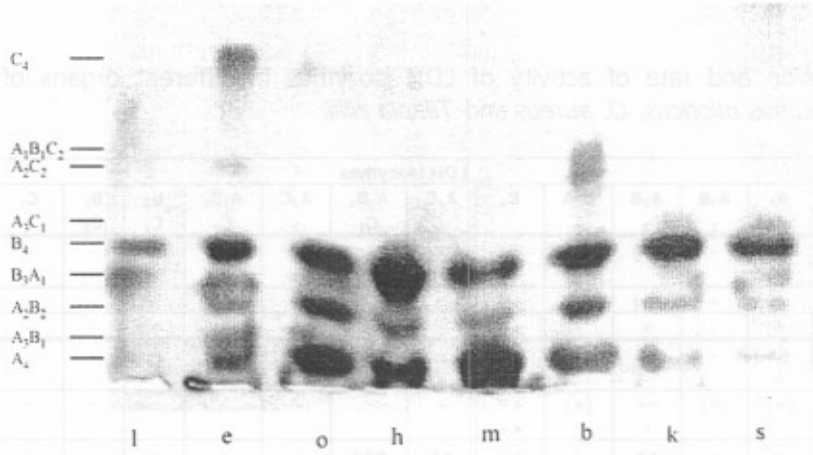

Fig. 1. Tissue distribution of LDH isozymes of *O. niloticus* (l, liver; e, eye; o, ovary; h, heart; m, muscle; b, brain; k, kidney; s, stomach).

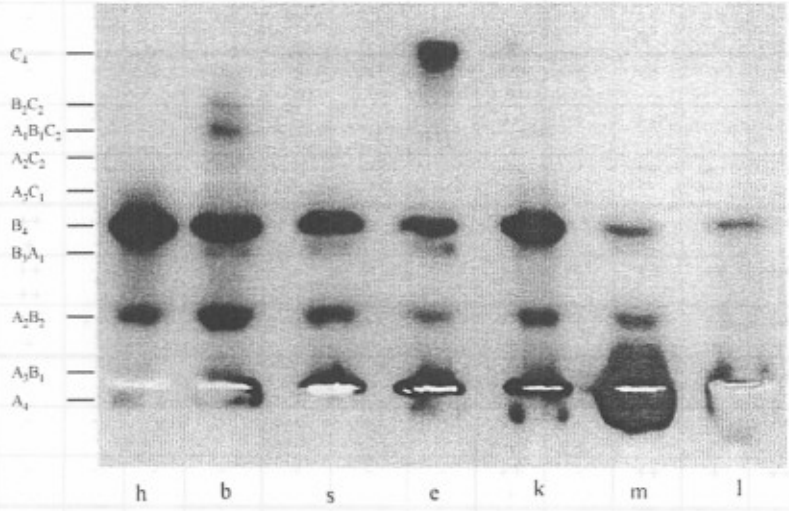

Fig. 2. Tissue distribution of LDH isozymes of *O. aureus* (h, heart; b, brain; s, stomach; e, eye; k, kidney; m, muscle; l, liver).

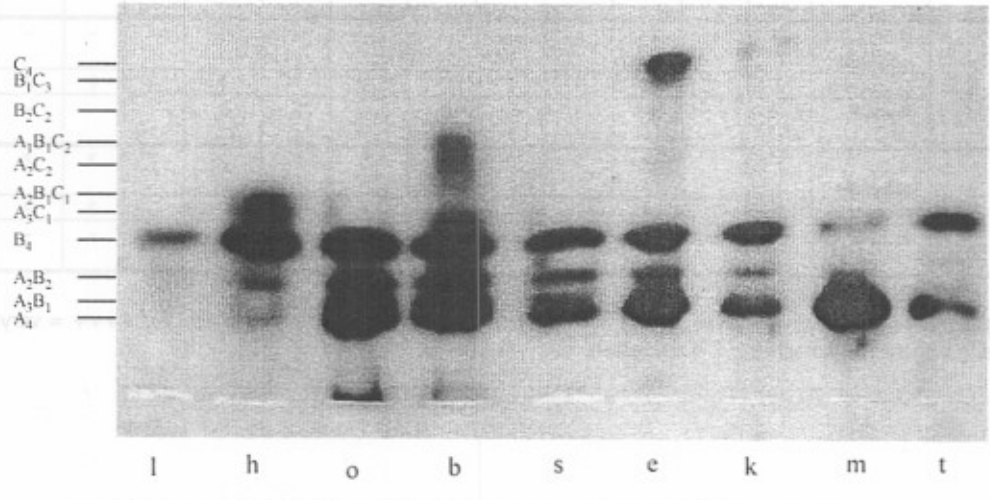

Fig. 3. Tissue distribution of LDH isozymes of *T. zillii* (l, liver; h, heart; o, ovary; b, brain; s, stomach; e, eye; k, kidney; m, muscle; t, testis).

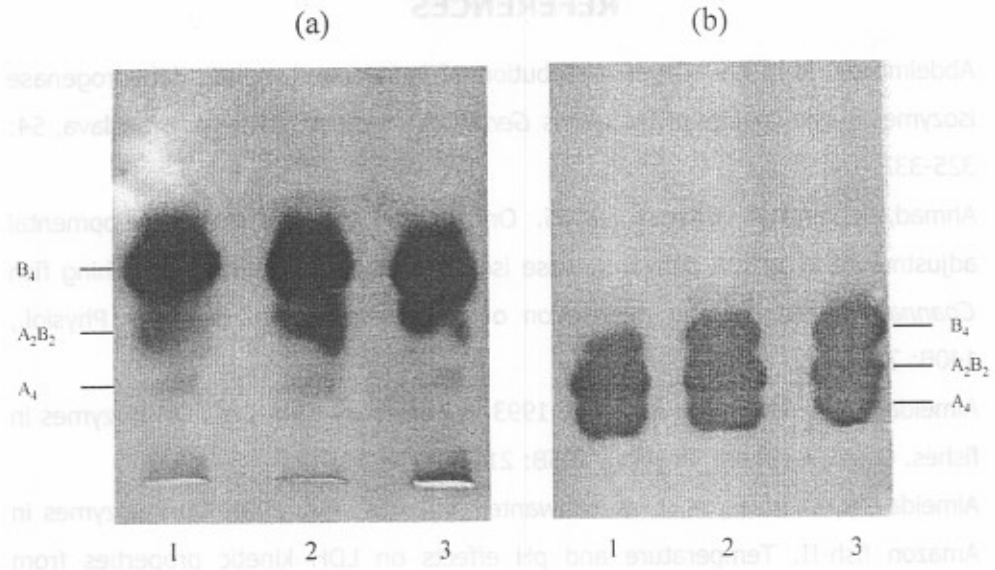

Fig. 4. LDH patterns in heart (a) and skeletal muscle (b) of tilapiine fishes (1, *O. niloticus*; 2, *O. aureus*; 3, *T. zillii*).

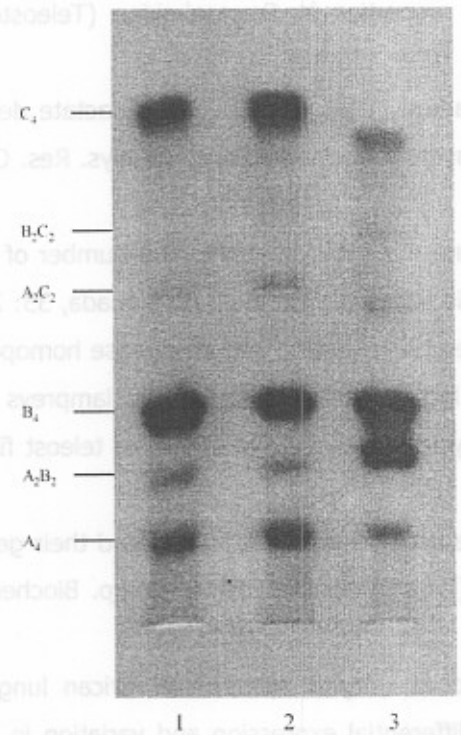

Fig. 5. LDH patterns in eye of tilapiine fishes (1, *O. niloticus*; 2, *O. aureus*; 3, *T. zillii*).

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الصور الإنزيمية لإنزيم اللاكتيت ديهيدروجينيز في أسماك البلطي (العائلة السايكليديه): أنماط التعبير النسيجي والإختلاف الوراثي

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درست في هذا البحث أنماط الصور الإنزيمية لإنزيم اللاكتيت ديهيدروجينيز (LDH) الخاصة بأعضاء الجسم المختلفة لأسماك البلطي ذات الأهمية الإقتصادية: البلطي النيلي، البلطي الأزرق، البلطي الأخضر، باستخدام طريقة الفصل الكهربي النشوي الهلامي. وكما هو الحال في سائر الفقاريات، فقد أظهرت الصور الإنزيمية لإنزيم LDH في أسماك البلطي حركة معتادة تحت تأثير الفصل الكهربي أي أن الصورة A_4 كانت تحمل شحنات سالبة مع حركة بطيئة. أما الصورة B_4 فكانت تحمل شحنات سالبة عالية مع حركة سريعة. وقد سادت الرباعيات المكونة من سلاسل بيبتيدية من النوع A في العضلات الهيكلية بينما سادت الرباعيات المكونة من النوع B في العضلات القلبية. وقد تم التعرف أيضا على صور إنزيمية لإنزيم LDH مكونة من سلاسل بيبتيدية من النوعين A، B غير أن نشاط هذه الصور الإنزيمية كان واضحا في بعض الأعضاء وغائبا في أعضاء أخرى. لذلك برهنت الدراسة الحالية أن أسماك البلطي تعتبر من الأسماك ذات النمط الإنزيمي ٣-٥ لإنزيم LDH. ويرجع وجود هذه الصور الإنزيمية لإنزيم LDH المكونة من النوع A، B لنشاط موقعين وراثيين هما *Ldh-A*، *Ldh-B*. كما أظهرت أسماك البلطي نوعا رباعيا ثالثا هو $LDH\text{-}C_4$ ويحمل هذا النوع شحنات سالبة عالية جدا أدت إلى سريانه السريع تجاه القطب الموجب. وكان نشاط هذا النوع الرباعي المكون من وحدات C موجودا في مستخلص كل من العين والمخ. ويشير ذلك إلى وجود موقع وراثي ثالث رمز له بالرمز *Ldh-C* يتحكم في الرباعيات المكونة من سلاسل بيبتيدية من النوع C. كما وجد لهذا الأخير أي الجين C نشاط طفيف في عدد قليل من الأنسجة غير العصبية. كما بينت الدراسة الحالية إمكانية ارتباط السلاسل البيبتيدية المختلفة (A، B، C) الناتجة من المواقع الوراثية الثلاثة لإنزيم LDH. تم مقارنة النمط التعبيري لإنزيم LDH في ثلاثة أعضاء هي القلب والعضلات والعين بين الأنواع الثلاثة. وقد كان هناك تباين بين الأنواع في التعبير الجيني للموقع *Ldh-C* على مستوى الجنس. تم مناقشة التكوين المحدود للصور الإنزيمية المكونة من سلاسل بيبتيدية مختلفة أي من النوعين A، B وكذلك التباين الوراثي في النشاط الجيني عند الموقع *Ldh-C* بين أسماك البلطي محل الدراسة.