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Isozyme Variation of Nile Tilapia *Oreochromis niloticus* in China

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Abstract

The biochemical genetic characteristic of GIFT, Egypt 92, Sudan 78, Egypt 88 and America strains of Nile tilapia *Oreochromis niloticus* were analyzed using acrylamide gel electrophoresis. Eighteen loci, including four esterase (EST) loci were examined. All were monomorphic except for two EST loci. There were differences in allele frequencies of EST in the liver tissue among the different strains. The strains clustered into two groups, one containing GIFT and Egypt 92 and the other containing the remaining three strains. The EST-2 locus was found only in GIFT and Egypt 92. This could be used as a genetic marker to differentiate them from other farmed strains of Nile tilapia in China. Isozyme variation in Nile tilapia is low.

Introduction

Tilapias have gained recognition as one of the most important species in tropical and subtropical aquculture because of their production potential. Among these fishes, the Nile tilapia *O. niloticus* is the most widely farmed species (Pullin et al.1991), but poor breeds may have been derived from introductions of small numbers of fish from different countries. There is a need for a better farmed strain of *O. niloticus* in Asian countries as the tilapia industry continues to expand (Pullin and Capili 1987).

A new line of *O. niloticus*, GIFT was established by combining germplasm brought from Africa (Egypt, Ghana, Senegal and Kenya) with four farmed strains in the Philippines (Eknath et al. 1993). The GIFT line has faster growth rate and higher survival than the local strains in the Philippines (Eknath et al. 1998)

Low levels of intraspecific variation in morphology and hybridization among different species and genera in tilapias make it difficult to differentiate tilapia based on morphological characteristics only. Electrophoresis could reveal variation at the protein level, and provide differentiation through biochemical genetic approaches (Avtation 1976, McAndrew and Majumdar 1983, Galman 1985, Macaranas et al. 1981, Agnese et al. 1997, Li 1988, Yang 1988, Li and Cai 1995). To effectively differentiate the GIFT strain from the widely-farmed strains (Sudan 78, Egypt 88 and American strain) in China as well as to manage and preserve these broodstocks, a study on the variations of biochemical genetic characteristics of these strains was conducted.

Materials and Methods

Experimental fish

Sudan 78 was introduced to China from Sudan twice (27, 34 fish respectively) by the Yangtze River Fisheries Institute (Shashi City, Hubei Province) in 1978, and had become the largest farmed strain in China (Li 1993). Egypt 88 was introduced to China from Egypt by the Hunan Fisheries Bureau in 1988 (eight females and one male) (Li 1993). American strain was introduced to China from America in 1991, but detailed ancestry was unclear. GIFT line was introduced to China with a total of 4,000 fry from the Philippines in 1994 for the international collaborative research on the project "Dissemination and Evaluation of Genetically Improved Tilapia Species in Asia" (DEGITA) initiated by the International Network on Genetics in Aquaculture (INGA). Egypt 92 was introduced to China from the Philippines in 1994 with 4,000 fry.

Sample preparation

Samples of 30 individuals of the five strains of Nile tilapia were collected from experimental stations. Sudan 78, Egypt 88 and GIFT were collected from Shanghai Fisheries University, Egypt 92 from Huzhou Fish Farm, Zhejiang Province, and American from the National Tilapia Seed Farm of Qingdao. Live fish were dissected after bleeding, tissues of skeletal muscle and liver were collected and labeled individually and kept in liquid nitrogen in the field. These were taken to the laboratory and kept at -25^0C.

Electrophoresis

Electrophoresis was conducted with 4% polyacrylamide gels exept for the enzyme EST analyzed with 5% gel. The enzymes studied are in table 1. Genetic distance and genetic similarity were calculated according to Nei (1975). A dendrogram was constructed using this data.

Results

Isozyme analysis

Ten enzymes in the skeletal muscle and liver of five strains of *O. niloticus* were studied. The results were summarized in table 2. Among the 18 loci examined, only two loci for one enzyme were found be polymorphic. The five strains were monomorphic for all enzymes examined except EST. The electrophogram of EST in liver tissue showed some strain-specific expressions (Fig. 1). From the bands observed it could be coded into four loci (EST-1, EST-2, EST-3, EST-4), of which EST-1 and EST-3 were polymorphic. All populations were polymorphic for the same loci. EST-2 was found only in GIFT

Table 1. Enzymes, tissues and buffer systems examined for strains of Nile tilapia *O. niloticus*

Enzyme	Buffer*	Tissue
Alcohol dehydrogenase (Adh)	TBE	Liver
Glycerol-3-phosphate dehydrogenase (G3pdh)	TC	Muscle
Lactase dehydrogenase (Ldh)	TC	Muscle
Malic dehydrogenase (Mdh)	TC	Muscle
Malic enzyme (Me)	TC	Muscle
Isocitritrate dehydrogenase (IDH)	TC	Liver
Glucose-6-phosphate dehydrogenase (G6pdh)	TC	Liver
Esterase (EST)	TBE	Liver
Superodixide dismutase (Sod)	TBE	Muscle
Sobitol dehydrogense (Sdh)	HC	Liver

* EBT denotes tris-borate EDTA buffer, pH=8.6
TC denotes tris-citrate buffer, pH=8.0
HC denotes histine-citrate buffer, pH=8.5

Table 2. Allelic frequency of polymorphic loci from 18 loci examined for five strains of Nile tilapia *O. niloticus* (N=30)

		Strain				
Locus	Allele	Sudan 78	Egypt 88	GIFT	Egypt 92	America
		Allele Frequencies				
EST-1	100	0.50	0.53	0.84	0.57	0.50
	94	0.50	0.47	0.16	0.43	0.50
EST-2	100			1.00	1.00	
EST-3	100	0.97	0.87	0.72	0.43	0.97
	104	0.03	0.13	0.28	0.57	0.03
EST-4	100	1.00	1.00	1.00	1.00	1.00

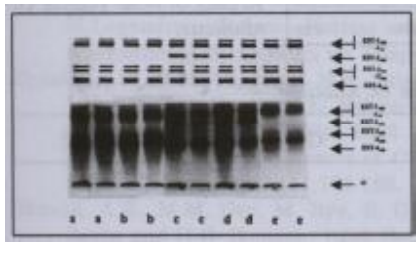

Fig. 1. EST in the liver tissue of five strains of Nile tilapia *O. niloticus*: a) Sudan 78, b) Egypt 88, c) GIFT, d) Egypt 92, e) America.

strain and Egypt 92 (one of the GIFT founder strains), and not observed in the other strains, that could be used as a genetic marker to differentiate them.

Genetic distance

Genetic distances of five strains of Nile tilapia is shown in table 3. A dendrogram was constructed from the genetic distances values among five strains of *O. niloticus* (Fig. 2). Sudan 78, Egypt 88 and American strain clustered forming one independent branch, while genetic distance (D=0.0087) of GIFT strain and Egypt 92 strain resulted in a divergent branch from the remaining strains (D=0.0389).

Among the five strains of Nile tilapia investigated, Egypt 92 strain, GIFT selected-breed from African strains that included Egypt 92 strain and Asian strains, displayed high similarity. Both strains that share all alleles were analyzed and had the EST-2 locus, only found in GIFT strain and Egypt 92 strain. EST-2 could be used as a genetic marker to distinguish these two strains from other farmed strains in China. Genetic similarity between GIFT strain and Egypt 92 strain was higher (I=0.9913) in these two strains and in other strains (I=0.9622-0.9659).

GIFT strain, that was select-bred on the mixed base population (synthetic breed), had inherited the affluent germplasm from African strains. Our investigation proved that GIFT strain is really a new genetically improved strain.

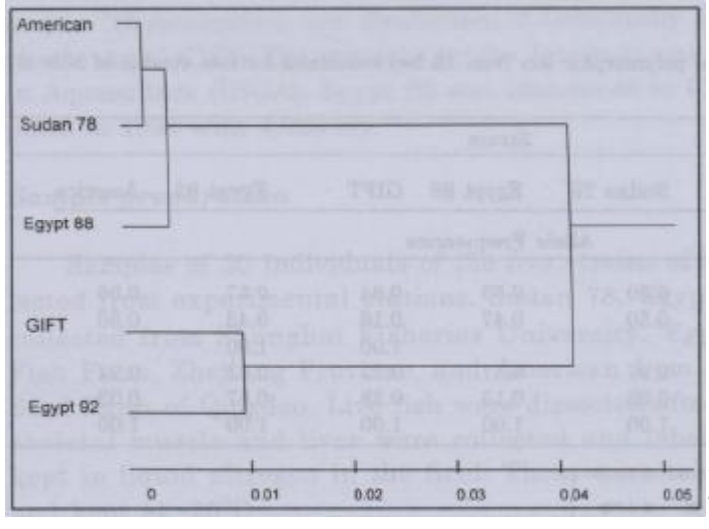

Fig. 2. Dendrogram constructed from genetic distance values of five strains of Nile Tilapia *O niloticus*.

Table 3 Genetic distances of five strains of Nile tilapia *O. niloticus*.

Strain	Sudan 78	Egypt 88	GIFT	Egypt 92
Sudan	78			
Egypt	88	0.0007		
GIFT	0.0378	0.0343		
Egypt 92	0.0449	0.0390	0.0087	
America	0.0001	0.0006	0.0377	0.0447

Yu (1995) found that the EST isoenzyme of silver carp *Hypophthalmichthys molitrix* in the Yangtze River has nine different phenotypes, and that different EST genotypes have a significant correlation with growth rate. Additionally, Hallerman et al. (1986) demonstrated that selection for increased body weight in channel catfish, *Ictalurus punctatus*, changed isozyme allele frequencies and reduced biochemical genetic variations. In this study, GIFT strain, Egypt strain, had a different genotype from the strains in China, and exhibited different growth performance (Li et al. 1997). The relationship between the genotype of EST and growth rate should be examined. Further studies on the culture properties of different strains of Nile tilapia are necessary to provide data for tilapia breeding, strain identification and conservation, and have important implications for genetic management of cultured stocks. Nile tilapia appears to have little isoyzme variation (Taniguchi et al. 1985, Abdelhamid 1988, Rognon et al. 1996), and the current research confirms this trend. However, increased genetic variation in some Nile tilapia populations is a result of interspecific introgression (Taniguchi et al. 1985, Rognon et al. 1996, Agnese et al. 1997, Macaranas et al. 1986, Abdelhamid 1988). Relationships between performance and proteins and DNA variations should be studied in Nile tilapia. Except for the extra locus found in some strains, isozyme variation does not appear to be a powerful approach for distinguishing strains of Nile tilapia in China.

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