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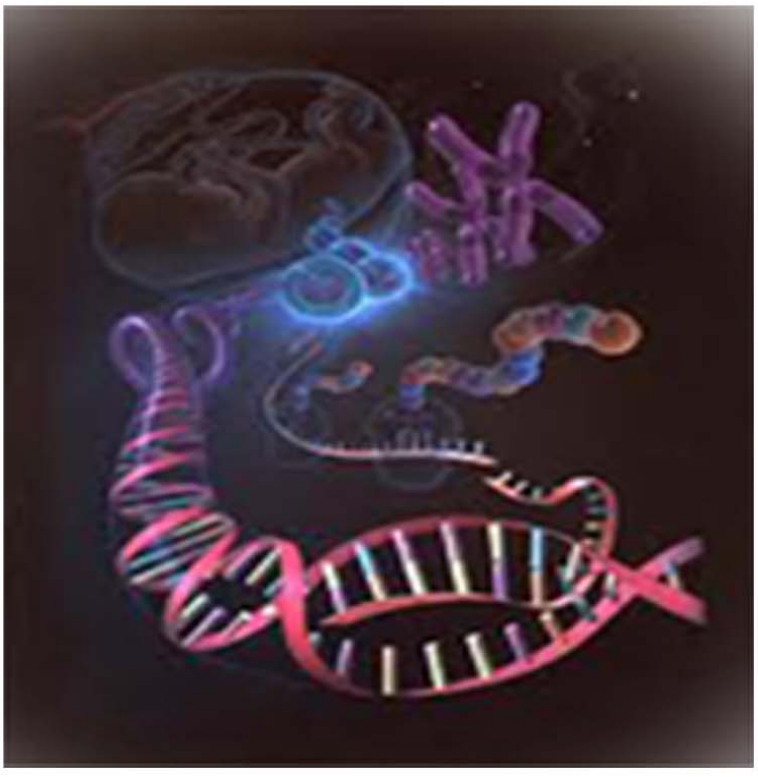

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Research Paper

GENETIC VARIATION BETWEEN TWO DIFFERENT STRAINS GIFU AND GIFT OF *NILE TILAPIA, OREOCHROMIS NILOTICUS* USING ALLOZYME MARKER

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Allozyme electrophoresis was accomplished to identify genetic variation in two different strains of *Nile tilapia*, GIFU and GIFT. Two months aged experimental fishes were collected from two different regions namely Noakhali and BFRI, Mymensingh. Five enzymes namely LDH, SOD, MDH, ODH and MDH (NADP+) were used in TC-1 buffer system to analyze the genetic variation. LDH and SOD provided clear resolution for muscle tissue. Two presumptive loci were identified where *Ldh-1** loci was found polymorphic in both populations. GIFT populations showed same mean proportion of polymorphic loci which was 50% whereas GIFU population showed highest mean number of alleles per locus which was 2.0. The highest mean number of effective alleles per locus was 1.47 and the mean proportion of heterozygous loci per individual was 24.31% which was found in GIFT population. Among two populations, observed heterozygosity (H_o) and expected heterozygosity (H_e) of GIFT population showed the highest values which was 0.333 and 0.254 respectively. The average observed heterozygosity (H_o) was 0.313 and expected heterozygosity (H_e) was 0.240 for GIFU populations. The average H_o/H_e value was 1.301 in *Oreochromis niloticus* population. The genetic differentiation (F_{st}) and the gene flow (N_m) over all two populations was 0.0239 and 10.1923, respectively. The genetic distance (D) between two populations was calculated and found to range from 0.0146 to 0.9855..

Keywords: Genetic variation, Allozyme marker, GIFT, GIFU, *Oreochromis niloticus*

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), is one of the most important exotic species in Bangladesh which is fast grower, suitable for culture in native water, has high tolerances of wide range of water salinity, resistance to pesticides and other toxicants and favored by most of the people of Bangladesh. Genetically Improved

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Farmed Tilapia (GIFT), an improved strain of *Nile tilapia*, was introduced by BFRI from the Philippines in 1994. Growth performance of GIFT is now 40% higher than that of the founder stock (Rahman, 2005). BFRI is doing research on genetically stock improvement through selective breeding technique at every generation. However GIFU, the new strain of *Nile tilapia* was recently introduced by the private hatchery owner at of Noakahli district of Bangladesh. He claimed that GIFU is the 11th generation of GIFT which is superior (20% more growth) to the GIFT in terms of growth performance. The hatchery owner also mentioned that the superiority of GIFU through its higher fillet ratio and attractive fillet color (light pink) than other strains. Tilapia, as aquaculture species, is now occupied highest position among all other aquaculture species and contributing significantly towards the enhancement of total fish production in Bangladesh. So ensuring the more productive strain is of prime importance for the sustainability of aquaculture. Therefore the present study was conducted to identify the genetic variation between GIFU and GIFT through allozyme electrophoresis.

MATERIALS AND METHODS

Fish Samples

Two months aged experimental fishes were collected from two different sources in Bangladesh namely GIFU from Noakhali and GIFT from Bangladesh Fisheries Research Institute (BFRI), Mymensingh. A small piece of muscle tissue was taken using scalpel and scissors maintaining cool condition. The scalpels and scissors were washed and soaked in ethanol and then dried with tissue paper (in time before cutting another individuals muscle tissue) to avoid protein contamination. Then the muscle tissues were stored in marked air tight small size plastic bag and this bags were transferred in a freezer (-18^{0}C) immediately. This storage system continued until electrophoretic analysis

Allozyme Electrophoresis

Horizontal starch gel electrophoresis method using tris citrate (TC-1) buffer (pH 7) (Shaw and Prasad, 1970) was used for allozyme work. The muscle sample was collected from each fish separately. The details of enzyme are shown in Table 2.

Table 1: Sources, Number of Specimen and Date of Collection of *Oreochromis niloticus* Population

S. No.	Strain	Source	No. of Individual
1	GIFU	Private fish hatcher at Noakhali	30
2	GIFT	BFRI, Mymensingh	30

Table 2: Details of Enzyme for Electrophoresis of GIFU and GIFT

Enzymes	Enzyme Abbreviations	Enzyme Patterns	* E C Number	Tissue
Lactate dehydrogenase	LDH	Tetramer	1.1.1.27	Muscle
Superoxide dismutase	SOD	Dimer	1.15.1.1	Muscle
Lactate dehydrogenase	LDH	Tetramer	1.1.1.27	Muscle
Malate dehydrogenase	MDH	Dimer	1.1.1.37	Muscle
Malate dehydrogenase(NADP+)	MDH(NADP+)	Dimer	1.1.1.40	Muscle

Note: * E.C – Enzyme commission.

After electrophoresis, enzyme staining recipes were followed by Whitmore (1990). Loci were numbered consecutively from the anodal to the cathodal side. Thus the most anodal locus was designated '1'. Gene nomenclature was followed by Shaklee *et al*. (1990). The electrophoretic bands corresponding to multiple alleles at each locus were alphabetically named as **a*, **b*, **c* etc. in order of detection.

Genetic Analysis

Allele frequencies were calculated directly from observed genotypes. Observed genotypes were compared with the expected, calculated from the Hardy-Weinberg equilibrium using χ^2 test. When the most common allele existed in a frequency less than or equal to 0.95 at a given locus this locus was regarded as polymorphic. The mean proportion of heterozygous locus per individual, mean proportion of polymorphic loci per population, mean number of effective allele per locus were calculated so as to show the extent of genetic variability (Lewontin and hubby, 1966; Lewontin, 1974). Expected (H_e) and observed(H_o) heterozygosity were also calculated (Nei, 1972). The co-efficient of gene differentiation (F_{ST}) and Gene flow (N_m) was calculated to estimate diversity. The analysis of allozyme data were performed using POPGENE (version 1.31) (Yeh *et al.,* 1999) computer package program.

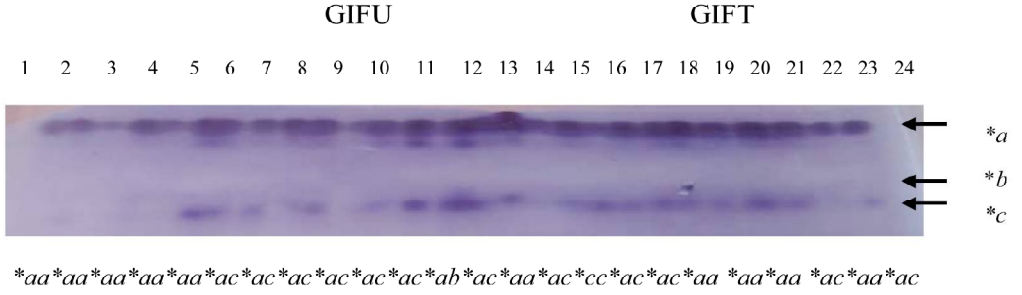
RESULTS AND DISCUSSION

In the present study five enzymes LDH, SOD, MDH, ODH and MDH (NADP+) were used in TC-1buffer system. Among them MDH, ODH and MDH (NADP+) did not show any resolution and LDH, SOD provided clear resolution for muscle tissue of the two strains of *Oreochromis niloticus.* According to khan (1999) the allelic enzyme activity varies from buffer to buffer, species to species and also tissue specific

Allele Frequencies

The electrophoretic patterns of muscle samples showed that the genes at two presumptive loci controlled by the enzymes. The *Ldh-1** was produced heterozygosity by the two alleles **a* and **c* with the frequency of 0.58 and 0.42 in the GIFT population and the GIFU population was produced heterozygosity by three alleles **a*, **b* and **c* with the frequency of 0.70, 0.04 and 0.26. On the other hand, the *Sod** loci was monomorphic with the allelic frequency of **a*=1.000 in two populations. In the present study, GIFU population showed three common alleles (**a*, **b* and **c*) and GIFT population showed two common alleles (**a* and **c*). The *Ldh-1** was showed four

Figure 1: Electrophoregram of Enzyme Lactate Dehydrogenase (LDH) of *Ldh-1 Locus Between GIFU and GIFT Populations**



Note: **a, *b, *c* indicate the three different alleles in *Ldh-1** locus. The respective genotypes are shown below in each lane for a particular individual.

genotypes, **aa, *ab,*cc,*ac* produced by three alleles, **a, *b,*c.* The allele frequency of **a* in *Ldh-1** was higher in all two populations. The allele frequency of **b* in *Ldh-1** was present in the GIFU populations whereas it was completely disappeared in the GIFT population.

The amount of genetic variation in a population can be estimated only if one has information about the number of LOCI at which variation occurs (polymorphic loci) depicted by Lewontin (1974). Electrophoretic data provided such information that could be successfully used to monitor levels of genetic variation in populations (Leary and Booke, 1990).

The chi-square was made in all the cases of polymorphic loci between observed and expected genotypes, based on Hardy-Weinberg equilibrium. The test was not effective in the most cases in which the expected values were <5.

Genetic Variability

The mean proportion of polymorphic loci per population was 50%. The mean proportion of heterozygous loci per individuals for all population ranging from 21.70% to 24.31% (Average 23.01%), which was higher in GIFT population and lower in GIFU population. The observed heterozygosity (H_o) and expected heterozygosity (H_e) obtained in the present study ranged from 0.2917 to 0.3333 (Average 0.3125) and 0.2264 to 0.2536 (Average 0.2400) respectively. The higher observed and expected heterozygosity (H_o= 0.3333 and H_e=0.2536) exhibited by the GIFT population indicated that the gene pool of the GIFT population was maintained effectively. Between two tilapia populations the H_o/H_e was higher in GIFT populations (1.3142) and lower in GIFU population (1.2884).

Sekino and Hara (2000) found that H_o and H_e values in *Anabas testudineus* ranging from 0.054 to 0.090 and 0.056 to 0.106. The observed heterozygosity and expected heterozygosity was higher than Sekino and Hara (2000). Observed heterozygosity (0.3125) was higher with that obtained (0.059) by Barua *et al.* (2004) and value (0.072) by Eunus (2004) for thai pangas, *P. Hypophthalmus*. Again Nevo (1978) reported that an average heterozygosity (H_o) value for bony fishes was 0.051. However, the H_e values obtained in the present study do not fall in the range of values (H_e=0.02 to 0.03) which are generally considered as the lower margin of the genetic variability for fishes (Nevo *et al.,* 1984; Kirpichnikov, 1992). The level of heterozygosity is related with the size of populations within a species. The practical interest of higher

Table 3: Allele Frequency at 2 Presumptive LOCI of *Oreochromis niloticus* Strains

	Allele Frequency		
Locus	Allele	GIFU	GIFT
*Ldh-1**	**a*	0.7	0.58
	**b*	0.04	0
	**c*	0.26	0.42
*Sod**	**a*	1.0	1.0

Table 4: Genetic Variabilities at 2 LOCI of *O. niloticus*

Population	Mean Proportion of Polymorphic LOCI (%)	Mean Number of Alleles (Na) per Locus	Mean Number of Effective Alleles (Ne) per Locus	The mean Proportion of Heterozygous LOCI Per Individual (%)	Heterozygosity		
					H_o	H_e	H_o/H_e
GIFU	50	2.00	1.38	21.70	0.291	0.226	1.288
GIFT	50	1.50	1.47	24.31	0.333	0.254	1.314
Average	50	1.75	1.425	23.01	0.313	0.240	1.301

heterozygosity (H_o) value of a population can be aimed at genetic breeding programs. The average heterozygosity (H_o or H_e) is considered as a good indicator of the genetic variability throughout the genome of the population (Leary and Brooke, 1990; Allendorf and Ryman, 1986)

Genetic Differentiation

The co-efficient of gene differentiation (F_{ST}) in all two *Oreochromis niloticus* populations examined (Nei's, 1972) for all loci was 0.0239, indicated the presence of population with a slight genetic differentiation and the number of individuals that migrate from one population to another is high (N_m=10.1923). The F_{ST} value (0.3667) was higher than obtained value for other fishes such as loach (0.774) (Khan and Arai, 2000) and freshwater Gobi (0.698) (Shimizu *et al*., 1993). Yet, the present F_{ST} value point towards the existence of slight genetic differentiation between the strains.

CONCLUSION

The result of the present study would be used to know the genetic structure and variation of different strains of the studied species and identify the distinct population groups existing in Bangladesh. It would also be useful for undertaking any stock improvement and conservation program. However, present study had some limitations in terms of limited number of individuals and populations, sophisticated equipments and enzymes.The result of the present study might be used as a guideline for further study with more sample lots and enzymes.

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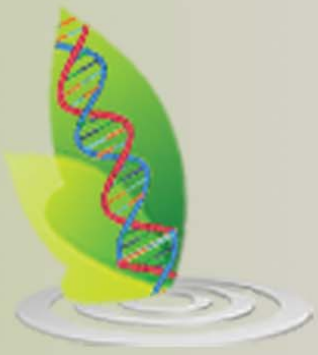

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