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Microsatellite Diversity in Rainbow Trout (*Oncorhynchus mykiss*) Stocks of Different Origin

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Abstract: Variation of 5 microsatellite loci was analyzed in 3 groups rainbow trout strains reared in North of Iran of different origin. In all 3 strains, the level of variability, measured as the average allele observed heterozygosity, was similar to each other while that of Norway exhibited markedly higher variability and decrease level of inbreeding. Only 8% of the total genetic variation in farmed strains was accounted for differences between the countries of origin. The farmed strains were moderately differentiated (average FST = 0.050). Some allele of locus Otsg249 are present in T1-origin and some of Otsg474 N1-origin strains which have not detected in others, therefore the individual fish could be assigned to their strain of origin with an average of 86% accuracy. The F1-origin strains were genetically more similar to the T1-origin strain than to the N1-origin.

Key words: Rainbow Trout % Microsatellites % *Oncorhynchus Mykiss* % Genetic Diversity

INTRODUCTION

Rainbow trout, *Oncorhynchus mykiss*, is among the most important cultivated fish species in the world with the total annual production exceeding 600 thousand tons [1]. During the last 4 decade, rainbow trout were extensively introduced into fish farms and lakes outside their native habitat and this fish is currently expanding its range through dispersal into adjacent waters in different farms in different countries. The rainbow trout is not a native trout species in Iran, therefore, there is not a program to preserve or conserve their population and has been growing rapidly. Neither the source of this introduction nor the number of founders is known exactly and documented. Recently the eyed eggs are introduced to fish farm from several European countries and from West Asia. This has been occurred in many countries introduced rainbow trout as cold fish for aquaculture and very few data are available about the biology of this fishbiochemical but not much information are exist in the levels of genetic variability and differentiation among the rainbow trout strains of different countries or if the strains within the Iran are genetically more related to each other than the strains between the countries.

It assume microsatellite genetic variation could help answer these questions, but no microsatellite loci have been specifically developed and available for loci identified of rainbow trout and some recommended showed modest levels of genetic variation. In this study, we used at least 9 microsatellite loci isolated from other fish, among them 5 was highly polymorphic. Examination of microsatellite variation at several polymorphic loci is a widely accepted method for determining parentage and examining genetic diversity within rainbow trout (*Oncorhynchus mykiss*) breeding programs [2].

DNA markers are more likely to detect small differences between populations due to their higher levels of allelic variation, for example, minisatellite DNA markers have been shown to detect significant differences in both overall heterozygosity and mean number of alleles between wild and farmed strains [3,4].

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Microsatellite have been shown to be highly polymorphic in teleost fish, this variability makes for suitability in a variety of applications in fisheries and aquaculture, particularly where genetic differentiation between populations may be limited. Potential applications in aquaculture include monitoring changes in genetic variation, as a consequence of different breeding strategies, parentage assignment and estimation of relatedness between potential breeding pairs [5]. Therefore, the aim of the present study was the analysis of the Genetic Variation of rainbow trout strains introduced from different countries and detect any genetically relation between the strains of the countries by using microsatellite.

MATERIALS AND METHODS

Sampling: The microsatellite locus of Rainbow trout of French origin, 30 specimens (F1), Norway origin, 60 specimens (N1) and Turkish origin, 30 specimens (T1) were carried out. Fishes were caught from the Northern part of the Iran. The specimens were collected from a fish farm located at Mazandran. The characteristics of the fishes, including length (28±5 cm), weight (1985±120 g) at two years old, were recorded.

Microsatellite DNA: Five primers producing microsatellite bands of OTSG3, OTS100, OTSG249, OMYF, OTSG474 were selected for analysis the microsatellite locus. The fin samples of 2 to 3 g were stored in 1.5 ml Eppendorf tubes with 96% ethanol. Total genomic DNA extracted by phenol-chloroform [6]. The quality and quantity of DNA were assessed by 1% agarose gel electrophoresis and spectrophotometer (model Cecil DE2040) and then stored at -20°C till used.

The quality of extracted DNA was checked by TBE buffer on 0.1% agaros gel [7]. Polymerase chain reaction (PCR) conditions, especially the annealing temperatures, for each of the primer sets were optimized. The PCR reactions were performed in a 25 µl reaction volume containing 100 ng of the template DNA, 0.5 to 1pµ of primer, 200 mM of dNTPs, 1U *Taq* DNA polymerase, 1.5 µl reaction buffer (10X), 1 to 2.5 mM MgCl2 and distilled water to final volume of 25 µl. The samples were subjected to an initial denaturation step at 95°C for 3 min, followed by 30 cycles of 95°C for 30 s, at annealing temperature for 45s, 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products were electro-phoresed on 8% polyacrylamide gel [7] at 150V for 3h with ladder marker pBR322 DNA/AluI Marker, 20, MBI Fermentas) and the DNA fragments were stained by silver nitrate.

Three micro litter of the PCR product was loaded with gel-loading buffer [8]. Gel was run for 3h until front bands reached the end of the gel. After electrophoresis, the DNA fragments were visualized by silver staining [9].

Data Analysis: Microsatellite alleles were sized by using UVI DOC Version V.99.04 software. In order to calculate allelic and genotype frequencies, observed (Ho) and expected (He) heterozygosity, deviations from Hardy-Weinberg expectations (HWE), Fst value, analysis of molecular variance (AMOVA) were conducted by using the GenAlex 6.2 software [10]. The genetic distance and the genetic identity between the populations was estimated from Nei standard genetic distance and genetic similarity index [11], unweighted pair-group mean analysis (UPGMA) computed in TFPGA version 1.3 and the presence of null alleles was checked by using Microcheker (Version 2.2.3).

RESULTS

A total of 46 alleles were observed in all studied samples across the 5 microsatellite loci, ranging from three alleles at Otsg474 to 12 alleles at Otsg100 (Table 1). Partition of farmed strains according to the origin of introducing the allele numbers of 34 for Norway strains, 27 for strains from Turkish and only 26 for the French strains. These differences in variability were observed also in allelic frequency (Table 2). The highest number of alleles was observed in French strain with some of them occurring at very high frequency, e.g. at locus OTSG249, occurred with a frequency 0.545, while 0.333 and 0.254 in Turkish and Norway strain, respectively. Both Norway and French populations shared alleles 168, 176 and 189 at locus Otsg100 which were not observed in Turkish stain. Significant deviations from Hardy-Weinberg equilibrium (Pb=0.05) at least in one locus were observed in three strains (Table 3) which are most probably due to the use of limited number of breeders and/or unequal sex ratio.

The genetic variability (as estimated by the observed heterozygosity, H_O) of N1 that are farmed in Iran was similar and did not differ significantly from the strains of F1 and T1 as well: H_O ranged from 0.015 (OTSG474) to 0.864 (OMYF) in Norway and French strain that results in high standard deviation in contrast to the Turkish strains

Table 1: Characteristics of the studied microsatellite loci (A-number of observed alleles, H_o-average observed heterozygosity, H_e-average expected heterozygosity; Fis -inbreeding coefficient and pair-wise Fst and Fit values

Locus	Size range (bp)	Sequence of the forward (F) and reverse (R)	A	Ho	He	Fis	Fit	Fst	Nm
Otsg3	142-158	F-GGGTTGAGTAGGGAGGCTTG	4	0.142	0.327	0.215	0.396	0.311	1.505
		R-TGGCAGGGATTTGACATAAC							
Otsg100	115-197	F-AGGTGGGTCCTCCAAGCTAC	12	0658	0.075	0.029	0.075	0.048	8.470
		R-ACCCGCTCCTCACTTAATC							
Otsg249	115-191	F-TAGTGTTCCGTGTTCGCCTG	10	0.741	0.742	0.089	0.089	0.001	2.569
		R-ACCTTCCATCTCTCATTCCAC							
Omyf	149-197	F-CCAGCAGTAAACCTTAGGTTG	7	0.756	0.791	0.014	0.058	0.044	18.022
		R-GTCAAAGGAGACGTAGAGCTT							
OTSG474	156-164	F-CCTGTGAGTGTTAATTCGACCTGT	3	0.005	0.010	0.008	0.058	0.494	32.10
		R-GCCAAATACTTTCGCAAGG							

Table 2: Allele Frequencies by strains

Locus	Allele	N1	F1	T1	Locus	Allele	N1	F1	T1
OTSG3	1	0.069	0.295	0.381		6	0.031	0.091	0.024
	2	0.000	0.000	0.024		7	0.231	0.114	0.214
	3	0.931	0.705	0.429		8	0.008	0.000	0.071
	4	0.000	0.000	0.167		9	0.000	0.250	0.071
OTS100	1	0.031	0.000	0.000		10	0.000	0.000	0.024
	2	0.008	0.045	0.024		11	0.000	0.000	0.071
	3	0.215	0.091	0.143		12	0.031	0.000	0.000
	4	0.246	0.023	0.119	OMYF	1	0.038	0.091	0.024
	5	0.000	0.114	0.000		2	0.338	0.273	0.310
	6	0.085	0.068	0.119		3	0.262	0.114	0.238
	7	0.031	0.114	0.000		4	0.077	0.182	0.167
	8	0.008	0.068	0.095		5	0.169	0.182	0.190
	9	0.377	0.477	0.500		6	0.077	0.023	0.048
OTSG249	1	0.085	0.000	0.000		7	0.038	0.136	0.024
	2	0.146	0.000	0.333	OTSG474	1	0.008	0.000	0.000
	3	0.208	0.000	0.024		2	0.985	1.000	1.000
	4	0.254	0.545	0.167		3	0.008	0.000	0.000
	5	0.008	0.000	0.000					

Table 3: Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium

Locus	T1	F1	N1
OTSG3	*	ns	ns
OTS100	*	*	***
OTSG249	ns	ns	***
OMYF	ns	**	***
OTSG 474	Monomorphic	Monomorphic	***

Table 4: Genetic variability within the studied rainbow trout strains: sample size (n), totalof alleles (A), expected (H_e) and observed (H_o) heterozygosity, Real (Na) and Effective number of allele (Ne)

Strain	n	A	Ho	He	Na	Ne
F1	30	26	0.53(0.36)	O51(0.32)	4.4(3.0)	2.9(1.8)
N1	60	34	0.49(0.38)	050(0.38)	5.8(3.1)	3.1(1.9)
T1	30	27	0.49(0.28)	0.58(0.33)	5.4(3.0)	3.3(1.6)

Table 5: Pairwise Population Fst Values and Estimates of Nm and Matrix of Nei Genetic Distance

Strain1	Strain2	Fst	Nm	Nei
N1	F1	0.076	3.040	0.105
N1	T1	0.080	2.883	0.120
F1	T1	0.062	3.788	0.115

had lower estimates of variability (Table 4). Differences between allele frequencies across all loci were highly significant for all population pairs (Pb 0.001). The level of differentiation among the farmed rainbow trout strains was moderate (average F_{ST}=0.072) and ranged from 0.062, between Norway and French strain to 0.080, between Norway and Turky strain (Table 5). Pairwise Population

Estimates of Nm and Matrix of Nei Genetic Distance also indicate of moderate genetic distance between the strains (Table 5).

DISCUSSION

The genetic variation in the rainbow trout strains that are farmed in Iran, introduced from European countries did not differ significantly between the countries. The analysis of molecular variation reviled high variability among loci used in present work. We used a variety of microsatellite that previously have been used for common carp and Salmonids [12-14]. In spite of using different microsatellite loci The results showed the same obtained by Ward *et al* please rewrite. [15], investigated microsatellite diversity in rainbow trout introduced to Western Australia.

The number of microsatellite alleles observed in rainbow trout of Norway was very similar to that observed in French and Turkey for similar sample sizes but almost for all samples presented at least one specific allele. These specificities at some loci represent a useful tool for further evolutionary studies, selection program and identical purposes.

The average number of allele in all strain of the present study was 5.8 and average observed heterozygosity of 0.50. In previous Genetic variability and differentiation of rainbow trout strains in Northern and Eastern Europe and two wild populations from Canada and a farmed strain from USA have been studied [16] using 10 microsatellite loci. In majority of European strains, the average number of allele ranged from 5.4 to 7 and observed heterozygosity ranged from 0.6 to 0.74 was similar between the European country with the exception of two Polish strains and wild Canadian populations which exhibited significantly lower variability (average allele:3.7, 4.5 Heterosigosity: 0.47, 0.45 respectively). Reduce allelic number and heterosigosity in our work may be is due small number of sample size, elevation level of inbreeding, differences of primer used, origin of sample fish. Small effective population sizes and poorly managed breeding programs are generally blamed for loss of genetic diversity in farmed stocks. Cross *et al.* [17] demonstrated that, when measures are taken to avoid inbreeding as part of a breeding program, genetic variation need not be reduced despite small effective population sizes.

These results also show that allelic diversity is a more sensitive measure of differences in genetic variation. This results is in agreement with those previously observed in hatchery salmon and farmed rainbow trout [18,19]. This is likely to be a result of the loss of low frequency alleles when new populations are created from larger founder ones. Differences in genetic variation observed between farmed stocks also may be a result of different breeding regimes and population sizes [5].

Comparing average number of alleles per locus and observed heterosigocity in the present work with those data assessed variation among three domesticated strains of rainbow trout in USA at nine microsatellite loci and found an average of 14 alleles and 0.72 heterozygosity [20], we can accept that the strains in our study are significantly less variable than those reported of domesticated strains in USA. A heterozygote deficiency also can be attributable to other phenomena including population admixture or the presence of a nonexpressed allele. Reduced genetic variability is characteristic also for domesticated/captive stocks as compared to wild populations (e.g. in common carp, 21 & 22). In another example farmed salmon showed less genetic variability than wild salmon in terms of allelic [5]. A number of studies of genetic variation in wild and farmed strains of Atlantic salmon have demonstrated a significant difference between allele frequencies of farmed and wild Atlantic salmon and a reduction in genetic variation in the reared strains [23].

One of the most parameter in study of genetic variation is the use of Fst value. The average level of differentiation among the three European strains in our study (Fst=0.073) is comparable with that of the three domesticated strains in USA (Fst=0.09; 20), but less than among northern and Eastern European countries (Fst=0.14; 16).

In total the limitation in number of alleles, in all 3 strains, the level of variability, measured as the average allele observed heterozygosity, was similar to each other while that of Norway exhibited markedly higher variability and decrease level of inbreeding. Only 8% of the total genetic variation in farmed strains was accounted for differences between the countries of origin. To illustrate the exact and more correctly the differences it is also recommended using morphological parameter [24] and biochemical differences [25] of these fish.

CONCLUSION

In spite of very few differences detected between the genetics of rainbow trout stocks of different origin, yet stocks of French fish perform better than the others. It

seems due to specific program some locus related to growth performance have been selected and pronounced in French fishes. The presence of some allele in French rainbow trout and lack in others confirm this clam. For further understanding more study with microsatellite techniques and higher number of primer should be done to find the correlation and linkage between genes and growth performance.

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