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Full Length Article

Characterization of TCR ß-chain Locus genotypes of rainbow trout (*Oncorhynchusmykiss*) by SSCP analysis

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ABSTRACT
T cells play important regulatory roles in the immune responses of jawed vertebrates. In the present study T cell receptor (TCR) coding genes of the Rainbow trout (Oncorhynchusmykiss) as a main aquaculture species of Iranian cold waters was analyzed. So five farms of this species were selected in Harazriver and 50 fin sampleswere collected and screened for TCR ß chain locus with the single strand conformation analysis method. Results of this research indicated limited alleles number (3-6 alleles) with higher observed heterozygosity (75%) than expected heterozygosity (68%). In addition, results imply the effect of aquaculture farms effluent that increases the pollution of pathogens and abiotic materials with increase of allele numbers in next farms. These data could be very useful in immunogenetic assessment of aquaculture farms.
Key words:TCR ß-chain, Locus genotypes, rainbow trout, Oncorhynchusmykiss, SSCP analysis

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INTRODUCTION

T cells play important regulatory roles in the immune responses of jawed vertebrates. In mammals, TCRs are heterodimeric that consisting of either α/ß or γ/δ polypeptide combinations. The T cells are active when the heterodimer TCR on the surface of a T cell, specifically recognizes an externally presented Ag, in conjunction with the CD3 receptor complex [5]. The structure and expression of genes encoding molecules homologous to mammalian αß TCR have been identified from almost all vertebrate classes, including birds [6], amphibians (Chretien et al, 1997), teleosts[3], and elasmobranches [2]. However, γδTCR homologues have not been identified in teleosts. Even though, the molecular structure of TCR proteins, presumed from their cDNA sequences, is appropriately conserved in all vertebrates, although there is very little information on the genomic organization and the genetic loci that encode the TCR molecules in primitive vertebrates. With increasing the importance of aquaculture production and immunity considerations, the structural and genomic organization of TCR ß chain locus have been analyzed in different fish species such as horned shark [2] and the rainbow trout [1]. Hence, the TCR α locus has been characterized in Japanese puffer fish (*Takifugurubripes*) with cosmidcolnes[4].
Several methods are available for genetic analysis of the polymorphic protein coding loci including direct sequencing, restriction fragment length polymorphism (RFLP) analysis, denaturing gradient gel electrophoresis (DGGE), reference strand–mediated conformational analysis (RSCA), and single-stranded conformation polymorphism (SSCP) analysis. As, T cells with MHC molecules have very important role in innate immunity and pathogen identification and there is complementary process between them, higher diversity of these receptors buffer the species against disease. Therefore, it is critical to characterize genomic structure and diversity of TCR genes. The aim of this study is to analysis the genotype diversity of TCR ß locus of the main cold-water aquaculture species, rainbow trout (*O. mykiss*), with the SSCP method.

MATERIAL AND METHOD

Genomic DNA was extracted from the fin clips of 50 rainbow trout brood stocks of randomly selected three farms located in Harazriver, IRAN (figure 1). Alcohol preserved samples was used for DNA extraction according to standard phenol-chloroform procedures (Sambrook et al, 1989). Quality and

quantity of purified genomic DNA assessed by 1 percent agarose gel and nanodrop (Thermo 2000), respectively. High quality DNA samples selected for PCR amplification.

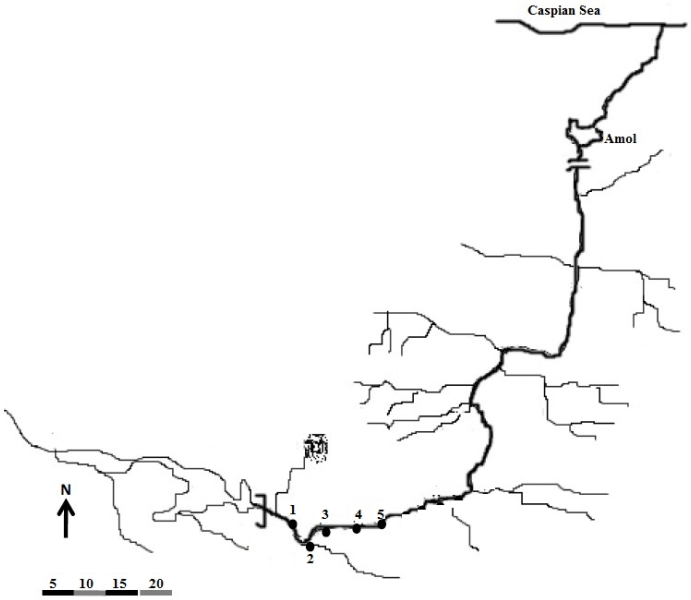

Figure 1- location of sampled aquaculture farms of rainbow trout (*O.mykiss*) in haraz river, Iran.

For PCR amplification of TCR ß chain locus, the specific primers (F: 5- GACTCTGTGACTGAGCCACA-3 ; R: 5- GAGACGAATACGAGCCGT-3) have been used[8]. PCR reaction was performed in a total volume of 25 ul containing approximately 100-50 nggDNA, 1.25ul of 10X PCR buffer, 1.5 mM MgCl2, 200 mMdNTPs, 200 nM of each primer and 1 unit of Taq polymerase. Amplification was performed in a BIOER thermal cycler using the following cycling profile: 10 min at 95°C, 35 cycles of 30s at 94°C, 30s at 64°C and 30s at 72°C, and a final extension step for 5 min at 72°C. Results of the amplifications were visualised in 2% agarose gel electrophoresis and was stained with 0.5 ug/ulethidium bromide. For genotyping, we use Single Strand Conformation Polymorphism (SSCP) method according to method of Sunnucks et al (2000). SSCP pattern analyzed by Gel Scannr program (Version 6) and genotype data export to POPGENE program (ver, 1.32) for further population statistic analysis such as allele frequency, heterozygosity.

RESULT AND DISCUSSION

Result of TCP ß chain locus amplification indicated in figure 2 with specific product that optimized for next step of SSCP genotype analysis. The SSCP method as a PCR-based technique allows amplification and separation of single-strand DNA fragments, representing both alleles at a single locus in an individual. Alleleswere scored according to the order in which they had migrated down the gel and represented alphabetically.

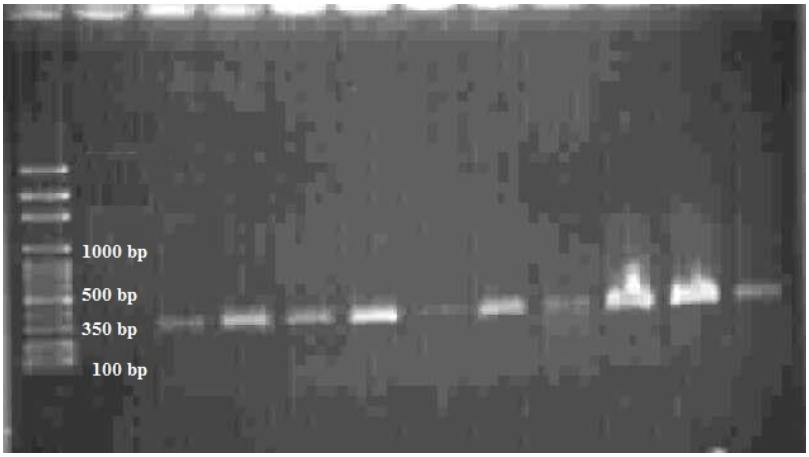

Figure 1- PCR product of TCR α chain locus of rainbow trout (*O. mykiss*)

In addition, the homozygotes and heterozygotes were identified and recorded. Since SSCP analysis yields two bands for homozygotes and four bands for heterozygotes, only the lower set of bands were used for

scoring genotypes (Figure 2). The genotype and alleles frequency criteria for SSCP analysis are presented in Table 1.

Figure 2- SSCP patterns of TCR α chain locus of rainbow trout (*O. mykiss*)

Result of TCP ß chain screen indicated, rainbow trout farms have limited diversity with3-6 alleles. As, this gene has very important immune-competency function, this condition could increase sensitivity to diseases and stress. Also, result of this study imply the effect of aquaculture farms effluent that increase the pollution of biotic pathogens such as bacteria and abiotic materials with increase of allele numbers in next farms (farms 4 and 5 have more alleles). Calculated observe and expected heterozygositywas 75% and 68% respectively.The immune system genes are under the balancing selection that increase the heterozygosity in a population, so decrease the expected heterozygosity in these farms are result of low alleles diversity.These results could be very useful in immunogenetic assessment of aquaculture farms.

Table 1- Allele frequency of TCP chain locus of rainbow trout (*O.mykiss*). N: allele number, K: alleles richness, Ho: observed Heterozygosity, He: expected Heterozygosity

Farms	1	2	3	4	5	Total
SSCP alleles						
TCPα001	0.12	0.25	0	0.14	0.6	0.114
TCPα002	0.54	0	0.50	0.65	0.24	0.386
TCPα003	0	0.62	0.35	0	0.15	0.224
TCPα004	0.34	0.05	0	0.8	0.31	0.156
TCP^b005	0	0	0.15	0.13	0.10	0.076
TCPα006	0	0.8	0	0	0.14	0.044
n	3	4	3	4	6	6
A	2.21	1.98	2.54	3.11	4.9	2.94
Ho (%)	70	72	64	78	85	75
He (%)	68	66	68	64	73	68

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