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Studies of cytochrome b protein modelling sequence in the *Salmo trutta fario* and comparing with other *salmonids*

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ABSTRACT

The present study represents a preliminary analysis of the cytochrome b protein sequences in the *salmo trutta fario*, *salmo trutta caspius*, *salmo trutta trutta* and *salmo salar* has been determined. The cytochrome b gene consisting 1141base pairs that encoding 380 amino acids and has been five origins ORF sites from nucleotides number of 125 to 718 which placed number of 72, 40, 37, 67, 32, respectively from first to end of gene. The amino acids sequences were compared with those of other salmonids such as *salmo salar, salmo trutta* and *salmo trutta caspius*, in this regards, the sequences aligned by DNAMAN program computer, results are shown regards *salmo salar, salmo trutta trutta* has 380 amino acids and same homology almost 99% but regards *salmo truttta caspius*, has 78% homology. There were more variation from 20 first amino acids than other parts of segments, however there is not full length of cytochrome b gene in s*amlo trutta caspius*. The sequence features of the 3 RD structure protein accessed and compared between species of *salmonids*, The results are shown there are same homology between structures of protein.

Keywords: *Salmo trutta fario*, sequencing, protein, cytochrome b gene

INTRODUCTION

The *salmonid* fishes are one of the best studied native in the world. At present there are major studies on the phylogenetic *salmonid* population in Norway, Chile, Canada, Scotland (Bernatchez *et al*., 1992), Iran (Rezaei *et al*., 2011, a, b,) and elsewhere). In related to, *salmonid* fishes also are commercially important and the farming of these fish represents a major economics in the world. In Iran *Salmo trutta fario* and *salmo trutta caspius* is widely spread in a large number of water streams from the north of Iran, also they are living in South of Caspian Sea that those are endemic in Caspian Sea, these fishes for reproduction migrates to rivers connected to Sea. Vertebrate mtDNA is a circular genome approximately 16 kb in size in most taxa and contains 37 genes that encode 13 proteins, 22 tRNAs, and 2 rRNAs, compared to other animals and plants, mtDNA among vertebrates is unique in that it lacks introns, consequently genes are tightly packed together(Bernatchez *et al*., 1992). Recently, have been found the complete sequence of mitochondrial DNA (mtDNA) in human genomic by (Anderson *et al*., 1981), also, was found the complete sequence in other animals, fungi, plant and other vertebrates and invertebrates (Wolstenholme, 1992). Regards fishes, Wilhelm *et al*., had reported that 67 sequences deposited in GeneBank, ((NCBI: www.ncbi.nlm.nih.gov/PMGifs/

Genomes/ 7898.html). Mitochondrial DNA is great interest parameter for identification, conservation and population genetics studies ((Phillips and Oakley, 1997). To investigate and characterized the genetic differentiation and phylogenetic relationships among between salmonid population at the mtDNA level. However the previously were amplified and has been analyzed for other genetic techniques including, RFLP, and allozymes as well. (Karakousis & Triantaphyllidis, 1990; Apostolidis *et al*., 1996; (Avise, 1994; Gyllenstein & Wilson, 1987; Hynes *et al*., 1989; Bembo *et al*., 1994; Hall & Nawrocki, 1995), but also was followed by the sequence of mtDNA of salmonids, including, cytochrome c oxidase (three subunits), 16S rRNA, 12S rRNA, ND(six subunits) and cytochrome b. Regards cytochrome b were found sequence of protein in *salmo salar*,(Hurset *et al*., 1999, Accession number:U12143) *salmo trutta caspius* (Jamshidi and Kalbasi, 2009, Accession number: ACN25134), *salmo trutta trutta* (Duc *et al*., 2007, Accession number: YP-001568983.1) and *salmo trutta fario* (Hao *et al*., 2006, Accession number: ABD52296). Cytochrome b protein in full length has 380 amino acids that including one exon and without intron in full length. Cytochrome b however is important in the respiratory system of cell, has major function belong to marker genetics and studies of population in *solmonids* (Bernatchez *et al*., 1992). In this study we examined the codon site of protein coding gene of the cytochrome b to identify what role natural selection may have played in divergence of the cytochrome b encoded protein among *salmonids*, what changes, if any, correlate with specific structures within the cytochrome b protein in other *salmonids*. In related to was found 380 amino acids from length of cytochrome b in *salmo trutta fario*, that also same number and has high homology with other *salmonids* reported in GeneBank system.

MATERIALS AND METHODS

The *salmo trutta fario* samples:

The samples of *Salmo trutta fario* has taken on the July 2011 from the Rivers of Tonekabon- Iran, these samples including Bloods and Muscles has taken from three old age females.

Extraction of DNA:

Genomic DNA was isolated from blood samples following the phenol-chloroform extraction method described by Sambrook (1989). Following this method, first, after adding proteinase k to phenol-chloroform reaction, was segregated upper supernatant solution, then added Ethanol 95% for precipitation of DNA genomic, then, DNA was dissolved in TE buffer and was kept in a water bath at 60°C for 2 h to dissolve pellet properly in buffer. The quality of DNA also was checked through spectrophotometry instrument. DNA samples with O.D. ratio between 1.7 and 1.9 were considered and also the wave length of 260/280 as good and used for further study. The samples also for getting more DNA were re-extracted by the phenol-chloroform extraction method. Then DNA quality was loaded on the gel 1.5 percent agarose for observation quality of band. The DNA samples devoid of smear were used for further study.

The PCR reaction:

A 1191 bp from cytochrome b gene in the *salmo trutta fario* by Forward and Reverse primers that were designed by DNAMAN program genetics and blast Network system in NCBI was amplified. These primers including, Forward Primer 5' GAC TTG AAA AAC CAC CGT TG 3'. Reverse Primer 5' CTC CGA TCT CCG GAT TAC AAG AC 3', these primers also were used for sequencing of the cytochrome b gene. For providing PCR reaction in volume of 5o µl, we used, 100 ng DNA template , 2.5 µl, 10X sigma PCR buffer, 2.0 µl dNTP mix (2.5mM each), 1.0 µl Forward primer (25pm/ µl), 1 µl Reverse primer (25pm/ µl), 0.25µl Sigma Taq DNA polymerase enzyme (5U/ µl). 17.5 µl. DNA nuclease free water. After that, the PCR-reactions were carried out in a thermocycler in two stages. In the first stage, initial denaturation at 95°C for 10 minutes, denaturation at 95°C for 1 minute,

annealing at 55°C for 1 minute, extension time at 72°C for 1 minute and 10 minute for 35 cycles.

The Sequencing project:

The PCR products were concentrated to50 ng/µl by pooling several tubes to precipitate by the isopropanol procedure. In order to obtain clean fragment for sequencing, the PCR products were separated by electrophoresis in a TAE agarose gel containing ethidium bromide using standard protocols (Sambrook *et al*., 1989). The desired PCR product band was excised using a clean, sterile razor blade or scalpel (band was visualized in a medium or long wavelength (e.g., ≥300 nm) UV light, and excised quickly to minimize exposure of the DNA to UV light). The minimum agarose slice was transferred to a 1.5 ml micro centrifuge or screw cap tube and then purified by using commercially available gel extraction kits (Qiagen). Quantification was done by loading one µl of eluted sample in 1.5% agarose gel and comparing with standard molecular marker (Phi X 174 DNA ladder or 500 bp DNA ladder-). Only samples with good concentration (>50 ng/µl) were selected and subjected to sequencing. Regards the sequencing, Products of PCR reaction sequenced directly using either the Thenosequenase sequencing kits (Amersham United States Biochemicals). Sequencing, electrophoresis and autoradiography were performed according to the manufacturer's instructions.

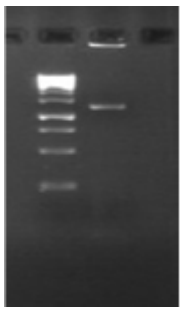
Deposited of DNA sequence in GeneBank:

The cytochrome b gene was sequenced and deposited in GeneBank by accession number (JN995186.1). The sequence of cytochrome b gene analyzed by DNAMAN program and transferred to Protein structure. The protein structures also analyzed by jigsaw program protein and compared with other structures proteins from other *salmonids* that reported in GeneBank- NCBI Network system.

RESULTS

PCR products:

The PCR product amplified and full length of gene cytochrome b (1191bp.) was runned and put on the 1.5 percent gel electrophoresis. After getting bands, the gel was take photo by Gel DOC instrument. (USA company) and are shown in (Fig.1).



M A

Fig. 1: PCR amplification of high quality *salmo trutta fario* using the primers of designed Samples were separated by electrophoresis in 1.5% gel electrophoresis. **A**: DNA amplified 1191bp. **M.** Size marker 1000bp.

Variable of nucleotide and amino acid sequences:

Polymorphism of the genotype of *salmo trutta fario* cytochrome b was confirmed by nucleotide sequencing. The nucleotide sequence as well as the derived amino acid sequence of cytochrome b gene of *salmo trutta fario* by DNAMAN program computer. Nucleotide sequence variations and amino acid variations were observed at the cytochrome b protein between *salmo trutta caspius* , *salmo salar* and *salmo trutta trutta* reveals almost 98 to 99 % nucleotide identity. (Fig. 2).

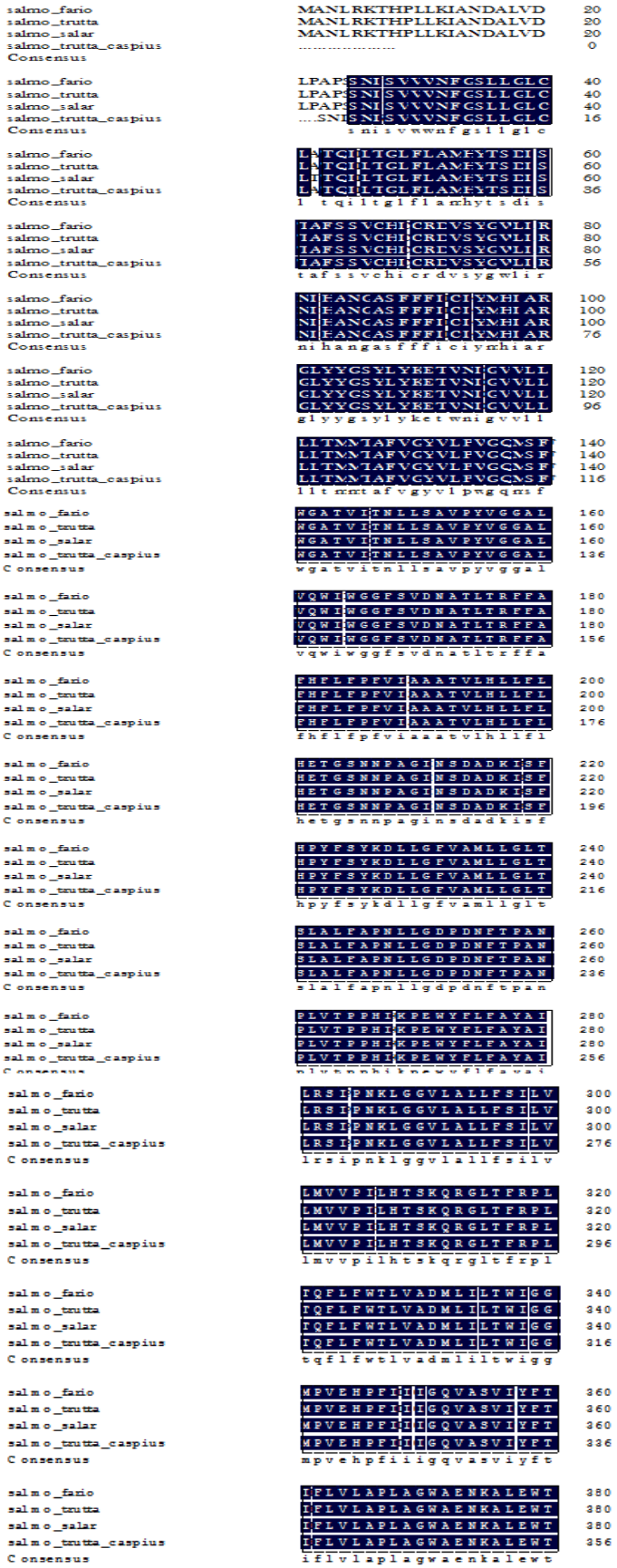


Fig. 2: Sequence alignment of cytochrome b protein for four important species of salmons The amino acids in gray color bold and white bold background The gray background color amino acids were same homology between *salmonids*, but there were some single nucleotides that different homology.

Prediction of Three-dimensional protein structure:

However, the number of ways that a protein can fold appears to be limited, there is considerable optimum that it will be possible to predict the fold of any protein, just given its amino acid sequence, structural alignment studies have revealed on the *salmo trutta fario*. In threading, the amino acid sequence of a cytochrome b protein is examined for compatibility with the structural of a known protein structure in other salmonids, including, *salmo trutta trutta, salmo salar* and *salmo trutta caspius*. In related to we observed from protein core is made up of α-helix, β-strands and other structural elements folded into a protein. In this regards, Twenty-four nucleotide sites (from 1 to 26) were variable among the four species examined. (Figs. 3,4,5). Also single mutation including, Threonine (T) and Alanine (A) occurred at nucleotide 42 for *salmo trutta trutta, salmo salar* and position of 18 in *salmo trutta caspius,* in fact these mutations are in first part of length. Regards third type of structure that called tertiary protein structure, there is a variety of bonding interactions between the chains that occurred on the amino acids, however these chains may be stronger than hydrogen bonds between side chains. In general, there is a high homology of sequences at the nucleotide and amino acid level when they are compared with other *salmonids* species.

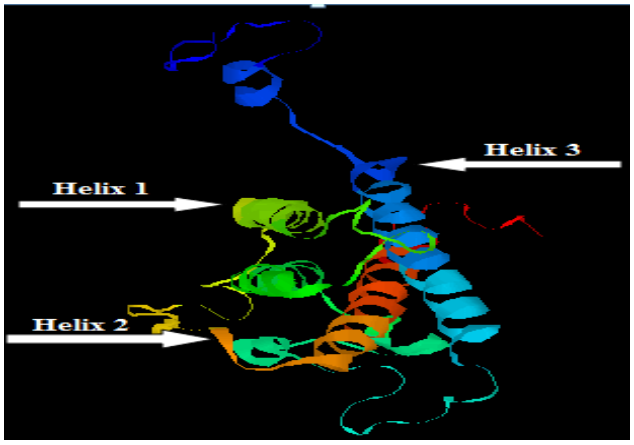


Fig. 3:3-dimensional crystal structure of Complex I from *salmo trutta fario*. The chains side compared, almost 70 percent from amino acids are involved in α-helix formation, which run anti-parallel to each other. These helices attribute to the typical 3- α- helix bundle protein conformation, the characteristic 3-D conformation of cytochrome b protein.

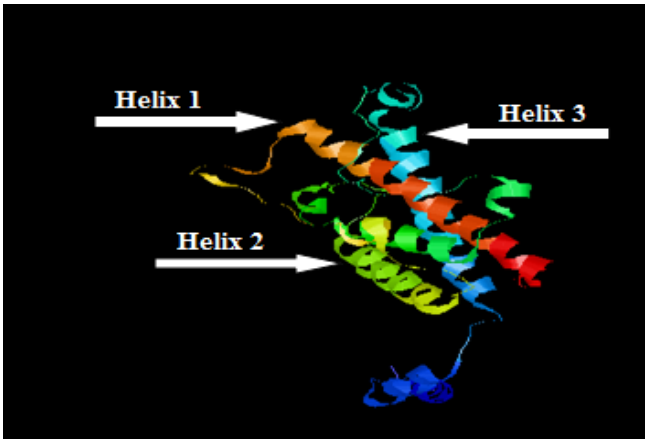


Fig. 4: 3-dimensional crystal structure of Complex I from *salmo trutta trutta*, The chains side compared, almost 70 percent from amino acids are involved in α-helix formation, which run anti-parallel to each other. These helices attribute to the typical 3- α- helix bundle protein conformation, the characteristic 3-D conformation of cytochrome b protein.

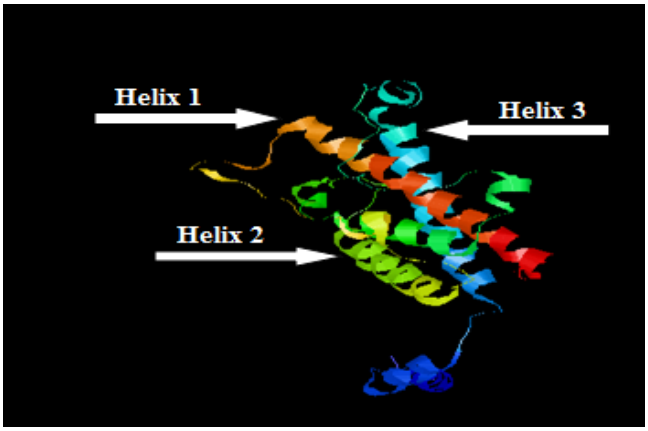


Fig. 5: 3-dimensional crystal structure of Complex I from *salmo salar*, The chains side compared, almost 70 percent from amino acids are involved in α-helix formation. Which run anti-parallel to each other. These helices attribute to the typical 3- α- helix bundle protein conformation, the characteristic 3-D conformation of cytochrome b protein.

DISCUSSION

Cytochrome b gene in Salmons:

Salmo trutta fario present some interested for biological characteristics for the study of genetic differentiation, they live in the upper part of rivers, and exhibit homing behavior. Therefore the different populations are relatively isolated but can be connected by migration (Aurelle *et al*., 1998). I the last decade was studied regards genetic variation by allozymes. (Fergusen 1989; Guyomond 1981 and 1989; Hamilton 1989; Pateaux 1995; Garcia-Marin and Pla 1996). Growth hormone gene marker (Rezaei *et al*., 2011; Gross *et al*., 1999 and 1995), that determined paternal traits in *salmonids*, also, mitochondrial genomic (Bernatchez *et al*., 1992) and microsatellite markers (Aurreli and Berrebi, 1998). All of those markers confirmed the rate of variation in salmonids. Following the discovery of cytochrome b genes in the salmonids (Hurst *et al*., 1999; Rezaei *et al*., 2011), the hypothesis of an ancestral mitochondrial genome in bony fishes gained rapid popularity (Bernatchez *et al*., 1992). This is the first study that analyzed the coding sequences in the cytochrome b protein, contains 380 bp. in the length. In this regards, *salmo trutta trutta, salmo salar* had been same homology with *salmo trutta fario*, there is one mutation in amino acid number of 42 between *salmo trutta trutta* and *salmo trutta fario* (Alanine) with *salmo salar* (Threonine). Also there were variation between first 20 amino acids between salmonids and other segment of protein there were not any variation.

The third structure of protein of cytochrome b in *Salmo trutta caspius* and other Salmons:

In this study were analyzed the protein structures of the cytochrome b gene from *Salmo trutta fario* with *Salmo salar* and *salmo trutta trutta.* Regards *salmo trutta caspius* there were not complete sequence in GeneBank, so we didn't compare with *salmo trutta fario*. 3D-structure of the *Salmo trutta fario* cytochrome b protein is some variation with *Salmo salar* and *salmo trutta trutta*. In this regards, in all the known crystallographic and theoretical cytochrome b structures, more than 70% of the residues are involved in the formation *a*-helix in anti-parallel twisted helical bundle. Sequence comparison indicates that the *a*-helical regions are more conserved than other parts of the molecule, suggesting the possibility of similar conformation in all the other proteins of the cytochrome b family. The cytochrome b in three species, *salmo trutta trutta*, *salmo salar* and *salmo trutta fario* had been three chains helix, but the shape of structure between *salmo trutta fario* was different with *slmo trutta trutta* and *salmo salar* however the results were shown that there were high homology between *salmo salar* and *salmo trutta trutta* more than *salmo trutta fario*. Regards the conformation of Cysteine residues 10 numbers, however the cytochrome had maternal traits, regards paternal traits there were five Cysteine residue in growth hormone protein in the *salmo salar* (Gross *et al*., 1995 and Gross *et al*., 1999), *salmo trutta caspius* (Rezaei *et al*., 2011)and *salmo trutta trutta* (Agelon 1998). Regards Open Reading Frame,(ORF sites)The *salmo trutta fario* cytochrome b protein contains 5 fragments Open reading frames, that started from nucleotide 125 is methionine and ended to nucleotide of 718 that started codon methionine from position of 135 that was stop codons contains, TAG, TAA, TGA, in the length (Fig. 6).

Strand	RF	AA Num	Position	Sequence
Plus	2	72	125-343	aacttgaccgATGatgat...ttgcgTAGgcgaa
Plus	3	40	615-737	gcaataacgaATGggaat...gtttgTAAttaca
Plus	1	37	883-996	ctcgggcgatATGtatat...gctgtTGAgatat
Minus	3	67	420-623	acgttcttccATGaggac...ttacaTGAaaccg
Minus	2	32	620-718	ctatttttacATGaaacc...attcgTAGctata

Fig. 6: Open Reading Frame (ORF) nucleotides in the cytochrome b gene in *salmo trutta fario.* There are five segments ORF. Was started from nucleotide of 125.

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