Phylogenetic analysis of NADH dehydrogenase subunit 1 (NADH 1) gene in Salmo trutta caspius

Abolhasan Rezaei

Abstract
The NADH 1 gene in the Salmo trutta caspius has been sequenced, characterized and deposited in Genbank, Accession Number LC011387. In this study we compared NADH 1 gene between Salmo trutta caspius, Salmo trutta trutta, accession number (AM910409.1), Salmo salar (KF792729.1) and Oncorhynchus mykiss (DQ288268.1) by DNAMAN program and NCBI Network system separately. According to DNAMAN program analysis, we found 23 SNPs and 98% similarity between Salmo trutta caspius and Salmo trutta trutta, 67 SNPs and 92% similarity between Salmo trutta caspius and Salmo salar, 126 SNPs and 86% similarity between Salmo trutta caspius and Oncorhynchus mykiss. Amino acid sequences also were determined by DNAMAN program. Between Salmo trutta caspius and Salmo trutta trutta, 3 SNPs, between Salmo trutta caspius and Salmo salar 14 SNPs, and between Salmo trutta caspius and Oncorhynchus mykiss, 17 SNPs were observed. In this study we concluded that the rate of homology between Salmo trutta trutta and Salmo trutta caspius is more than its homology with Salmo salar and Oncorhynchus mykiss, so these results show that Salmo trutta caspius had originated from Salmo trutta.

Keywords: Salmo trutta caspius, Salmo salar, Salmo trutta, Oncorhynchus mykiss, NADH 1 gene

1. Introduction
NADH dehydrogenase (ubiquinone) is an enzyme of the respiratory chains in myriad of organisms from bacteria to humans. It catalyzes the transfer of electrons from NADH to its coenzyme and, in eukaryotes, it is located in the inner mitochondrial membrane. NADH 1 gene was investigated and characterized in Hucho populations [27]. They used two mitochondrial loci (control region and NADH 1) in 47 Huchen samples to study genetic diversity and phylogeographic structure of Huchen populations. Neave, 1958 [12] proposed that the common ancestor of rainbow trout was the first to diverge from the protoOncorhynchus evolutionary line, which then radiated to form the seven extant Pacific salmon species. They found a relationship between brown trout and Oncorhynchus mykiss when compared different subunits of NADH genes and growth hormone genes [14], using polymerase chain reaction, reported that two mtDNA loci, cytochrome and NADH 1, were amplified in 14 individuals. A similar comparison with the NADH 1 sequences revealed a similar ratio in rainbow trout, chum, sockeye and Salmo salar. Generally, NADH in mitochondria of Salmons, especially Salmo trutta caspius has seven subunits (NADH 1, NADH 2, NADH 3, NADH 4, NADH 5 and NADH 6), which have different size in full length. The full length of NADH 1 gene in Salmo trutta, Salmo salar and Oncorhynchus mykiss was amplified, sequenced and deposited in GenBank (NCBI-Network system). According to their sequences, NADH 1 have been approximately 970-1000 bp in full length. To compare the phylogeny in brown trout, three methods were used to compare NADH 1 gene in Salmo salar and Oncorhynchus mykiss (parsimony, maximum likelihood, and neighbour-joining distance analyses) which found a close relationship between them. mtDNA NADH 3 gene region is useful as a genetic marker for stock identification and phylogeographic study because NADH 3 gene is conserved across all organisms and has a fast rate of sequence evolution [10, 28]. Salmo trutta caspius was originated from south of Caspian Sea, Iran. They are on the IUCN Red List of Threatened Species. Therefore, studies on the Salmo trutta caspius are very important. The aim of this research was to study the phylogenetic of Salmo trutta caspius by comparing its NADH 1 gene with other Salmonids. NADH 1 gene was suitable for finding the relationships within Salmo trutta caspius species and between Salmo trutta caspius and other species of Salmonids such as Salmo trutta, Salmo salar and Oncorhynchus mykiss.
2. Materials and Methods
2.1 Fish samples and DNA extraction
In June of 2014, blood samples were excised from Salmo trutta caspius collected from the rivers of Tonekabon, Iran, for a total of 30 fish including male and female samples that were three years old. Samples were stored at -80°C, or kept in 80% ethanol at room temperature until DNA extraction. DNA was extracted from approximately 70 mg of the stored specimens with a QIAGEN Blood and Cell Culture DNA Midi Kit (Qiagen, Germany) according to the manufacturer’s instruction. A UV spectrophotometer (Eppendorf, Germany) was used to determine the quantity and quality of the isolated DNA. DNA concentration was estimated by measuring the absorbance at 260 nm wave length. Protein contamination was estimated by the ratio of absorbance at 260 nm and 280 nm wave lengths [20].

2.2 PCR amplification
NADH 1 gene was amplified with polymerase chain reaction (PCR) technique in 50 µl reaction mixture containing 1-2 ng DNA, PCR Buffer 1 X, 1 unit Taq DNA polymerase (Roche, Germany), 0.2 mM dNTPs and 10 pMol forward and Reverse primers. The condition of PCR amplification using a thermo cycler instrument (BioRad, USA) was as follow: denaturation at 94°C for 5 min, following by 35 cycles of 59°C for 1 min, and 72°C for 5 min, with final-extension at 72°C for 7 min. The amplified DNA fragments were determined for the size and quality with 1.5% agarose-gel electrophoresis and ethidium bromide staining, then purified by the QIAquick PCR Purification Kit (Qiagen, Germany).

2.3 Sequencing and phylogenetic analysis
The amplified DNA fragments sequenced which carried out by ABI PRISM dye terminator cycle sequencing kit (PE Biosystem, USA) according to the manufacturer’s instruction. After sequencing, the data were deposited in GenBank, accession number LC011387. Bioinformatics analysis: The sequence of NADH 1 gene was analysed by DNAMAN Bioinformatics analysis: accession number LC011387. The sequence of NADH 1 gene was analysed by DNAMAN program, and our data shows that between Salmo trutta caspius and Salmo salar and Oncorhynchus mykiss, 23 SNPs and 92% similarity between Oncorhynchus mykiss (DQ288268.1) and Salmo salar (KF792729.1) and Oncorhynchus mykiss (DQ288268.1) in GenBank.

We compared the NADH 1 gene between Salmo trutta caspius and Salmo trutta trutta (accession number AM910409.1), Salmo salar (KF792729.1) and Oncorhynchus mykiss (DQ288268.1) by DNAMAN program and NCBI Network system separately (Figures 2-4). According to DNAMAN program and NCBI Network system analysis, we found 23 SNPs and 98% similarity between Salmo trutta caspius and Salmo trutta trutta, 67 SNPs and 92% similarity between Salmo trutta caspius and Salmo salar, and 126 SNPs and 86% similarity between Salmo trutta caspius and Oncorhynchus mykiss. Amino acid sequences also were determined by DNAMAN program, and our data shows that between Salmo trutta caspius and Salmo trutta trutta 3 SNPs, between Salmo trutta caspius and Salmo salar 14 SNPs, and between Salmo trutta caspius and Oncorhynchus mykiss, 17 SNPs are identified (Figures 5-7).
Fig 2: NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Salmo trutta trutta* (S.t. trutta) were analysed with DNAMAN program and NCBI Network system. There were 23 SNPs and 98% similarity between them.
Fig 3: NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Salmo salar* (S. salar) were analysed with DNAMAN program and NCBI Network system. There were 67 SNPs and 92% similarity between both sequences.

~ 188 ~
Fig 4: NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Onchorhynchus mykiss* (O. mykiss) were analysed with DNAMAN program and NCBI Network system. There were 126 SNPs and 86% similarity between both sequences.
Fig 5: The amino acid sequences of NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Salmo trutta trutta* (S.t. trutta) with DNAMAN program. There were 3 SNPs between both sequences.

Fig 6: The amino acid sequences of NADH 1 dehydrogenase gene in *Salmo trutta caspius* (S.t. caspius) and *Salmo salar* (S. salar) with DNAMAN program. There were 14 SNPs between both sequences.
4. Discussion

4.1 Why phylogenetic analysis of Salmo trutta caspius is important?

Salmonid species populations are native of some of the rivers draining to the Mediterranean and Black Sea (at least in upper Danube drainage) and the Caspian Sea (at least in upper Volga drainage). List Category and Criteria (red list) of Salmo trutta species was reported [3] which is a widespread species. However, anadromous part of populations (sea trout) and many lacustrine stocks have in many cases markedly declined because of pollution (and possibly from impacts from salmon farming). The phylogeographic structure is almost destroyed by stocking. However Freyhof [3] identified different species on the basis of morphological characters, but here we aimed to identify different species of Salmonids such as Salmo trutta trutta, Salmo salar and Oncorhynchus mykiss by comparing their NADH 1 gene. In other species of Salmonids like Salmo trutta fario, full length of their mitochondrial genome, specially their NADH 1 gene have been reported, but here we randomly selected Salmo trutta trutta, Salmo salar and Oncorhynchus mykiss, because the rate of homology between them is high.

4.2 Why NADH 1 gene were used for phylogenetic analysis?

In this study we used NADH 1 gene for analysis of phylogenetic of Salmonids. However in salmonids phylogenetic have been reconstructed from morphology, physiology, ontogeny, DNA-DNA hybridization, protein electrophoretic mobility variation, karyology, DNA polymorphism and sequence analysis [1, 2, 8, 11, 13, 21-26]. Regarding NADH dehydrogenase, its genes have been examined previously in Salmonid species and their sequences have been deposited in GenBank (Accession nos. U28345, U28364, U28365, U28366 and etc.). In this study we sequenced full length of NADH dehydrogenase 1 in Salmo trutta caspius for making it possible to examine the relationship among Salmo trutta caspius, Salmo trutta, Salmo salar and Oncorhynchus mykiss. For getting good quality, the specimens were analysed by DNAMAN program and NCBI Network system. In this regards, we selected 20 samples from Salmo trutta caspius. After sequencing of NADH 1 gene, they were analysed within and between sequences, and we did not find any variation between them, so we selected one sequence from examined samples for comparing with Salmo trutta trutta, Salmo salar and Oncorhynchus mykiss populations.

4.3 The situation of SNPs between sequences of NADH1 in Salmo trutta caspius, Salmo trutta and Oncorhynchus mykiss

Single nucleotide polymorphism (SNP) is a suitable technique for analysis of NADH 1 gene sequences, because the homology between sequences was high, so in this study we aimed for finding single nucleotide variations that were between these different sequences from different species, from the first to the last nucleotide of NADH 1 gene. According to DNAMAN program analysis, when comparing NADH1 gene, 23 SNPs were found between Salmo trutta caspius and Salmo trutta trutta for, 67 SNPs between Salmo trutta caspius and Salmo salar, and 126 SNPs between Salmo trutta caspius and Oncorhynchus mykiss. Amino acid sequences also were determined by DNAMAN program, according to its program settings, and between Salmo trutta caspius and Salmo trutta...
trutta 3 SNPs, between Salmo trutta caspius and Salmo salar 14 SNPs, and between Salmo trutta caspius and Oncorhynchus mykiss 17 SNPs were observed. The situations of located mutations in nucleotides showed that there were not any single mutation on the start codon like ATG for three species cited above. Moreover, amino acid sequences also were studied and subjected to analysis by DNAMAN and its result showed three single mutations: G to S (Glycine to Serine), N to S (Asparagine to Serine) and S to Q (Serine to Glycine). Salmo trutta caspius and Salmo salar also showed mutations of I to V, V to I, V to I, V to I, I to V, I to M, I to M, I to V, G to S, I to M, L to F, N to S and I to M and T to M respectively. Salmo trutta caspius and Oncorhynchus mykiss also were observed that have I to M, T to L, V to I, I to V, I to M, I to V, I to V, G to S, F to T, A to I, V to L, F to V, L to K, W to N, K to K, T to I, T to L, S to Q mutations.

According to the results of comparison between Salmo trutta caspius and Salmo trutta trutta, there were not any start codon like M (Methionine), but between Salmo trutta caspius and Salmo salar we found five M (Methionine) that were mutated from NADH 1 gene of Oncorhynchus mykiss to Salmo salar. In Salmo trutta caspius and Oncorhynchus mykiss we also found two Ms (Methionines) that mutated from NADH 1 gene of Salmo trutta caspius to Oncorhynchus mykiss. Hence, except for Salmo trutta trutta, both species of Salmo salar and Oncorhynchus mykiss had start codon mutations. At the level of nucleotides, we observed single nucleotide mutations between Salmonids of cited species. The percent of homology of Salmo trutta caspius was 98% with Salmo trutta trutta, 92% with Salmo salar and 86% with Oncorhynchus mykiss (Figures 4-6). According to those results, we concluded that Salmo trutta caspius has a higher homology with Salmo trutta trutta compared to Salmo salar and Oncorhynchus mykiss respectively. The sequence from start codon to stop codon is called the reading frame, and since synthesis of all polypeptide chains in eukaryotic cells begins with amino acid methionine, mutations on this region is very important for the organism. According to Figures 4-7, similarity and variety of sequences of NADH 1 gene between salmon species that cited in this study is important because Berg \(^5\) had reported that Salmo trutta species originated from Atlantic Ocean and had migrated to White Sea and then left to Russia by the rout of Caspian Sea. Hence Salmo trutta caspius originated from Salmo trutta by morphological documents, however had reported between and within high homology on the Salmonid species such as Salmo trutta fario and Salmo trutta caspius when baised to 16S rRNA gene, cytochrome C oxidase, ATPase subunit 6 and cytochrome b for population genetics.\(^7,8\). Finally, we concluded that based on the sequence of NADH 1 gene, there is SNPs between Salmo trutta caspius and other Salmonid species, but in order to get the exact results about the rate of homology between salmon species, we have to study the full length of mitochondrial gene and also use other molecular techniques such as microsatellites and RFLP, RAPD.

5. Acknowledgments

This work was financially supported by the Research Council of Islamic Azad University Tonekabon Branch, Iran.

6. References


