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Short Communication

5.8S rRNA Sequence and Secondary Structure in *Parabronema skrjabini* and Related Habronematidae Species

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Abstract

Background: Genomic DNA was isolated from *Parabronema skrjabini*. rRNA region was amplified and sequenced.

Methods: The RNA secondary structure was predicted using mfold software (http://mfold.rit.albany.edu). The secondary structure with bulge, hairpins, helices, interior, external and multi loops was predicted for 5.8srDNA of our sequence of *P. skrjabini* and a sequence *of P. skrjabini* and two species of *Habronema* (*H. microstoma* and *H. muscae*) in GenBank. RNA motifs were predicted by MEME program version 4.10.2.

Results: The length of 5.8S rRNA sequence for *P. skrjabini#1*, *P. skrjabini#2*, *H. microstoma* and *H. muscae* was 158, 156, 127 and 127bp, and the DG required for the formation of the secondary structure was -70.50, -56.40, -41.50 and -41.40 kcal/Mol, respectively. Common structural elements were initially recognized with the help of mfold by screening for thermodynamically optimal and suboptimal secondary structures (default settings, with T = 37 °C). The energy levels of the presumptive secondary structures were then calculated with mfold at the DNA level. Both motifs and the sequence of *P. skrjabini#1* were completely different from the other analyzed samples. This difference might be due to the differences in host and geographical area.

Conclusion: This is the first molecular study of *P. skrjabini* in sheep, which could be further used in the structure modeling across Habronematidae.

Introduction

Parabronema skrjabini is one of the nematodes that affect the abomasum of ruminants. Abomasum is one of the most important sites for nematodes, which could be harmful to the health of infected animal and causes economic losses due to reduce weight

gain and other production losses. The prevalence of *P. skrjabini* in Iran has been reported 4.2% in goat, 5.43% in sheep (1, 2), and 0.8% in wild sheep (1, 3).

The primary sequences of rDNA were molecular data traditionally applied in phylogenetic studies. Researchers could analyze the divergences and genetic distances among taxa in the rDNA nucleotide sequences to perform phylogenetic relationships analysis and species identification researches. The rDNA secondary structures are predicted from the corresponding primary sequences according to base pairing, containing all the sequence information. Although, there are significant variations in rDNA sequences across different taxa (4), the corresponding secondary structures of the transcribed rRNA are highly conserved during evolution (5), perhaps due to the important role of the rRNA folding in holding the structural RNA functions (6). In addition, the secondary structures are more conserved than the primary sequences for the semi-compensatory or compensatory mutations, and therefore when the multiple sequence alignments look less reliable due to deletion or insertion, the structures can help to make more reliable assignment of nucleotide homology with important role in the phylogeny (7). In addition, some changes, like expansions and deletions, of a certain helix could be specific to a taxon to help in species identification. "So, the secondary structures have drawn a lot of attention from phylogenetic scientists" (8).

However, the study on secondary structures takes a slower step than that primary sequences because of the limited sequence data in GenBank suitable for structure prediction. In this study, the secondary structures of 5.8SrRNA of *P. skrjabini* were comprehensively investigated and compared with secondary structure of a *P. skrjabini (*EU375510.1*)* and two species of *H. muscae (*AY251024.1*)* and *H. microstoma* (AY251023.1) sequences, which are located in the same family retrieved from NCBI. Such case studies are suitable to provision of basic data, both for reconstructing molecular evolution in expansive phylogenetic contexts and for analyzing function in ribosome biogenesis. However, only one study could be derived on *Parabronema* at a molecular level (9).

The aim of this study was to analyze the 5.8S rRNAgene sequence and to study the possible effect of nucleotide substitutions on the topology of the secondary structure of the 5.8S rRNA molecule in *P. skrjabini* and related Habronematidae species. Consequently, we studied the 5.8S rRNA gene sequence of the members of the family Habronematidae and determined the probable secondary structure of the5.8S rRNA molecule for the first time.

Material and Methods

DNA Extraction and PCR

DNA extraction from worms was performed using an extraction kit (MBST, Iran) according to the manufacturer's instructions. The rRNA gene was amplified using the primer pairs based on the r DNA genome sequence (9). The forward primer was PS-F: 5'-GTA GGT GAA CCT GCG GAA GG -3' and reverse primer was PS-R: 5'-TTAGTTTCTT-TTCCT CCGCT -3'. The PCR reaction was carried out in a total volume of 100µL containing 1×PCR buffer, 100 mMol $MgCl_2$, 100 µM dNTP mix (Cinaclone, Iran), 20 µMol of each primer (Cinaclone Co.), 5 unit/µL Taq DNA polymerase (Cinaclone) and 1µl of template DNA (100 ng DNA) in an automated thermocycler. The PCR was performed using the following protocol: 5 min incubation at 94 °C, 33 cycles of 45 s at 94 °C, 45s at 59 °C, and 45 s at 72 °C, with an additional extension step for 5 min at 72 °C. Samples without genomic DNA were used as negative controls. The PCR products were analyzed on 1% agarose gels in 0.5× TBE buffer and visualized using Sybersafe staining (Cinaclon, Iran) and a UV illuminator. The PCR product was purified using a quick PCR product purification kit (MBST, Iran) according to the manufacturer's instructions.

Isolation of DNA, PCR and Sequencing

Genomic DNA sequencing using the Sanger method was performed in both directions on the PCR product by the Kawsar Biotech Co. Iran. The sequences were analyzed using the Geneious 5.1.6 software and compared against GenBank (www.ncbi.nlm.nih.gov/) using the 'Basic Local Alignment Search Tool' (BLAST). The probable secondary structure of the 5.8S rRNA molecule was constructed using mfold software (http://mfold.rit.albany.edu/). RNA motifs were predicted by MEME program version 4.10.2. Common structural elements were initially recognized with the help of mfold(10, 11) by screening for thermodynamically optimal and suboptimal secondary structures (default settings, with T = 37 °C). Energy levels of the presumptive secondary structures were then calculated with mfold (10, 11). The completed sequence was uploaded into GenBank with accession number of KT339317. Alignment, analysis of the derived nucleotide sequences, and cluster analysis were performed using MEGA 6.0 software (12).

Results

The length of 5.8S rRNA sequence for *P.skrjabini#1*, *P.skrjabini#2*, *H. microstoma* and *H. muscae* was 158, 156, 127 and 127bp, and the DG required for the formation of the secondary structure was -70.50, -56.40, -41.50 and -41.40 kcal/Mol, respectively. The stem loop structures were folded using the mfold web server (http://mfold.rna.albany.edu/) Zuker 2003 (Fig. 1).

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H.microstoma  g------------------------------AGTCGATGAAGAACGCAGCTAGCTGCAA
H.muscae      .-----------------------------.....................G.T
p.skrjabini_1 tTTTCTTTTAGGGGGGGATCCCTTGGCTTGGGGA..A..A...C.CT....T...GT
p.skrjabini_2 t-TACTCTTAGCGGTGGATCACTTGGCTCGTGGA.....................G.

H.microstoma  TAAATAGTGCGAATTGCAGACGCATTGAGCACAAAGATTTCGAACGTACATTGCACCATC
H.muscae      ...................................G.....................
p.skrjabini_1 ....A....G.........G.C.C...CA.C..............CCC.C....C..C.G
p.skrjabini_2 ..........................................C.............

H.microstoma  GGGTTGCATCCCGATGGTACGTCTGGCTGAGGGTCGAT
H.muscae      ...................................
p.skrjabini_1 ......AT.....GG...GTC.T...GA.G...G....
p.skrjabini_2 ......AT..........................-
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Fig. 1: The 5.8S rRNA gene sequences in the Habronematidae samples analyzed

Maximization of the hydrogen bonding formed solid stems, and the largest negative delta g value (free energy). We predicted the motifs for 5.8s rDNA of *P. skrjabini* and three other sequences in genebank (*P. skrjabini#2*, *H. muscae* and *H. microstoma*). Three motifs were identified for 5.8s of *P. skrjabini#1*, the first motive- AGGGGG (6bp), the second motive- TAAAAA (6bp) and the third motive- CAAAGA (6bp). The first motive is repeated in two positions [10 and 148]. In addition, the second motive is repeated in two positions [39 and 61]. For *P. skrjabini#2* three motifs were identified, the first motive – GATAAA-TAGTGCGAATTGCA (20bp), the second motive- GTGGAT (6bp) and the third motive- CCATCGGG (8bp). The second motive is repeated in two positions [13 and 29]. For *H. muscae* there are three motifs, the first motive – CATCCCGATGGT (12bp), the second motive – GTCGAT (6bp) and the third motive – CAGACG (6bp). The second motive is repeated in two positions [3 and 122]. For *H. microstoma* we identified three motifs, the first motive – CCCGATGGT (9bp), the second motive – AGCTGC (6bp) and the third motive – CAGACG (6bp). The number of different loops, motifs and the DG for formation of the secondary structure is shown in Table 1, Fig. 2.

Table 1: Statistical information of the predicted secondary structure of *Parabronema skrjabini* rRNA

Sequence name	Sequence length (in bases)	GC Content %	Bulge loop	Helix (number)	Interior loop (number)	Hairpin loop (number)	External loop (number)	Multi loop (number)	Motifs (in bases)	DG kcal/mol
P.skrjabini#1	158	54	4	9	2	3	1	0	3	-70.50
P.skrjabini#2	156	49	0	9	2	4	1	3	3	-56.40
H. microstoma	127	49.6	0	8	3	3	1	2	3	-41.50
H. muscae	127	50.4	0	6	3	2	1	1	3	-41.40

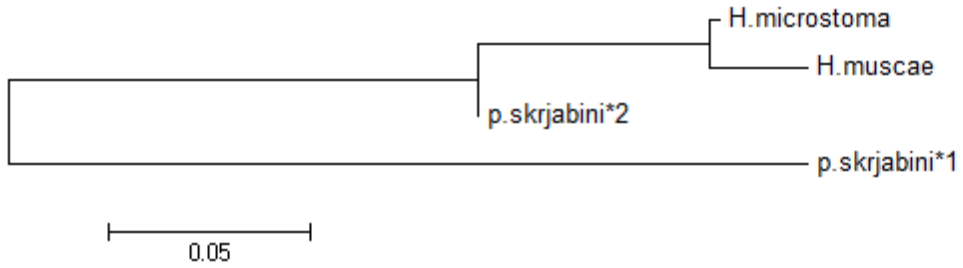

Fig. 2: Phylogenetic tree of Habronematidae family

Discussion

The secondary structures contain more information than the primary sequences and are the bases of rRNA function; have gained a lot of attention in phylogenetic analysis. In this study, the secondary structure of 5.8S rRNA of *P. skrjabini* was predicted and the structure comparison performed by predicating the secondary structures for a *P. skrjabini* and two species of *Habronema* (*H. muscae* and *H. microstoma*) sequences, which is located in the same family retrieved from NCBI.

5.8S ribosomal RNA (5.8S rRNA) is a non-coding RNA component of the large subunit of the eukaryotic ribosome that plays an important role in protein translation. In this study secondary structure with bulge, hairpins, helices, interior, external and multi loops of 5.8S rRNA sequence of *P. skrjabini* was reconstructed under specific settings for base pairing and compared with secondary structure of a sequence of *P. skrjabini,* and two species of *Habronema* (*H. muscae* and *H. microstoma*) retrieved from NCBI. The predicted SSU rRNA secondary structure in the present study was the first model for *P. skrjabini*. However, the prediction of the secondary structure was impaired due to a few complete sequences of limited species of *Parabronema*.

In total, 41 variable sites in the 5.8S rRNA gene sequence were detected in the samples analyze (Fig.1). *P. skrjabini*#2 was more similar to *H. muscae* and *H. microstoma* and was more invariable than *P. skrjabini*#1. *P. skrjabini*#1 differed from the related species *P. skrjabini#2* by 31 specific nucleotide substitutions. There were only two nucleotide substitutions for *H. muscae*. There was a 31-nucleotide absence at the beginning of the 5.8s rRNA gene of *H. muscae* and *H. microstoma*. However, this insertion is present in the *P. skrjabini*#1and this presence is 30 for *P.skrjabini*#2. The motive 2a and 2b for *P. skrjabini#2* and the motive 2a and 2b for *H. muscae* are different in only one nucleotide (G-C). The motive 3a in *H. muscae* and 3a in *H. microstoma* is common and motive 1a in *H. muscae* and *H. microstoma* had only three-nucleotide difference. The motifs in *P. skrjabini#1* are completely different from the other analyzed samples. This difference might be is due to the differences in host and geographical area. We isolated *P. skrjabini#1* in sheep and from Sanandaj (west of Iran), but *P. skrjabini#2* is in camel and China, *H. muscae*

and *H. microstoama* are in horse and Italy. The phylogenetic tree (Fig. 3) proves this result.

Moreover, some changes, such as expansions and deletions, of a certain helix could be specific to a taxon to help a lot in species identification. Using mFold software, the probable secondary structure of the 5.8S rRNA gene was constructed. When constructing the secondary structure of the 5.8S rRNA gene sequence, the sequence of *P. skrjabini* was used as a consensus sequence. Fig. 3 shows the identified motifs and Fig. 1 shows the nucleotide substitutions found in other analyzed representatives of the family Habronematidae.

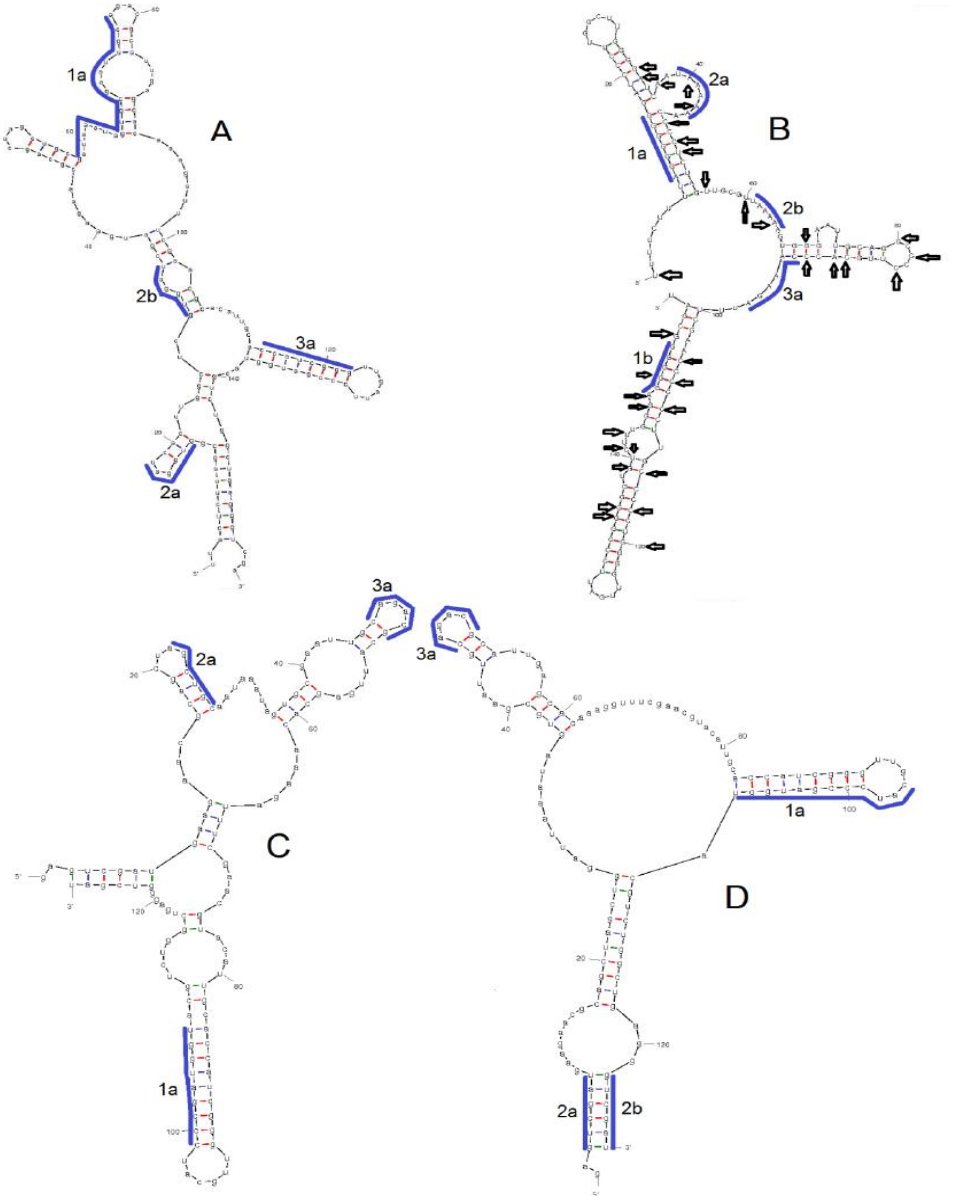

Fig. 3: Secondary structure of 5.8 rDNA for family Habronematidae. A: *P. skrjabini#2*(EU375510.1). B: 5.8s of this study*P. skrjabini#1* (KT339317). C: *H. microstoma* (AY251023.1). D: *H. muscae* (AY251024.1).The position of motifs is shown on the secondary structures. The nucleotide substitutions are shown by arrows

Conclusion

The secondary structure of the 5.8S rRNA gene has not been described for family Habronematidae, specially the genus of *P. skrjabini*. In our study, for the first time we determined the secondary structure of 5.8S rRNA in the *P. skrjabini* and related Habronematidae species. We made a multiple alignment and found structural differences among the analyzed samples, *Parabronema* and *Habronema*, which could be further used in the structure modeling across Habronematidae.

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