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Molecular Characterization of Subunit G of the Vacuolar ATPase in Pathogen Dermatophyte *Trichophyton rubrum*

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Abstract

Trichophyton rubrum is an anthropophilic fungus causing up to 90% of chronic cases of dermatophytosis. Several properties of this fungus have been investigated so far. However, a few studies were carried out in the field of molecular biology of this fungus. In the present study, we tried to identify the subunit G of its vacuolar ATPase (V-ATPase). Pairs of 21 nt primers were designed from highly conserved regions of the V-ATPase subunit G genes in other fungi. Mentioned primers were utilized in PCR using isolated genomic DNA template as well as cytoplasmic RNA of *T.rubrum* and the PCR and RT-PCR fragments were then sequenced. About 469 nucleotides were sequenced which encoded a polypeptide with 119 amino acids. Nucleotide sequence comparison in gene data banks (NCBI, NIH) for both the DNA and its deduced amino acid sequence revealed significant homology with V-ATPase subunit G genes and proteins of other eukaryotic cells. The amino acid sequence of the encoded protein was about 84% identical to the sequence of V-ATPase subunit G from other fungi. In summary, we have cloned the first V-ATPase subunit G of dermatophytes and characterized it as a member of this gene family in other eukaryotic cells.

Keywords: *Dermatophyte,* Trichophyton rubrum*, Fungal RNA, Fungal DNA, Iran*

Introduction

The vacuolar ATPases (V-ATPase) are a family of ATP-dependent proton pumps that are responsible for acidification of intracellular compartments in eukaryotic cells (1-7). In addition to their role in intracellular compartments, V-ATPases play an important role in the plasma membrane of various cells (2, 8). The G subunit of V-ATPases is a soluble subunit that seems to be essential for V-ATPase activity (1, 4, 5, 9).

In the field of Mycology, the V-ATPase subunit G gene family of yeast has recently been investigated intensively (3, 5), whereas little information is available on V-ATPase subunit G of the filamentous fungi, especially of those which are involved in human infections such as dermatophytes.

Dermatophytes are a group of keratinophylic filamentous fungi infecting the skin and skin appendages of humans and animals (10). The vast majority of chronic dermatophyte infections of humans are caused by *T. rubrum* (10, 11). Investigation of the molecular characteristics of this fungus as well as of all other dermatophytes, have only recently begun (11, 12). In the present study, we have explained the molecular characterization and analysis of a gene encoding the V-ATPase subunit G of this dermatophyte.

Materials and Methods

Isolation of nucleic acids Total RNA from *T. rubrum* was isolated by a method, which we had been previously developed (13). The poly

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$(A)^+$ RNA was obtained from total RNA and used for cDNA synthesis.

High molecular weight DNA from *T.rubrum* was isolated by a modification of the method of Rezaie et al. (12). Briefly, the harvested mycelial mass was flash-frozen in liquid nitrogen and ground to a fine powder in a porcelain mortar. The mycelial powder was suspended in DNA extraction buffer containing: 50 mM Tris-HCl (pH 8.0), 50 mM EDTA, 3% SDS, 1% β-mercaptoethanol and 50 µl of proteinase-K (20 mg/ml). The suspension was then incubated at 65 °C for 1 h and the cellular debris was removed by centrifugation at 2500-x g for 15 min. After addition of 25 µl RNase-H (10 mg/ml), the suspension was incubated at 37 °C for 30 min, extracted once with phenol-chloroform-isoamyl alcohol (25:24:1) and once with chloroform-isoamyl alcohol (24:1). The DNA was precipitated by addition of an equal volume of isopropanol, followed by centrifugation at 15000-x g for 30 min. The DNA pellet was rinsed with 70% ethanol and resuspended in distilled water.

PCR analysis PCR and reverse transcriptase PCR (RT-PCR) analysis of genomic DNA derived from *T. rubrum* were performed according to a standard protocol (14) using synthetic oligonucleotide primers including: kh1; 5'CACCAGGCAAGACAGACCGAC3' as sense and kh2; 5'GTCAAGTCTCAACCGAGCCGT3' as reverse primers. Briefly, 20 pM of each primer was added to a volume of 50 µl containing: 20 mM $(NH4)_2$ SO_4, 75 mM Tris-HCl (pH. 8.8), 1 mM $MgCl_2$, 0.2 µM dNTP mix, 1.2 units of thermostable DNA polymerase (Advance Biotechnologies, UK), and 1 µl of template (genomic or plasmid DNA). The PCR cycle employed was 95 °C for 60s, 52 °C for 60s, and 72 °C for 120s, with 35 cycles. PCR products were analyzed by electrophoresis through a 1% agarose gel.

Sequencing of the RT-PCR fragments Sequencing of the amplified cDNA fragment was performed with the Dye Terminator Cycle Sequencing Kit (MWG, Germany), by using, the amplified double stranded cDNA as template and synthetic 21-meric primers designed according to the obtained DNA sequence fragments from other fungi. Sequencing of each part was repeated at least three times for both strands. The nucleotide sequence of DNA was compared with the sequences in gene data banks in National Centre for Biotechnology Information (NCBI, NIH).

Results

Isolation and characterization of V-ATPase subunit G DNA (TrATPase-G) After amplification of the gene by PCR and RT-PCR, it was identified as a DNA with the approximate molecular weight of 0.78 kbp. However, the molecular weight of amplified cDNA was approximately 0.6 kbp (Fig. 1). This revealed the presence of intron in the genomic DNA of the amplified gene.

The nucleotide sequence of the cDNA insert is presented in Fig.2. The amount of 469 bp of the cDNA has been sequenced. The sequenced cDNA contains an open reading frame of approximately 361 bp encoding a 119 amino acids protein. Nucleotide sequence comparison in gene data banks (NCBI, NIH) for the cDNA and its deduced amino acid sequence revealed significant homology with members of the eukaryotic V-ATPase subunit G family. The amino acid sequence of the encoded protein was about 69 to 88% identical to the sequence of V-ATPase subunit G from *Neurospora crasa* and *Aspergillus nidulans*. The amino acid composition of this protein indicates that it is rich in lysine (18.70%), and glutamic acid (14.30%). In contrast, a lot content of tyrosine, phenylalanine, propylene (1.68%) and metionin (0.84%) was deduced from the sequenced gene.

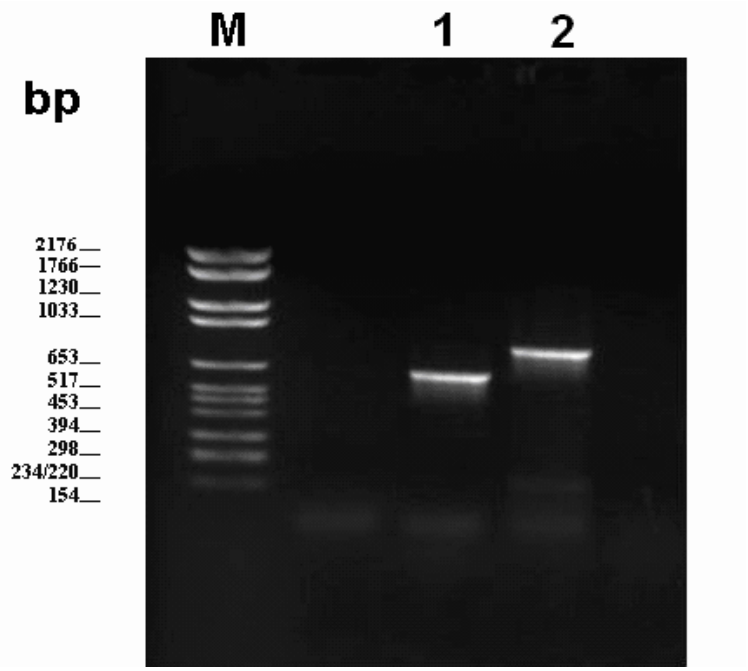

Fig. 1: PCR products of the *Tr-ATPase G*. M: molecular weight marker VI (Roch, Germany), Lane 1: RT-PCR product, Lane 2: PCR product.

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  1   GGCACCAGGCAAGACAGACCGACGAGACACAAC  33

  1    M   A   A   Q   N   S   A   G   I   Q   T  L   L   D   A   E   R   E   A   Q     20
 34   ATG GCT GCA CAA AAT TCC GCC GGA ATC CAG ACC CTC CTC GAT GCG GAA AGG GAA GCT CAG    93

 21    K   I   V   Q   T   A   R   E   Y   R   T   K   R   I   K   D   A   K   T   E    40
 94   AAG ATA GTT CAG ACT GCT AGA GAA TAC CGC ACG AAG CGC ATA AAG GAC GCC AAG ACA GAG  153

 41    A   Q   K   E   I   E   D   Y   K   K   Q   K   E   E   E   F   R   K   F   E    60
154   GCA CAG AAG GAG ATT GAA GAC TAC AAG AAA CAA AAG GAA GAG GAA TTC CGA AAA TTC GAA  213

 61    A   E   H   S   S   G   N   Q   K   A   E   N   D   A   N   K   D   A   E   A    80
214   GCT GAG CAC TCG AGC GGA AAC CAG AAA GCC GAA AAT GAT GCA AAC AAA GAT GCA GAA GCC  273

 81    Q   L   L   E   I   K   K   S   G   K   E   K   G   N   K   V   V   E   D   L   100
274   CAG CTC CTT GAA ATC AAG AAA TCT GGG AAA GAA AAG GGC AAC AAG GTC GTC GAA GAT CTC  333

101    I   K   T   V   L   D   V   N   P   Q   V   P   E   K   L   A   K   K   A   *   119
334   ATT AAG ACT GTC CTA GAT GTC AAC CCC CAG GTC CCA GAG AAG CTA GCT AAA AAA GCT TAA  393


394   ATTACGCTCAGTGACCTCTTCTATGTTTAACGGCTCG                                          430
431   GTTGAGACTTGACATTCTTCTTCCTGTGTTCGGGGTCTT                                        469
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Fig. 2: Complete nucleotide sequence of cDNA and deduced amino acid sequence of the Tr-ATPase G (Genbank accession number: AY834222). The initiation codon (ATG) and the stop codon (TAA) are indicated as underlined.

Discussion

In the present study, we report the identification and molecular characterization of a *T. rubrum* gene encoding a protein belongs to the V-ATPase subunit G family, which will here be referred to as *TrV*- ATPase- G. Analysis of the amino acid sequence of this gene revealed a considerable identity with other eukaryotic V-ATPase subunit G such as those of *N.crasa* (69%), *A.nidulans* (88%), and *S.cerevisiae* (57%) (1, 15). Sequence conservation was the highest at the N-terminus and decreased towards the C-terminus, which was in accordance with other results (6, 9, 15). The presence of initiation and stop codons at real positions of the TrV-ATPase-G DNA sequence, together with the information deduced from the alignment with other V-ATPase subunit G, indicated that it might encompassed the full-length gene coding sequence.

To our knowledge, TrV-ATPase-G is the first V-ATPase gene of the dermatophyte fungi characterized so far. Identification of possible roles of this newly characterized gene in the physiological function of *T.rubrum* is still under investigation. The molecular characterization of TrV-ATPase-G gene, which has been performed for the first time worldwide and described here, may open the way to the disclosure of the functional characteristics of TrV-ATPase-G and to the assessment of its possible role in the pathogenesis of dermatophyte infections due to *T. rubrum*.

Nucleotide and amino acid sequences of TrV-ATPase-G have been submitted to the National Centre for Biotechnology Information GenBank and are available for public access under the accession Number: AY834222.

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