Full Length Research Paper

Molecular characterization of 26S proteasome regulatory subunit in dermatophyte pathogen *Trichophyton verrucosum*

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*Trichophyton verrucosum* is a zoophilic dermatophyte, which causes dermatophytosis infection in human as well as animals. 26S proteasome is an important protein in eukaryotic cells that is involved with degradation of unneeded or damaged proteins, when tagged with ubiquitin. In this study, we characterized the 26S Proteasome regulatory subunit gene in dermatophyte pathogen *T. verrucosum*. High molecular weight DNA has been isolated from *T. verrucosum* and utilized with pairs of specific nucleotides primers, designed from highly preserved regions of the 26S proteasome regulatory subunit genes. Obtained DNA fragments were sequenced and the results were analyzed in GenBank. This DNA fragment, which contains no intron within its open reading frame, encodes a polypeptide with 332 amino acids. The characterized PCR fragments revealed significant homology with other 26S proteasome regulatory subunit genes in GenBank.

Key words: Dermatophyte, *Trichophyton verrucosum*, fungal DNA, nucleic acid sequencing, 26S proteasome.

INTRODUCTION

*Trichophyton verrucosum* is a zoophilic dermatophyte. This fungus is an agent of ringworm disease in human and domestic animals like camel, cow and cattle (Kane and Smitka, 1978; Oborilova and Rybnikar, 2005). Direct contact with this fungus causes of infection of nail, skin and hair in human. The infection is usually with high inflammation such as in tinea mannum bullosa (Aste et al., 2005). *T. verrucosum* also makes economical lose in domestic animals (Cabanés, 2000). Identification and categorization of fundamental genes in this dermatophyte may help in the treatment of infections caused by *T. verrucosum*. One of the most important proteins in *T. verrucosum* as well as other eukaryotic cells is the 26S proteasome.

The function of the 26S proteasome is to degrade non-functional proteins. It destroys proteins tagged with ubiquitin for degrading by the 26S proteasome/ubiquitin pathway (Zeng et al., 2006). Ubiquitin is a spherical protein that has 76 amino acids. It is highly preserved (Hanna and Finley, 2007). Ubiquitin occupies many cellular processes like protein degradation, DNA repair and apoptosis (Yerlukaya, 2004). It serves only as a label that marks proteins for degradation and 26S proteasome degrades proteins that are tagged with it (Sullivan et al., 2003).

The 26S proteasome is the essential protease in non-lysosomal ubiquitin-dependent protein dilapidation (Wakata et al., 2004), and also involved in transcription, oxidative stress, the regulation of gene expression,
an improvement of the method of Rezaie et al. (2000). We cultured T. verrucosum in sabouraud's glucose broth 2% for 14 days. Then T. verrucosum mycelia gathered and washed with PBS buffer three times, flash-frozen in liquid nitrogen and ground to soft powder. The mycelial powder was suspended in a buffer consisting of 50 mM Tris-HCl (pH 8.0), 50 mM EDTA, 3% SDS, 1% β-mercaptoethanol and 50 µl of protease-K (20 mg/ml). This suspension was incubated at 65°C for 1 h and centrifuged at 2500×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 one time with phenol-chloroform-isoamyl alcohol (25:24:1) and one time with chloroform-isoamyl alcohol (24:1). The DNA was precipitated by addition of an equal volume of isopropanol, followed by centrifugation at 15000×g for 30 min. The DNA pill was washed with 70% ethanol and re-suspended in distilled water.

PCR analysis

PCR analysis of the genomic DNA isolated from T. verrucosum was done according to a standard protocol (Rezaie et al., 1999). Oligonucleotide primers have been designed by homology search of highly conserved areas within 26S proteasome regulatory subunit genes from other eukaryotic cells in gene data bank. From several pairs of primers which have been synthesized (Sinna gene, Iran), a pair including Nas1 \textsuperscript{5′} - CGAGAAGCCGGACGTGACATAC - 3′ as sense and NaAs1 \textsuperscript{5′} - CGGTCTTGACCTGAGCAGCATAGGC -3′ as reverse primers were selected for amplifying the gene in T. verrucosum. 20 µM of each primer was added in a volume of 50 µl that consists of 10X buffer with MgCl\textsubscript{2} 10 µl, dNTP 1 µl, genomic DNA 1 µl, sense primer 1.5 µl, reverse primer 1.5 µl and thermo stable DNA polymerase 1.5 µl (Roche, Germany). The PCR program was 94°C for 30 s, 60°C for 90 s and 72°C for 150 s with 35 cycles. PCR products were analyzed by electrophoresis through a 1% agarose gel.

Sequencing of the PCR product

Sequencing of the amplified DNA fragments was done with the Dye Terminator Cycle Sequencing Kit (MWG, Germany). 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 the 26S proteasome regulatory 6B/Rpt3 subunit was completed by amplification of this gene with using synthetic primers (Figure 1). Almost 996 bp of the DNA was sequenced; the nucleotides encode a polypeptide with 332 amino acids (Figure 2). The nucleic acid sequence has considerable homology with other eukaryotic 26S proteasome regulatory subunit 6B/Rpt3 subunits, including Trichophyton rubrum (77%), Ajellomyces capsulatum (72%), Drosophila melanogaster (71%), Neurospora crassa (65%), Coccidioides immitis (65%), Aspergillus clavatus (64%), Aspergillus terreus (64%) and Aspergillus fumigatus (64%). The amino acid sequence of the encoded protein has homology with T. rubrum (53%), A. capsulatum (53%), N. crassa (52%), C. immitis (52%), A. clavatus (51%), A. terreus (51%) and A. fumigatus (51%) and...
Figure 2. Complete nucleotide sequence of DNA fragment and its deduced amino acid sequence of the *T. verrucosum* 26S proteasome regulatory subunit 6B.
D. melanogaster (44%). Nucleotide and amino acid sequences of this newly characterized gene have been submitted to the National Centre of Biotechnology Information Gene Bank and are available for public access under the accession number EU836237 for Genomic DNA.

**DISCUSSION**

In this study, we report the molecular characterization of a *T. verrucoum* gene encoding a protein that belongs to 26S proteasome family, which is hereby referred to as Tv26S-Proteasome. Analysis of the amino acid sequence of this gene shows a significant homology with other eukaryotic 26S proteasome family such as those of *T. rubrum* (Naeimi et al., 2007), *A. capsulatum* (Birren et al., 2008 a), *N. crassa* (Galagan et al., 2008), *C. immitis* (Birren et al., 2008 b), *A. clavatus* (Nierman, 2008) and *Penicillium marneffei* (Fedorova et al., 2008). Investigation of amino acid composition in 26S proteasome family revealed arginine and aspartic acid as common amino acids in these proteins. The amino acid composition of the 26S proteasome regulatory 6B/Rpt3 subunit in *T. verrucoum* indicates the amount of arginine and aspartic acid as 8.70 and 9.00%, respectively. Besides, the 26S protea-some regulatory 6B/Rpt3 subunit in *T. verrucoum* is rich in lysine (11.10%) and glutamic acid (7.20%). In contrast, the amount of methionine and phenyl alanine (1.50%) was poor and the amounts of tyrosine, tryptophan and glutamine were zero.

In addition, there was no *intron* identified during sequencing of all PCR fragments of 26S proteasome regulatory 6B/Rpt3 subunit in *T. verrucoum*. To the best of our knowledge, Tv26S-Proteasome is the first 26S proteasome regulatory 6B/Rpt3 subunit gene of this fungi characterized. Recognition of potential roles of this recently characterized gene in the physiology of *T. verrucoum* is still under exploration. The molecular characterization of Tv26S-Proteasome gene may reveal the practical individuality of Tv26S-Proteasome and its probable role in the pathogenesis of dermatophyte infections due to *T. verrucoum*.

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