

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 (Cabanes, 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,

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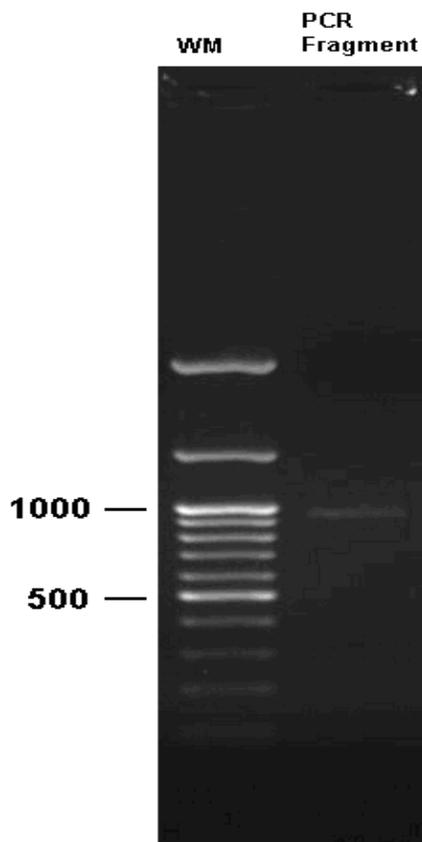


Figure 1. PCR product of the *T. verrucosum* 26S proteasome regulatory subunit 6B. WM: Molecular weight markers.

homeostatic control, cell cycle and cell differentiation (Hilt and Wolf, 1995; Sweder and Madura, 2002). Its structure has a core (20S) and one or two caps (19S) (Wolf and Hilt, 2004; Chouduri et al., 2008; Kurepa and Smalle, 2008). The core consists of four rings. The cap or regulatory particle (RP) has two parts: lid and base (Isono et al., 2007; Velichutina et al., 2004). Lid consists of Rpn3-8, 11 and 12 (regulatory particle none ATPase) and base contains Rpt1-6 (regulatory particle triple A), Rpn1, 2 and 10. RPs have the other names, for example Rpt3 also named 6B, S6, Tbp7, Yta2/Ytn1, P48 and mts2 (Yang et al., 2004; Li and Ching, 2002).

Characterization of the gene encoding 26S proteasome may offer more information for controlling these infections. In the present study, we report the identification and characterization of 26S proteasome regulatory particle 6B/Rpt3 subunit gene in *T. verrucosum*.

MATERIALS AND METHODS

Isolation of nucleic acids

High molecular weight DNA from *T. verrucosum* was extracted by

an improvement of the method of Rezaie et al. (2000). We cultured *T. verrucosum* in sbouraud'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 μ l of proteinase-K (20 mg/ml). This suspension was incubated at 65°C for 1 h and centrifuged at 2500 \times g for 15 min. After addition of 25 μ l 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 \times 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 5'-CGAGAAGCCGGACGTGACATAC-3' as sense and NaAs1 5'-CGGTCTTGACCTGAGCAGCATAGGC-3' as reverse primers were selected for amplifying the gene in *T. verrucosum*. 20 pM of each primer was added in a volume of 50 μ l that consists of 10X buffer with $MgCl_2$ 10 μ l, dNTP 1 μ l, genomic DNA 1 μ l, sense primer 1.5 μ l, reverse primer 1.5 μ l and thermo stable DNA polymerase 1.5 μ l (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 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%), *A. fumigatus* (51%) and

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aat atc gag ccc cgg aaa ggc gcg aaa act gac cga gat gat gac aat act acg ccg aag      60
  N   I   E   P   R   K   G   A   K   T   D   R   D   D   D   N   T   T   P   K      20
agg tta caa aga cat cta aag ccc caa gag aaa gaa atc aag gat aag cag aac agc ttc      120
  R   L   Q   R   H   L   K   P   Q   E   K   E   I   K   D   K   Q   N   S   F      40
aag cga gaa atc ctc cag ccc aag aag aaa tta aac gaa tac aat gtg ttg ccc agg aca      180
  K   R   E   I   L   Q   P   K   K   K   L   N   E   Y   N   V   L   P   R   T      60
gcg gca aat tca aag aag caa tcg acc aaa aac acc ggg atc gta aaa tcg tca aca ggc      240
  A   A   N   S   K   K   Q   S   T   K   N   T   G   I   V   K   S   S   T   G      80
tcc aat gat aac atc aaa atc cga tct aca ctt gac cgc gaa ctg gag aaa cca gcc tcc      300
  S   N   D   N   I   K   I   R   S   T   L   D   R   E   L   E   K   P   A   S      100
tcc gta gcc cga aaa cgg aat tcc aaa tcc ctc gta gac ata ctg aca ccg aaa aat aat      360
  S   V   A   R   K   R   N   S   K   S   L   V   D   I   L   T   P   K   N   N      120
gaa tcc atg cca agc aaa cac aaa aag cca gac aaa aca tat gcg aac ata gga gag atg      420
  E   S   M   P   S   K   H   K   K   P   D   K   T   Y   A   N   I   G   E   M      140
gat aag cag aaa caa gag act aga gaa gcc atc gaa caa cca ata aaa cat ttc gac atg      480
  D   K   Q   K   Q   E   T   R   E   A   I   E   Q   P   I   K   H   F   D   M      160
tac aaa caa aat caa aac gac ccc ccg cgc ggt ggg atc aga cac cac ccc ccc gga aac      540
  Y   K   Q   N   Q   N   D   P   P   R   G   G   I   R   H   H   P   P   G   N      180
ggc aaa acc agg cct gtc aag gcg ggc gcg aac ggc tca aaa gcc aac gtc aac cgc gaa      600
  G   K   T   R   P   V   K   A   G   A   N   G   S   K   A   N   V   N   R   E      200
gcc agc aac cag ttc aga caa aac caa cca gga gaa acg cct cgc atc gtc cgc gac ata      660
  A   S   N   Q   F   R   Q   N   Q   P   G   E   T   P   R   I   V   R   D   I      220
ttc cac atg gcc cga gaa aaa gcc ccg gca gac gaa att gat gcc act gcc acc aag cga      720
  F   H   M   A   R   E   K   A   P   A   D   E   I   D   A   T   A   T   K   R      240
agc gac gcg cag aac ggt gcc aaa cga gag ctg caa cat atc cag ctg gaa ttg ctc aac      780
  S   D   A   Q   N   G   A   K   R   E   L   Q   H   I   Q   L   E   L   L   N      260
caa aac gaa gcc ttc gac cag acc aac aac gga aaa gtc atc ata gca aca gca ggt gcc      840
  Q   N   E   A   F   D   Q   T   N   N   G   K   V   I   I   A   T   A   G   A      280
gaa tgc ggc acc acc caa ccc cgt acc gct act cct gaa gat aag atc gac tcc ccg gag      900
  E   C   G   T   T   Q   P   R   T   A   T   P   E   D   K   I   D   S   P   E      300
ctg cga gat aaa cga gaa cga cga cga agc atc act act att gcc ggc aac atg tca cgg      960
  L   R   D   K   R   E   R   R   R   S   I   T   T   I   A   G   N   M   S   R      320
tcg cca gaa atc gac aac aac tcg ctc aat gtc cgc      996
  S   P   E   I   D   N   N   S   L   N   V   R      332

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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 marneffeii* (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 proteasome 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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REFERENCES

- Aste N, Pau M, Aste N (2005). *Tinea manuum bullosa*. *Mycoses*, 48(1): 80-81.
- Birren B, Lander E, Galagan J, Nusbaum C, Devon K, Ma LJ, Henn M, Jaffe D, Butler J, Alvarez P, Gnerre S, Grabherr M, Kleber M, Mauceli E, Brockman W, Rounsley S, Young S, LaButti K, Pushparaj V, DeCaprio D, Crawford M, Koehrsen M, Engels R, Montgomery P, Pearson M, Howarth C, Larson L, Luoma S, White J, Yandava C, Kodira C, Zeng Q, Oleary S, Alvarado L, Taylor J, Sil A, Goldman B (2008). *Ajellomyces capsulatus* NAM1 26S protease regulatory subunit 6B HCAG_00039) partial mRNA. *ACCESSION XM_001542943*. <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.
- Birren B, Lander E, Galagan J, Devon K, Nusbaum C, Henn M, Ma LJ, Jaffe D, Butler J, Alvarez P, Gnerre S, Grabherr M, Kleber M, Mauceli E, Brockman W, Rounsley S, Young S, LaButti K, Pushparaj V, DeCaprio D, Crawford M, Koehrsen M, Engels R, Montgomery P, Pearson M, Howarth C, Kodira C, Zeng Q, Yandava C, Alvarado L, Oleary S, Orbach MJ, Galgiani J, Mandel MA, Kellner EM (2008b). *Coccidioides immitis* RS 26S protease regulatory subunit 6B homolog (CIMG_03688) partial mRNA. *ACCESSION XM_001244246*. <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.
- Cabanes FJ (2000). Dermatophytes in domestic animals. *Revista Iberoamericana de Micología*. pp. 104-108.
- Choudhuri AU, Tokumoto T, Dohra H, Ushimaru T, Yamada S (2008). Functional and biochemical characterization of the 20S proteasome in a yeast temperature-sensitive mutant, rpt6-1. *BMC Biochem*. 9: p. 20.
- Fedorova ND, Joardar V, Maiti R, Schobel S, Amedeo P, Galens K, Inman JM, White OR, Whitty BR, Wortman JR, Nierman W (2008). *Penicillium marneffeii* ATCC 18224 proteasome regulatory particle subunit Rpt3, putative, mRNA. *ACCESSION XM_002143503*. <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.
- Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S, Rehman B, Elkins T, Engels R, Wang S, Nielsen CB, Butler J, Endrizzi M, Qui D, Ianakiev P, Bell-Pedersen D, Nelson MA, Werner-Washburne M, Selitrennikoff CP, Kinsey JA, Braun EL, Zelter A, Schulte U, Kothe GO, Jedd G, Mewes W, Staben C, Marcotte E, Greenberg D, Roy A, Foley K, Naylor J, Stange-Thomann N, Barrett R, Gnerre S, Kamal M, Kamysseles M, Mauceli E, Bielke C, Rudd S, Frishman D, Krystofova S, Rasmussen C, Metznerberg RL, Perkins DD, Kroken S, Cogoni C, Macino G, Catcheside D, Li W, Pratt RJ, Osmani SA, DeSouza CP, Glass L, Orbach MJ, Berglund JA, Voelker R, Yarden O, Plamann M, Seiler S, Dunlap J, Radford A, Aramayo R, Natvig DO, Alex LA, Mannhaupt G, Ebole DJ, Freitag M, Paulsen I, Sachs MS, Lander ES, Nusbaum C, Birren B (2008). *Neurospora crassa* OR74A 26S protease regulatory subunit 6B partial mRNA. *ACCESSION XM_954811*. <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.
- Hanna J, Finley D (2007). A proteasome for all occasions. *FEBS Lett*. 581(15): 2854-2861.
- Hilt W, Wolf DH (1995). Proteasome of the yeast *saccharomyces cerevisiae*: genes, structure and functions. *Mol. Biol. Rep.* 21(1): 3-10.
- Isono E, Nishihara K, Saeki Y, Yashiroda H, Kamata N, Ge L, Ueda T, Kikuchi Y, Tanaka K, Nakano A, Toh-e A (2007). The assembly pathway of the 19S regulatory particle of the yeast 26S proteasome. *Mol. Biol. Cell*. 18(2): 569-580.
- Kane J, Smitka C (1978). Early detection and identification of *Trichophyton verrucosum*. *J. Clin. Microbiol.* 8(6): 740-747.
- Kurepa J, Smalle JA (2008). Structure, function and regulation of plant proteasomes. *Biochimie*. 90(2): 324-35.
- Li Z, Ching CW (2002). Functional characterization of the 11 Non-ATPase subunit proteins in the Trypanosome 19 S proteasomal regulatory complex. *J. Biol. Chem.* 277(45): 42686-42693
- Naeimi B, Noorbakhsh F, Safari MR, Rezaie S (2007). *Trichophyton rubrum* 26S proteasome regulatory subunit 6B gene, partial cds. *ACCESSION EU304456*. <http://blast.ncbi.nlm.nih.gov/Blast.cgi>
- Nierman WC (2008). *Aspergillus clavatus* NRRL 1 proteasome regulatory particle subunit Rpt3, putative (ACLA_039290), partial mRNA. *ACCESSION XM_001271139*. <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.
- Oborilova E, Rybnikar A (2005). Experimental dermatophytosis in calves caused by *Trichophyton verrucosum* culture. *Mycoses*, 48(3): 187-191.
- Rezaie S, Ban J, Mildner M, Poitschek C, Brna C, Tschachler E (2000). Characterization of a cDNA clone, encoding a 70 kDa heat shock protein from the dermatophyte pathogen *Trichophyton rubrum*. *Gene*. 241(1): 27-33.
- Rezaie S, Pourmojib M, Tschachler E (1999). Isolation of total RNA

- from dermatophytes. *Mycoses*, 42(11-12): 615-7.
- Sullivan JA, Shirasu K, Deng XW (2003). The diverse roles of ubiquitin and the 26S proteasome in the life of plants. *Nat. Rev. Genet.* 4(12): 948-58.
- Sweder K, Madura K (2002). Regulation of repair by the 26S proteasome. *J. Biomed. Biotechnol.* 2(2): 94-105.
- Velichutina I, Connerly PL, Arendt CS, Li X, Hochstrasser M (2004). Plasticity in eucaryotic 20S proteasome ring assembly revealed by a subunit deletion in yeast. *EMBO J.* 23(3): 500-510.
- Wakata Y, Tokumoto M, Horiguchi R, Ishikawa K, Nagahama Y, Tokumoto (2004). Identification of α -type subunits of the *Xenopus* 20S proteasome and analysis of their changes during the meiotic cell cycle. *BMC Biochem.* 5(1): p. 18.
- Wolf DH, Hilt W (2004). The proteasome: a proteolytic nanomachine of cell regulation and waste disposal. *Biochem. Biophys. Acta* 1695(1-3): 19-31.
- Yang P, Fu H, Walker J, Papa CM, Smalle J, Ju YM, Vierstra RD (2004). Purification of the *Arabidopsis* 26S proteasome. *J. Biol. Chem.* 279(8): 6401-6413.
- Yerlukaya A (2004). Cellular functions of the 26S proteasome. *Turk. J. Biol.* 28: 31-38
- Zeng LR, Sanchez MV, Zhu T, Wang GL (2006). Ubiquitination-mediated protein degradation and modification: an emerging theme in plant-microbe interactions. *Cell. Res.* 16(5): 413-426.