Short Report

Molecular Characterization of a Squalene epoxidase Gene in Dermatophyte Pathogen Trichophyton tonsurans

Kamyar Motavaze*1, Zahra Namvar1, Massoud Emami1, Fatemeh Noorbakhsh2 and Sassan Rezaie2

1Dept. of Microbiology, School of Basic Sciences, Islamic Azad University, North Tehran Branch, Tehran; 2Division of Molecular Biology, Dept. of Mycology and Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background: Trichophyton tonsurans is one of the dermatophyte fungi which invades the skin and hair of human. 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 Squalene epoxidase gene which is related to synthesis of ergosterol in this fungus. Methods: Pairs of 23 and 24 nucleotides primers were designed from highly conserved regions of the similar genes in other fungi. Mentioned primers were utilized in PCR by using isolated genomic DNA of T. tonsurans whereas the PCR fragments were then sequenced. Results and Conclusion: Nucleotides (n = 558) have been sequenced from this new gene which encodes a polypeptide with 186 amino acids. Sequences comparison in gene data banks (NCBI, NIH) for this part of DNA and its deduced amino acid revealed significant homology with members of the eukaryotic Squalene epoxidase. Iran. Biomed. J. 12 (1): 55-58, 2008

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INTRODUCTION

Eukaryotic and prokaryotic cells have a membrane that isolates them from environment. The component of cell membrane is very important, because of contributing in survival of cell. Sterols are one of the necessary compounds in most eukaryotic cells but fungi have a unique kind of sterol named ergosterol. This compound is important in different actions such as regulation of cycle cell, enzyme activation and permeability [1].

Squalene epoxidase is a microsomal membrane-associated enzyme that acts as an important regulator in the sterol biosynthetic pathway. Ergosterol contributes to photooxidation in fungi. This enzyme needs oxygen, Nicotine amide Adenine Dinucleotide Phosphate Hydrogen) and Flavin Adenine Dinucleotide for activation [2].

In yeasts, Squalene epoxidase makes correspondence between lipid particles and endoplasmic reticulum [3]. The main target of some antifungal drugs is the mentioned enzyme. In recent studies, it has been proved that Trichophyton rubrum and Aspergillus fumigatus are resistant to terbinafine [4, 5]. Dermatophytes are filamentous fungi that are keratinophilic and invade the skin and hair of human. Trichophyton tonsurans is responsible for the vast majority of dermatophyte infections [6-8]. This fungus is an important agent of tinea gladiatorum [9]. In this study, the molecular identification of Squalene epoxidase gene in T. tonsurans was the main aim.

MATERIALS AND METHODS

Fungal Specimen Preparation. In order to perform this fundamental study, sampling was done

*Corresponding Author; Tel. (+98-21) 2271 6582; Fax: (+98-21) 2270 0102; E-mail: motavaze49@yahoo.com
from patients suspicious to dermatophytosis in Mycology Laboratory at School of Public Health, Tehran University of Medical Sciences (Tehran, Iran) in 2006. After macroscopical and microscopical examinations, the existence of *T. tonsurans* was proved.

**DNA isolation.** High molecular weight DNA from *T. tonsurans* was isolated by modified method of Choi *et al.* [10] 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: Tris-HCl (pH 8.0), EDTA, SDS, and proteinase-K. The suspension was then incubated at 65°C for 1 hour and the cellular debris was removed by centrifugation at 3000 ×g for 15 min. After extracted once with phenol-chloroform-isooamyl alcohol and once with chloroform-isooamyl alcohol, the DNA was precipitated by addition of an equal volume of ethanol and resuspended in distilled water.

**PCR analysis.** PCR analysis of genomic DNA was performed according to the standard protocol using synthetic oligonucleotide primers. Briefly, 2.5 µl of each primer (OD 20) was added to a volume of 45 µl containing: 10 × PCR buffer + MgCl₂, 10 mM dNTP mix, 1 unit/µl of Taq DNA polymerase and template (genomic DNA). The PCR program employed for amplification of the appropriate part of this gene was 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min and 30 s; whereas the program was repeated 35 cycles. PCR products were analysed by electrophoresis through a 1% agarose gel.

**Purification and sequencing of PCR products.** The fragment deduced by PCR amplification should be purified in order to eliminate waste materials. For this purpose, desired fragment was cut and purified by Qiagen Kit (Qiagen Company, Germany) Sequencing of the PCR fragments. After purification of PCR fragments by Qiagen Kit, sequencing of the genomic DNA was performed with the Dye Terminator Cycle Sequencing Kit (MWG Biotech, Germany), by using the isolated double-stranded DNA as template and synthetic oligomeric internal primers were designed according to the highly conserved regions from other fungi. Sequencing of each part was repeated. The nucleotide sequence of DNA was compared with the counter parts in gene data bank (NCBI, NIH).

**RESULTS AND DISCUSSION**

**Isolation and characterization of a Squalene epoxidase gene from *T. tonsurans*.** After amplification of a part of this gene by PCR, it has been identified as a new *Squalene epoxidase* gene in dermatophyte pathogen *T. tonsurans*. The approximate length of this new gene was 600 bp according to the comparison with size marker (Fig. 1).

![Fig. 1. PCR product deduced from a part of Squalene epoxidase gene in T. tonsurans (lane 2). The length of size marker bands (lane 1) is 1300, 1200, 1100, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp (from top to bottom).](http://ibj.pasteur.ac.ir)
Fig. 2. Partial nucleotide and its deduced amino acid sequences of *Trichophyton tonsurans* Squalene epoxidase gene.

*A. fumigatus* and 65% to *Aspergillus niger*. The amino acid composition of this protein indicates that it is rich in glycine (9.13%) and leucine (8.6%); in contrast, the amounts of tryptophan (0.53%) and glutamine (1.07%) were poor.

In the present study, we report the identification and molecular characterization of the *T. tonsurans* gene encoding a *Squalene epoxidase* enzyme. Generally, most of the molecular investigations have been carried out on *Trichophyton* and *Microsporum* among three genera of dermatophytes. *T. tonsurans* is an anthropophilic pathogen which causes *tinea capitis*, *tinea corporis* and *tinea unguium* and considered as an important causative agent of dermatophytosis in human. According to the involvement of this pathogen in human infections, we decided to survey about one important molecular aspects of this fungus which probably influences its pathogenicity and resistance to antifungal drugs. Less molecular information is available about this fungus; however there are reports on genome sequencing of this fungus which has medium density. There are some surveys on internal transcribed spacer (ITS) sequencing, phylogeny of this fungus and similarity of this dermatophyte to other fungi [11]. A few restriction enzymes such as *Mva*I and *Hinf*I have been used for rDNA and ITS regions of mentioned fungus [12]. It is noteworthy that some molecular diagnosis methods i.e. RAPD, RFLP and DNA-fingerprinting were used in dermatophytes identification [13, 14]. *Squalene epoxidase* is associated with ergosterol synthesis which is one of the membrane components influences on permeability, photooxidation and activation of enzymes [1]. This enzyme has been detected in *T. rubrum*, *A. fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans*, *Neurospora crassa* and human [2, 5, 8, 15-17].

Comparison analysis of this new gene sequence and its deduced protein revealed a significant homology with other fungi e.g. 95% with *T. rubrum*, 75% with *E. nidulans*, 74% with *A. fumigatus* and 65% with *A. niger*. This is obvious that the maximum and minimum amounts of amino acids are the same among these proteins. Our studies reveals a significant similarity with those of Osborne et al. [5] on *T. rubrum* as leucine and glycine were major amino acids and tryptophan was minor [5]. In the Rocha et al. [18] survey, leucine and alanine were major whereas tryptophan and cysteine were in the
least. Gracia et al. [NCBI, NIH; Accession No: AY619002] concluded that A. fumigatus Squalene epoxidase was rich in leucine and valine whereas amounts of tryptophan, cysteine and methionine were poor which reveals a significant consistency with the present survey. Dave et al. [NCBI, NIH; Accession No: AJ748126] proved that in the Squalene epoxidase of A. niger, there is a great homology in amino acids amounts with T. tonsurans.

It can be concluded that there is a great similarity between nucleotide sequence of Squalene epoxidase gene and its deduced amino acids of T. tonsurans with other fungi and eukaryotes. It is desired to design new fungal drugs by further investigation on this new gene.

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REFERENCES