

Metadata Analysis

Metadata analysis of the squalene epoxidase gene in dermatophytes.

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Abstract

Background: Squalene epoxidase gene is an azole antifungal target enzyme in the ergosterol biosynthesis pathway in fungi—the inhibition of enzyme aggregate squalene, a type of ergosterol derivative depletion that leads to fungal growth inhibition. Squalene epoxidase widely diverges in three fungal groups of dermatophytes Trichophyton, Microsporum and Epidermophyton.

Methodology: The study has demonstrated a variety of squalene epoxidase genes in the dermatophyte genome. Squalene epoxidase gene was also studied for possible single nucleotide polymorphism (SNPs) in the Trichophyton group's homologs and found multiple SNP positions that induce non-synonymous mutation and change the sequence of amino acid into protein structure that can change phenylalanine to leucine.

Results: Squalene epoxidase is widely present in dermatophytes. Mutation in the squalene epoxidase gene can mislead normal sterol formation in the fungal cell membrane that induces fungal resistance against several drugs, including azoles and polyenes. Squalene epoxidase gene carries 2 - 3 transcripts and 2 - 3 number of exons. Squalene epoxidase gene has FAD-dependent oxidoreductases and NADP binding domain conserved domains in fungal groups of dermatophytes.

Conclusion: This study analyzed the abundance of the squalene epoxidase gene, suggesting that squalene epoxidase gene resistant mutants can occur naturally. Thus squalene epoxidase gene should be extensively studied in order to increase the potential of available antifungals.

Keywords

Squalene Epoxidase, Resistant Mutant, Trichophyton, Microsporum, Epidermophyton.



Introduction

Dermatophytes are a very specialized group of keratinophilic fungi responsible for superficial mycoses of humans and animals¹. Dermatophyte infections are a worldwide major health concern, specifically in an immunocompromised individual. They may pose severe risks. Dermatophytes are divided into three genera: *Microsporum*, *Trichophyton* and *Epidermophyton*, although the precise taxonomic associations of this fungal group are far from being fully determined. Regardless of the common occurrence of dermatophyte infections, little is known about these pathogens' molecular biology. Dermatophytes are slow-growing as compare to other more significant fungal pathogens. They are not very well studied for their metabolic reactions and stress adaptive mechanisms. However, several natural mutants are investigated in clinical and agricultural practices².

Squalene epoxidase is a monooxygenase that catalyzes the conversion of squalene to 2, 3-oxidosqualene. It is a microsomal membrane-associated enzyme that functions as a significant regulator in the biosynthetic pathway of sterols. Ergosterol helps in the photo-oxidation of fungi. This enzyme needs oxygen, Nicotine amide, Adenine dinucleotide, Phosphate hydrogen and Flavin adenine dinucleotide for stimulation³. Previously, terbinafine resistant isolates have been reported in increasing number. Terbinafine inhibits the Squalene epoxidase (SE) enzyme and interferes with the ergosterol biosynthesis, an integral cell membrane constituent. Mutations have been identified within the squalene epoxidase gene like clinical terbinafine resistant isolates⁴.

Squalene epoxidase gene is involved in ergosterol biosynthesis, which converts squalene to lanosterol in sterol biosynthesis. This enzyme functions as a major regulator in the biosynthetic pathway to sterols⁵. Mutation in this gene causes resistance against fluconazole, ketoconazole, itraconazole, voriconazole and terbinafine drugs because of the modification of the target enzyme by mutation induces increased drug efflux, stress adaptation and overexpression of drug efflux, which degrade the drug effects⁶.

Squalene epoxidase is an enzyme involved in the early steps of ergosterol biosynthesis, is inhibited by allylamines such as terbinafine. This inhibition contributes to squalene accumulation that is toxic for fungi. Resistance is due to single point mutations in the gene encoding squalene epoxidase. Both mutations introduced missense replacements of amino acids (Leu393Phe in one case and Phe397Leu in the other), leading to 100 fold higher MIC⁷. Terbinafine resistant mutants of *Trichophyton rubrum*, *Aspergillus nidulans* and *Aspergillus fumigatus* strains were identified. This mutant carries a mutation in the squalene epoxidase gene that contributes to the replacement of leucine by phenylalanine in the mutant of Erg1⁸.

ERG1 (squalene epoxidase) protein comprised of two flavin adenine dinucleotide domains (FAD) and one nucleotide-binding domain (NB). ERG1 required oxygen from energy molecules, NADPH and FAD. ERG1 protein alignment with other squalene epoxidase showed two strongly conserved FAD-binding domains, FAD1 and FAD 2¹⁰. Spontaneous PCR mutagenesis of the ERG1 gene identified one ERG1 allele that brings a mutation. It contributes to a single exchange of amino acids in the FAD1 domain near the ERG1 N-terminus¹¹.

Methodology

Squalene epoxidase gene sequence

Squalene epoxidase gene from the dermatophytic fungi *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton interdigitale*, *Microsporum canis*, *Aspergillus Versicolor*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans* and *Candida glabrata* obtained from NCBI gene sequences. Squalene epoxidase and related proteins were explored within acquired amino acid sequences of a FAD-binding domain. In the next step, FAD-binding proteins were searched against a transmembrane domain NADB. Squalene epoxidase contains a putative FAD-binding site and is a crucial enzyme in the sterol biosynthetic pathway. Putative transmembrane regions are found in the proteins C-terminus. For the detection

of some unpredicted genes, we explored raw genomic sequences using NCBI nucleotide blast. Sequences of all known Squalene epoxidase genes were disguised from genomic sequences. All amino acid sequences of previously identified Squalene epoxidase genes were used as queries in nucleotide sequence blast.

For all Trichophyton species, similarity ranges from 44.66%-45.34%. Query cover ranges from 95%-99%. For all *Microsporum* and *Aspergillus* species, similarity ranges from 37.34%-45.53%. The range of query cover is 84%-99%.

Squalene epoxidase protein prediction

The number of genes contained FAD and NABD binding domain, which was similar in all species of dermatophytes. These domains have an alpha-beta-alpha configuration. NAD binding involves numerous hydrogen and van der Waals contacts.

Transcript Exon and intron analysis

Several exons and introns of different species of dermatophytes were obtained from NCBI. A single gene can produce multiple different RNAs (transcripts). Similarly, the numbers of transcripts were also obtained from the CBI database (<https://www.ncbi.nlm.nih.gov/>).

Squalene epoxidase gene Phylogenetic Analysis

A sequence alignment was done with ClustalX. Squalene epoxidase genes were determined by a conserved domain database. Phylogenetic analysis was made in MEGA6. Phylogenetic analysis was done using maximum possibility and neighbourhood joining method.

Single nucleotide polymorphism

Single nucleotide polymorphism (SNPs) analysis was found in the Squalene epoxidase gene of the Trichophyton group of dermatophytes. SNPs were

obtained from NCBI and compared with the reported.

Results

Bioinformatics survey of Squalene epoxidase gene

The squalene epoxidase gene was analyzed using different bioinformatics tools. The comparison was made based on percentage similarity, exon count, conserved domains and query cover. Sequences were aligned, and phylogenetic trees were constructed. The squalene epoxidase gene in dermatophytes has an average sequence length of 489 amino acids.

Squalene epoxidase protein prediction

NAD binding involves numerous hydrogen-bonds and van der Waals contacts, particularly H-bonding of residues in turn between the first strand and the subsequent helix of the Rossmann-fold topology. Characteristically, this turn exhibits a consensus binding pattern. The first 2 glycines participate in NAD(P)-binding, and the third facilitate close packing of the helix to the beta-strand (<https://go.usa.gov/xARNY>).

Prediction of conserved domains of Squalene epoxidase

In this study, we used genomic sequences of all available dermatophyte species. The first step is the identification of all Squalene epoxidase genes within their genomes. Squalene epoxidase consists of two types of domain. FAD and NADB. The identified genes were containing FAD and NABD binding domain. The E value of $1e^{-135}$ was used. Query cover ranges from 95% to 99%. The length of the amino acid sequence was 489 bp. Percentage similarity was very low such as 45.34% for *Trichophyton rubrum*. *Trichophyton rubrum* has the highest percentage similarity to other Trichophyton species, as shown in table 1.

Table 1: Paralogs of Squalene epoxidase gene

S. no	Species	Accession no	Query cover	E-value	Percentage similarity	Amino acid sequence length	Conserved domains
1	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	APQ47162.1	99%	5e-136	44.90	489	FAD-dependent oxidoreductase, NADP binding proteins
2	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	ATA67033.2	99%	7e-136	44.90	489	FAD-dependent oxidoreductase, NADP binding proteins
3	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIJ32792.1	99%	1e-135	44.90	489	FAD-dependent oxidoreductase, NADP binding proteins
4	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIJ32790.1	99%	4e-135	44.90	489	FAD-dependent oxidoreductase, NADP binding proteins
5	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIJ32789.1	99%	5e-135	44.69	489	FAD-dependent oxidoreductase, NADP binding proteins
6	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	ATA67030.2	99%	6e-135	44.69	489	FAD-dependent oxidoreductase, NADP binding proteins
7	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIJ32791.1	99%	6e-135	44.69	489	FAD-dependent oxidoreductase, NADP binding proteins
8	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QHO61327.1	98%	1e-134	44.66	487	FAD-dependent oxidoreductase, NADP binding proteins
9	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QGA31127.1	95%	1e-129	44.82	453	FAD-dependent oxidoreductase, NADP binding proteins
10	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIB02548.1	95%	2e-129	44.82	453	FAD-dependent oxidoreductase, NADP binding proteins
11	squalene epoxidase [<i>Trichophyton</i>	XP_00323384 5.1	99%	5e-136	45.34	489	FAD-dependent oxidoreductase, NADP binding proteins

	<i>rubrum</i> CBS 118892]						
12	squalene epoxidase [<i>Trichophyton rubrum</i>]	AAQ18216.1	99%	1e-135	45.12	489	FAD-dependent oxidoreductase, NADP binding proteins
13	squalene epoxidase [<i>Trichophyton rubrum</i>]	AAZ08563.1	99%	4e-135	45.12	489	FAD-dependent oxidoreductase, NADP binding proteins
14	squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67047.1	99%	1e-135	44.90	489	FAD-dependent oxidoreductase, NADP binding proteins
15	squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67045.1	99%	2e-135	44.90	489	FAD-dependent oxidoreductase, NADP binding proteins
16	squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67048.1	99%	2e-134	44.69	489	FAD-dependent oxidoreductase, NADP binding proteins
17	squalene epoxidase [<i>Trichophyton interdigitale</i>]	AVR57463.2	99%	1e-135	44.90	489	FAD-dependent oxidoreductase, NADP binding proteins
18	squalene epoxidase [<i>Trichophyton interdigitale</i>]	AVU05317.1	99%	3e-135	44.69	489	FAD-dependent oxidoreductase, NADP binding proteins
19	squalene epoxidase [<i>Trichophyton interdigitale</i>]	AVU05318.1	99%	5e-135	44.69	489	FAD-dependent oxidoreductase, NADP binding proteins
20	squalene epoxidase [<i>Trichophyton interdigitale</i>]	QBL54211.1	98%	2e-134	44.66	487	FAD-dependent oxidoreductase, NADP binding proteins
21	squalene epoxidase [<i>Trichophyton interdigitale</i>]	QBL54209.1	98%	5e-134	44.66	487	FAD-dependent oxidoreductase, NADP binding proteins
22	squalene epoxidase [<i>Trichophyton tonsurans</i> CBS 112818]	EGD99021.1	99%	7e-136	44.90	489	FAD-dependent oxidoreductase, NADP binding proteins

Prediction of exon count and number of transcripts

An exon is a coding region of a gene that contains the information necessary to encode a protein. Numbers of exons of different species of dermatophytes were obtained from NCBI. A single gene can produce multiple different RNAs (transcripts). All squalene epoxidase genes generally contain 2 to 3 transcripts. Similarly, the numbers of transcripts were also obtained from NCBI's genomic regions, as shown in table 2.

Table 2: Exon count and number of transcripts of paralogs of Squalene epoxidase gene.

S. no	Species	Accession no	No of exons	Transcript no
1	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	APQ47162.1	2	3
2	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	ATA67033.2	1	2
3	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIJ32792.1	2	3
4	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIJ32790.1	2	3
5	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIJ32789.1	2	3
6	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	ATA67030.2	1	2
7	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIJ32791.1	2	3
8	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QHO61327.1	2	2
9	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QGA31127.1	2	3
10	squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	QIB02548.1	2	3
11	squalene epoxidase [<i>Trichophyton rubrum</i> CBS 118892]	XP_003233845.1	2	3
12	squalene epoxidase [<i>Trichophyton rubrum</i>]	AAQ18216.1	2	2
13	squalene epoxidase [<i>Trichophyton rubrum</i>]	AAZ08563.1	2	3
14	squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67047.1	2	3
15	squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67045.1	2	3
16	squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67048.1	2	3
17	squalene epoxidase [<i>Trichophyton interdigitale</i>]	AVR57463.2	1	2
18	squalene epoxidase [<i>Trichophyton interdigitale</i>]	AVU05317.1	2	3
19	squalene epoxidase [<i>Trichophyton interdigitale</i>]	AVU05318.1	2	3

20	squalene epoxidase [<i>Trichophyton interdigitale</i>]	QBL54211.1	2	2
21	squalene epoxidase [<i>Trichophyton interdigitale</i>]	QBL54209.1	2	2
22	squalene epoxidase [<i>Trichophyton tonsurans</i> CBS 112818]	EGD99021.1	2	3

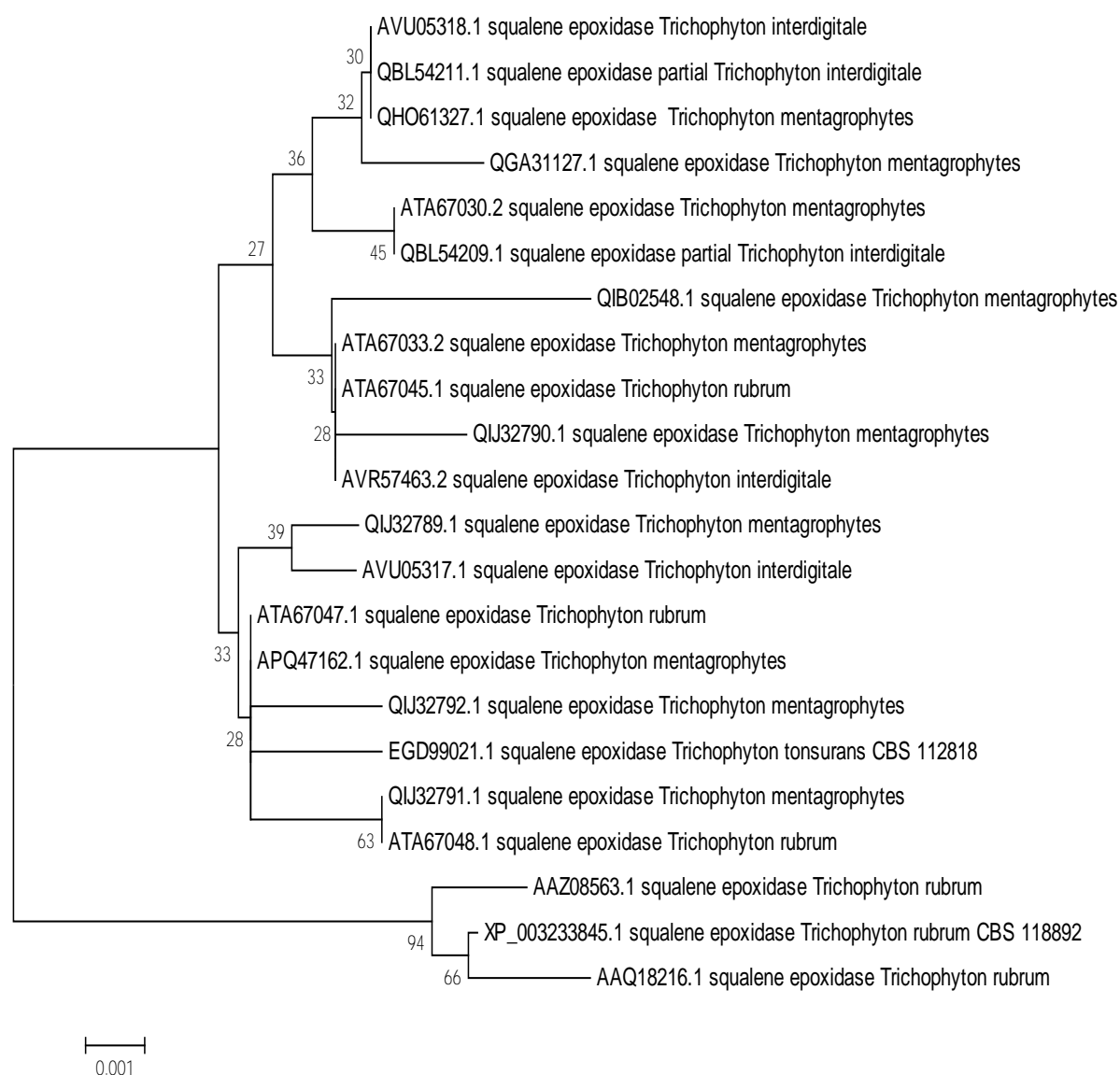


Figure 1: Multiple sequence alignment of amino acid sequences of 22 species were performed using clustalx. Phylogenetic tree of paralogs of squalene epoxidase protein sequences of various dermatophytes. The construction of the tree was performed with Mega 6. Sequences are annotated with their GenBank accession numbers.

Orthologs of Squalene epoxidase

Similarly for orthologs, Squalene epoxidase gene was compared with *Microsporum canis*, *Aspergillus versicolor*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans* and *Candida glabrata*. Conserved domain database search demonstrated the existence of conserved domain of squalene epoxidase gene. Another functional domain found was NADP binding domain, also recognized as Rossmann fold. Amino acid sequence length varies among different species from 405 bp to 512 bp. For orthologs, *Aspergillus fumigatus* has highest similarity for ERG1. Percentage similarity was low compared to paralogs of ERG1, and query cover was 98%, as shown in table 3.

Table 3: Orthologs of Squalene epoxidase gene.

S. no	Species name	Accession no.	Query cover	E-value	Percentage similarity	Amino acid sequence length	Conserved domains
1	squalene epoxidase [<i>Microsporum canis</i> CBS 113480]	XP_002849431. 1	84%	1e-106	42.86	425	FAD-dependent oxidoreductases, NADP binding proteins
2	squalene epoxidase [<i>Microsporum canis</i>]	BBC18146.1	84%	4e-105	42.35	425	FAD-dependent oxidoreductases, NADP binding proteins
3	squalene monooxygenase [<i>Nannizzia gypsea</i> CBS 118893]	XP_003170113.1	99%	2e-134	44.57	489	FAD-dependent oxidoreductases, NADP binding proteins
4	hypothetical protein ASPVEDRAFT_52898 [<i>Aspergillus versicolor</i> CBS 583.65]	OJJ02152.1	98%	1e-114	43.11	1427	FAD-dependent oxidoreductases, NADP binding proteins
5	squalene monooxygenase Erg1 [<i>Aspergillus flavus</i> NRRL3357]	XP_002378894. 1	98%	1e-128	42.98	480	FAD-dependent oxidoreductases, NADP binding proteins
6	squalene epoxidase [<i>Aspergillus flavus</i>]	RAQ67502.1	98%	2e-128	42.98	480	FAD-dependent oxidoreductases, NADP binding proteins
7	squalene monooxygenase Erg1 [<i>Aspergillus flavus</i> AF70]	KOC12150.1	98%	4e-128	42.76	480	FAD-dependent oxidoreductases, NADP binding proteins
8	squalene epoxidase [<i>Aspergillus flavus</i>]	RAQ47162.1	98%	8e-128	42.76	480	FAD-dependent oxidoreductases, NADP binding proteins
9	squalene monooxygenase Erg1	XP_753858.1	98%	2e-130	44.93	481	FAD-dependent oxidoreductases,

	[<i>Aspergillus fumigatus</i> Af293]						NADP binding proteins
10	squalene monooxygenase Erg1 [<i>Aspergillus fumigatus</i> Z5]	KMK59426.1	98%	4e-130	44.71	481	FAD-dependent oxidoreductases, NADP binding proteins
11	hypothetical protein CNMCM8812_000993 [<i>Aspergillus fumigatus</i>]	KAF4275551.1	98%	5e-128	44.93	481	FAD-dependent oxidoreductases, NADP binding proteins
12	squalene epoxidase 1 [<i>Aspergillus fumigatus</i>]	AAS60234.1	79%	5e-106	45.53	405	FAD-dependent oxidoreductases, NADP binding proteins
13	squalene epoxidase [<i>Aspergillus niger</i> CBS 513.88]	XP_001390258.1	98%	2e-130	45.41	512	FAD-dependent oxidoreductases, NADP binding proteins
14	SE-domain-containing protein [<i>Aspergillus niger</i> CBS 101883]	XP_025456429.1	98%	8e-131	45.41	478	FAD-dependent oxidoreductases, NADP binding proteins
15	squalene monooxygenase Erg1 [<i>Aspergillus fischeri</i> NRRL 181]	XP_001259905.1	98%	1e-126	44.93	481	FAD-dependent oxidoreductases, NADP binding proteins
16	squalene monooxygenase [<i>Candida albicans</i> P94015]	KGQ90519.1	99%	3e-115	38.71	496	FAD-dependent oxidoreductases, NADP binding proteins
17	squalene monooxygenase [<i>Candida albicans</i> SC5314]	XP_711894.1	99%	3e-115	38.71	496	FAD-dependent oxidoreductases, NADP binding proteins
18	squalene monooxygenase [<i>Candida albicans</i> Ca529L]	RLP62132.1	99%	3e-115	38.71	496	FAD-dependent oxidoreductases, NADP binding proteins
19	uncharacterized protein CAGL0D05940g [[<i>Candida glabrata</i>]	XP_445667.1	98%	1e-106	37.34	489	FAD-dependent oxidoreductases, NADP binding proteins
20	squalene monooxygenase [<i>Candida tropicalis</i> MYA-3404]	XP_002551185.1	98%	9e-115	38.98	497	FAD-dependent oxidoreductases, NADP binding proteins

Predicting exon count, a number of transcripts and chromosome location.

Numbers of exons were obtained from NCBI from a genomic context. All aspergillus species have only one gene transcript. Numbers of transcripts were also obtained from genomic regions from NCBI. The chromosome of almost all species was unknown except *Aspergillus fumigatus*, located on chromosome no 5 and *Candida albicans* are located on chromosome no 1 as shown in table 4.

Table 4: Exon count, number of transcripts and chromosomal location of orthologs of Squalene epoxidase gene.

S. no	Species	Accession no	No of exons	No of transcripts	Chromosome no
1	squalene epoxidase [<i>Microsporum canis</i> CBS 113480]	XP_002849431.1	3	2	Unknown
2	squalene epoxidase [<i>Microsporum canis</i>]	BBC18146.1	3	2	Unknown
3	squalene monooxygenase [<i>Nannizzia gypsea</i> CBS 118893]	XP_003170113.1	2	2	Unknown
4	hypothetical protein ASPVEDRAFT_52898 [<i>Aspergillus Versicolor</i> CBS 583.65]	OJJ02152.1	3	1	Unknown
5	squalene monooxygenase Erg1 [<i>Aspergillus flavus</i> NRRL3357]	XP_002378894.1	2	1	Unknown
6	squalene epoxidase [Aspergillus <i>flavus</i>]	RAQ67502.1	2	1	Unknown
7	squalene monooxygenase Erg1 [<i>Aspergillus flavus</i> AF70]	KOC12150.1	2	1	Unknown
8	squalene epoxidase [Aspergillus <i>flavus</i>]	RAQ47162.1	2	1	Unknown
9	squalene monooxygenase Erg1 [<i>Aspergillus fumigatus</i> Af293]	XP_753858.1	2	1	Chromosome 5
10	squalene monooxygenase Erg1 [<i>Aspergillus fumigatus</i> Z5]	KMK59426.1	2	1	Chromosome 5
11	hypothetical protein CNMCM8812_000993 [<i>Aspergillus fumigatus</i>]	KAF4275551.1	2	1	Unknown
12	squalene epoxidase 1 [<i>Aspergillus fumigatus</i>]	AAS60234.1	2	1	Unknown
13	squalene epoxidase [Aspergillus <i>niger</i> CBS 513.88]	XP_001390258.1	2	1	Unknown
14	SE-domain-containing protein [<i>Aspergillus niger</i> CBS 101883]	XP_025456429.1	2	1	Unknown
15	squalene monooxygenase Erg1 [<i>Aspergillus fischeri</i> NRRL 181]	XP_001259905.1	2	1	Unknown
16	squalene monooxygenase [<i>Candida albicans</i> P94015]	KGQ90519.1	1	1	Chromosome 1

17	squalene monooxygenase [<i>Candida albicans</i> SC5314]	XP_711894.1	1	1	Chromosome 1
18	squalene monooxygenase [<i>Candida albicans</i> Ca529L]	RLP62132.1	1	1	Chromosome 1
19	uncharacterized protein CAGL0D05940g [[<i>Candida</i> <i>glabrata</i>]	XP_445667.1	1	1	Chromosome D
20	squalene monooxygenase [<i>Candida tropicalis</i> MYA-3404]	XP_002551185.1	1	1	Unknown

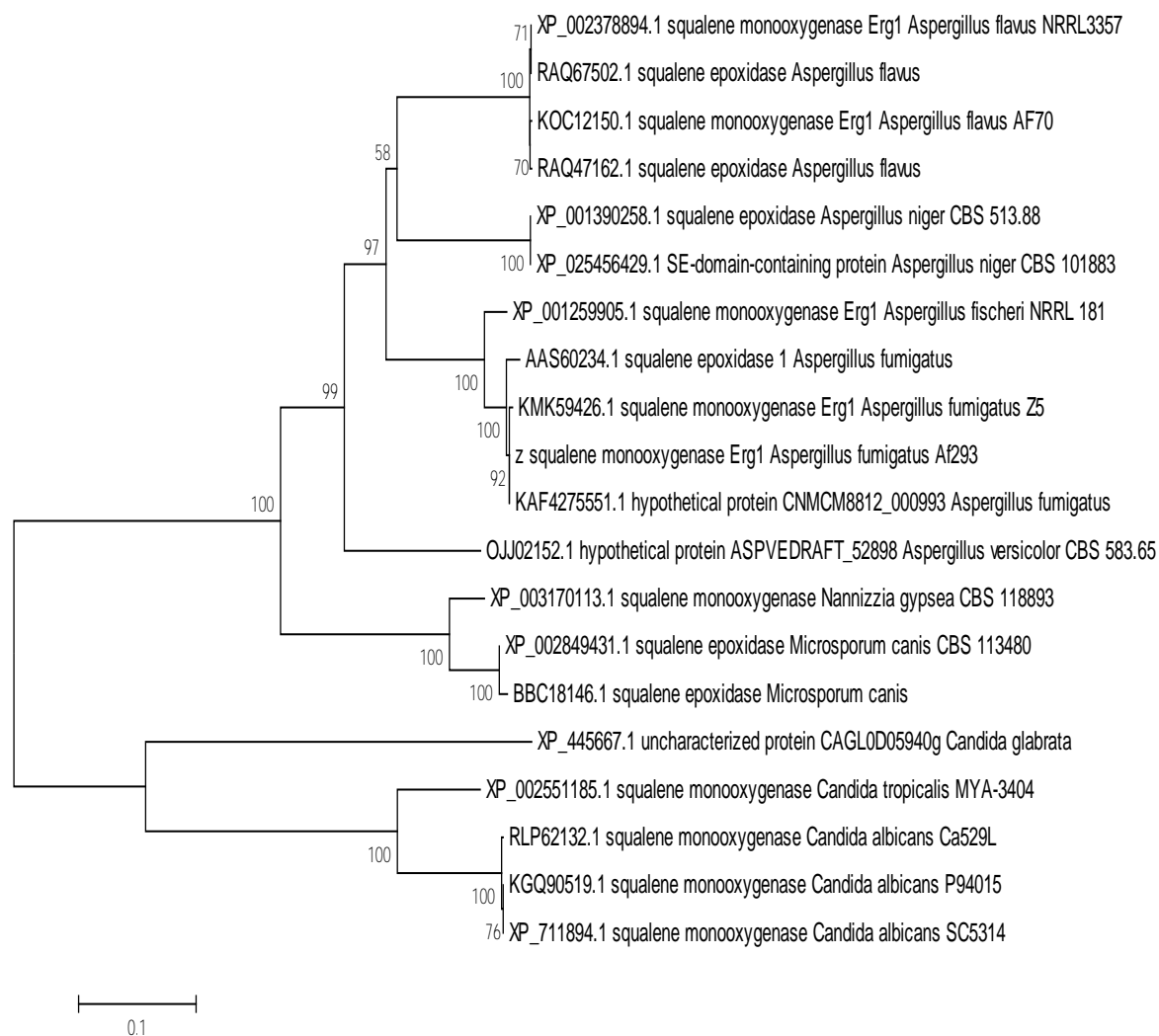


Figure 2: Phylogenetic tree of orthologs of squalene epoxidase amino acid sequences. The tree was constructed using the neighbour-joining method in MEGA6 software. Protein sequences were obtained from the Gene bank.

Single nucleotide polymorphisms (SNPs) in Squalene epoxidase gene

Single nucleotide polymorphisms (SNPs) have been identified in the squalene epoxidase gene of the Trichophyton group. Resistance in the gene encoding squalene epoxidase is attributable to point mutations. The mutation was observed in *Trichophyton interdigitale* and *Trichophyton rubrum* isolates. *Trichophyton interdigitale* nucleotide substitution in the Squalene epoxidase gene was CTC, which was substituted into TTC at 1184. Similarly, another substitution was found at position 1185, leading to the replacement of TTC by CTC. For *Trichophyton rubrum*, a substitution occurred at position 1380, leading to the replacement of CAT by TAT. Another substitution was at position 1175, leading to the replacement of TTC by TTA in the squalene epoxidase gene, as shown in table 5.

Table 5. SNPs in Squalene epoxidase gene of Trichophyton group.

Species	Accession no	Nucleotide substitution in SQLE gene	SNPs in this study	Reported 12 (Burmeister, Hipler et al. 2020)
Squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	MT371234.1	TTG→TTC	1184	1189
Squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	MT371233.1	TTG→TTC	1184	1189
Squalene epoxidase [<i>Trichophyton mentagrophytes</i>]	MT371235.1	CTC→TTC	1184	1189
squalene epoxidase, partial [<i>Trichophyton interdigitale</i>]	QBL54211.1	TTC→CTC	1185	1189
squalene epoxidase, partial [<i>Trichophyton interdigitale</i>]	QBL54209.1	TTC→CTC	1185	1189
squalene epoxidase [<i>Trichophyton rubrum</i>]	AAQ18216.1	CAT→TAT	1380	1380
squalene epoxidase [<i>Trichophyton rubrum</i>]	AAZ08563.1	TTT→TTA	1197	1177
squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67047.1	TTC→TTA	1175	1189
squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67045.1	TTC→TTA	1175	1189
squalene epoxidase [<i>Trichophyton rubrum</i>]	ATA67048.1	TAT→CAT	1368	1380

Discussion

Squalene epoxidase is a monooxygenase that catalyzes the conversion of squalene to 2, 3 oxidosqualene. Terbinafine inhibits squalene epoxidase, a key enzyme in the ergosterol biosynthesis pathway. This inhibition contributes to squalene aggregation, ergosterol depletion and the inhibition of growth of fungus¹³. This study was focused on the abundance of squalene epoxidase

in dermatophytes. Gene sequence Percentage similarity ranges were suggested from 44.66%-45.34%. Query cover ranges from 95% - 99%. The percentage similarity of *Trichophyton rubrum* was higher than other species of dermatophytes such as Trichophyton. *Trichophyton mentagrophytes* have one exon, while all other species have two exons. The number of transcripts was 2-3 for all species. Chromosomal locations of all species were

unknown except *Aspergillus fumigatus* was located on chromosome no 5¹⁴.

The number of detected genes contained FAD and NABD binding domain, which was similar in all genomes. These domains have an alpha-beta-alpha configuration. NAD binding involves numerous hydrogen, and van der Waals contacts¹⁵. *Trichophyton rubrum* has a percentage similarity of 45.12 with the squalene epoxidase gene. For orthologs, *Aspergillus fumigatus* has the highest percentage similarity¹⁶. The bioinformatics survey of the squalene epoxidase gene revealed that dermatophytes comprise a closely-related and diverged lineage of the squalene epoxidase gene. A variety of divergence of squalene epoxidase gene was found in three fungal groups of dermatophytes, while squalene epoxidase homologs were not found in Epidermophyton¹⁷.

Single-nucleotide polymorphism (SNP) is a substitution of a single nucleotide at a specific genome position. Single nucleotide polymorphisms occur within coding sequences of genes, non-coding regions of genes, or in the intergenic regions between genes. SNPs in the coding regions are of two kinds synonymous and non-synonymous SNPs. Synonymous SNPs do not affect protein or DNA sequence, while non-synonymous SNPs change the amino acid sequence of protein or DNA¹⁸. SNPs have been identified in squalene epoxidase homologs of Trichophyton group¹⁹. Nucleotide substitution within the squalene epoxidase gene was observed in *Trichophyton interdigitale* all isolates that substitutes TTC with CTC at 1185 position²⁰. In *Trichophyton rubrum*, nucleotide substitution occurred at 1380, leading to the replacement of CAT with TAT⁸. Another substitution occurred at 1175 position leading to the replacement of TTC by TTA that was near related to previous study²¹ in which the nucleotide and amino acid substitution in the squalene epoxidase gene varies between homologs of the Trichophyton group.

Conclusion

Squalene epoxidase is an antifungal targeted key enzyme in the ergosterol biosynthesis pathway of

several fungal groups. This inhibition of the gene contributes to squalene aggregation, ergosterol depletion and the inhibition of fungal growth. Studies have demonstrated a variety of squalene epoxidase genes in a major group of dermatophytes. Squalene epoxidase has widely diverged in three fungal groups of dermatophytes Trichophyton, Epidermophyton and Microsporum. Mutation in the squalene epoxidase gene can mislead normal sterol formation in the fungal cell membrane that induces fungal resistance against several drugs, including azoles and polyenes. Squalene epoxidase gene carries 2 - 3 transcripts and 2 - 3 number of exons in dermatophytes. Squalene epoxidase gene has FAD-dependent oxidoreductases and NADP binding conserved domains similar in three major fungal groups of dermatophytes. Squalene epoxidase gene was also studied for possible single nucleotide polymorphism (SNPs) in the Trichophyton group's homologs and found multiple SNP positions on 1184, 1185 1380, 1175 and 1368 nucleotide positions of TTC→CTC. This position was the cause of non-synonymous mutation, which can change the sequence of amino acid into a protein structure that can change phenylalanine to leucine.

Conflicts of Interest

None.

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