Al-Kufa University Journal For Biology: 2023. Vol. 15 No. 3 P: 1-7
ISSN (Print)2073-8854 ISSN (Online): 2311-6544

Original Research Paper : *Journal hompage : https://journal.uokufa.edu.iq/index.php/ajb/index*

Molecular Identification Of Trichophyton *Interdigitale* From Patients With Dermatophytosis

[1]Ihsan Ali Alzamily
[2]Majid Kadhim Al-Shibly
[3]Azhar Noory Hussein Almousawi

[1,2,3] Department of Biology / College of Education / Al-Qadisiyah University / Iraq

Article history
Received: 4 / 6 /2023
Revised: 10 / 8 /2023
Accepted: 15 / 8 /2023
DOI:

**Corresponding Author:*

Emails:
ihsan@biotech.uoqasim.edu.iq

Abstract: *Trichophyton interdigitale is a significant pathogen responsible for dermatophytosis, including tinea unguium & tinea pedis.A fungal genome contains numerous copies of ribosomal DNA (rDNA). (ITS) region can be used to analyze relationships between closely related taxa.The genetic variants of the ribosomal sequences were investigated to assess the pattern of biological diversity of three isolates (assigned S4, S6 and S7) collected in Karbala province.Our results indicated the exact identity of the amplified samples. Sequencing reactions indicated that the identity of S4, S6, and S7 samples was Trichophyton interdigitale. phylogenetic analyses also confirmed their positions within their corresponding clades accordingly.*

Keywords: Trichophyton interdigitale , DNA Sequencing , phylogenetic tree, NCBI, dermatophytosis, Trichophyton mentagrophytes

1.Introduction

Dermatophytosis is a common disease that infects (20-25%) of populations of human every year [1-3].The common pathogen causes dermatophytosis. *Trichophyton interdigitale*, is a strictly anthropophilic species that is a member of the *Trichophyton mentagrophytes* complex [4].In comparison to other species of Trichophyton, *Trichophyton interdigitale* which is clonal offshoot of *Trichophyton mentagrophytes* be exceptional because they have a substantial number of genotypes in (ITS) region, which together make about 34% of the diversity of the genus. Thus, the same DNA sequences can be utilized for achieving both molecular strain type and species identification. The differences in epidemiological origins and clinical pictures between special genotypes serve as a base for species delineation [5]. *Trichophyton interdigitale* is reserved in the current classification for anthropophilic isolates, which are mostly detected in tinea pedis & tinea unguium cases, in comparison with zoophilic isolates of *Trichophyton mentagrophytes*, it is also present in clinical cases other than infections of nail & foot [6]. *Trichophyton interdigitale* have white colonies with consistency of cottony obverse and a beige to brown reverse. Branching septate hyphae contain many spherical microconidia organised

in grape clusters، spiral hyphae and macroconidia that are cigar shaped [7]. The objectives of study is to use molecular methods to identify *Trichophyton interdigitale*, as well as to determine the phylogenetic relationship between isolates and other isolates from around the world and submission of sequences gotten in NCBI database.

2.Methodology

Sample collection:

Samples of (skin, nails, and hair) were collected from outpatients suspected of dermatophytosis and referred to the mycology laboratory of Imam Husain Medical City and Imam AL-Hassan AL-Mujtaba Teaching Hospital in Karbala.

Sequencing methods

One specific PCR fragment partially covering the IS1, 5.8S rRNA, and ITS rRNA was amplified.To analyze the pattern of genetic polymorphism of fungal samples, amplified PCR fragments were subsequently subjected to Sanger sequencing experiments. A specific comprehensive tree was constructed for the evaluation correct genotyping of identified variations, including their phylogenetic distribution.

PCR amplicons nucleic acids sequencing

Following the manufacturer's instructions, the resolved PCR amplicons were commercially sequenced in both forward and reverse directions by Macrogen Inc. Geumchen, Seoul, South Korea. For confirmation that variations & annotation are not the results of sequencing artifacts or PCR, additional analysis was performed on clear chromatographs obtained from ABI (Applied Biosystem) sequence files.Virtual positions and information of obtained PCR fragments were determined by comparing the retrieved nucleic acid sequences with observed nucleic acid sequences of local samples.

Sequencing data interpretation

The sequencing findings of the PCR products of the targeted samples were edited, aligned, and assessed using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA) alongside the corresponding sequences in the reference database. The identified variations in each sequenced sample were assigned. numbers of both their corresponding position in referring genome's PCR amplicons. The detected nucleic acids were assigned numbers in both their respective roles in the reference genome and in PCR amplicons. SnapGene Viewer version 4.0.4 (https://www.snapgene.com) annotated each noticed variant within sequences.

Sequences deposition to GenBank

Both of the investigated and analyzed sequences were submitted to the NCBI Bankit portal and all the instructions described by the portal were followed as described by the server. The proposed series was provided as nucleic acid sequences in the NCBI to get a unique GenBank accession number for the investigated sequences.

Comprehensive phylogenetic tree construction

A specific comprehensive tree was constructed using neighbour-joining protocol provided via [8].Utilzing NCBI-BLASTn server [9], variations observed were matched to their neighbor sequences of homologous reference. Then, using the iTOL suit [10], a full inclusive tree, which includes observed variation, was constructed using the neighbour-joining method and visualized as rectangular and circular cladograms. Sequences of each categorized phylogenetic group were coloured differently in the complete tree..

3. Results & Discussion

Phenotyping of *Trichophyton interdigitale* isolates

White colonies with cottony consistency and powdery surfaces on the obverse and beige to brown on the reverse. Over time, the character became granular .Direct microscopy reveals hyaline septate hyphae with numerous giant forms of microconidia and chlamydospore-like structures as in Figure 1 [6.7].

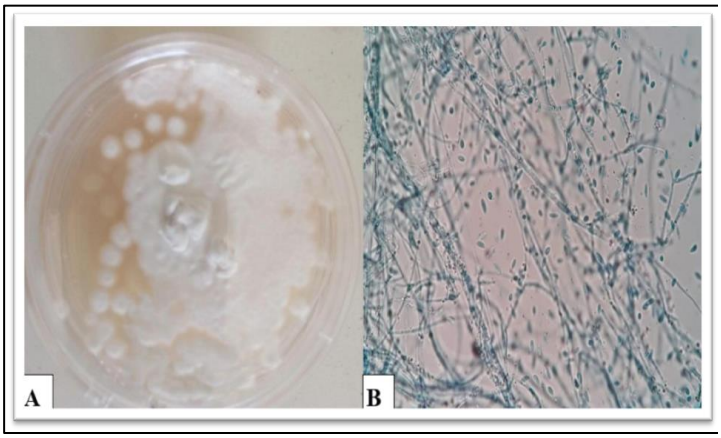

Fig (1) Morphological and microscopic characteristics of Trichophyton interdigitale (A)colonies are flat, white to cream in color, and have a powdery to suede-like surface. (B) several subspherical to pyriform microconidia and a few spiral hyphae.

Polymerase chain reaction (PCR) product

The results of the polymerase chain reaction (PCR) analysis showed that all the isolates under study that were characterized phenotypically contained a single band of extracted DNA utilizing (DNA Ladder) of (250) Pb which was used as an indicator of the size of DNA fragments that may appear after replication via Polymerase chain reaction PCR as in Figure (2).

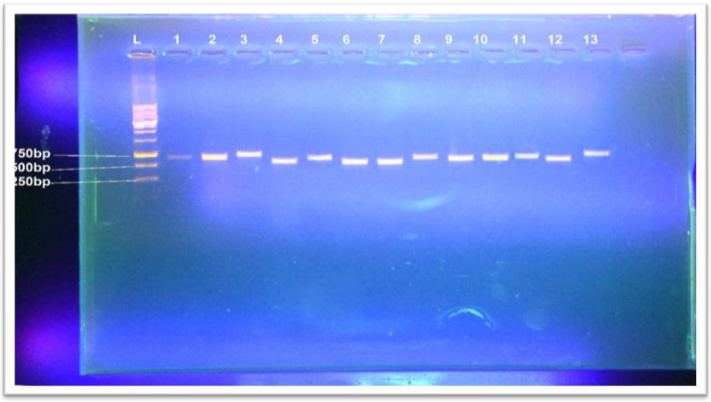

Fig (2) Electrophoresis of PCR product. Amplification of ITS region of different fungal isolates.The letter L indicates the ladder marker 250 bp (Korean Intron Biotechnology Company), while the numbers from 1 to 12 indicate the PCR products of fungal isolates. Electrophores conditions: 100 V / for 30 minutes, Gel concentration: 1% (w/v) (w/v), Buffer used: TBE buffer (1X), ethidium bromide dye.

Sequencing results

Eleven samples were used in the current analysis within the targeted locus. The ribosomal sequences of the examined fungal species were partially amplified utilizing these samples as a screening tool.This is because the capacity of the ribosomal sequence variation to adapt to changing genetic diversity makes it useful for genotyping. Sequencing procedures confirmed their exact identity following NCBI blastn of these PCR amplicons [11]. The NCBI BLASTn engine revealed (99-100%) sequence similarity between sequenced samples and targeted reference target sequences for the ribosomal amplicons of S4, S6 & S7.

Exact positions and other features of the retrieved PCR fragments were discovered by comparing the observed nucleic acid sequences of these studied samples with the retrieved nucleic acid sequences (GenBank acc. KM822820.1).The overall length of the targeted locus was determined on the NCBI server, and the target's start and end positions were verified within the most homologous Trichophyton interdigitale target as in Fig (3).

Trichophyton interdigital

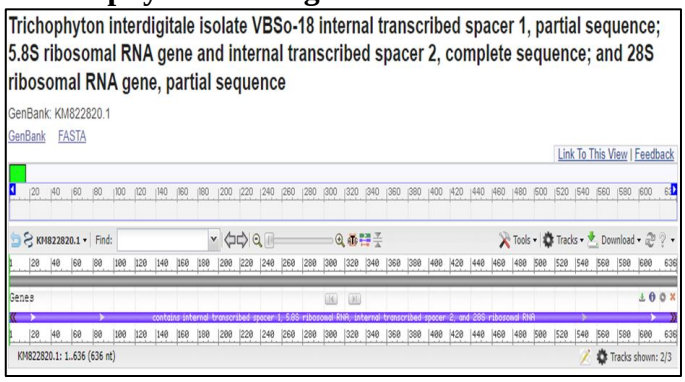

Fig (3) Retrieved PCR amplicon exact position partially covered ribosomal portions of variable fungal genomic sequences (GenBank acc. no. KM822820.1).

Particular sequences' details had been highlighted, and overall length of the amplified amplicons was determined as well after the ribosomal amplicons' sequences were positioned within the genomic sequences of amplified fungal sequences as in Table (1). This table shows position & size of PCR amplicons utilized for partially amplifying ITS1, 5.8S, and ITS2 ribosomal sequences within the amplified fungal genomic sequences (KM822820.1).

Table (1) PCR amplicons length & position that are utilized for partially amplify the ITS1, 5.8S, and ITS2 ribosomal sequences within the amplified fungal genomic sequences

Amplicon	Reference locus sequences (5′ - 3′)	length
Ribosomal sequences of *Trichophyton interdi*	GGAATTTTGCCGCAGGCCGGAGGCTGGCCCCCCACGATAGGGCCA AACGTCCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCATTCTT GTCTACCTTACTCGGTTGCCTCGGCGGGCCGCGCTCTTCCAGGAGAGCCGT TCGGCGAGCCTCTCTTTAGTGGCTAAACGCTGGACCGCGCCCGCCGGAGGA CAGACGCAAAAAAATTCTTTCAGAAGAGCTGTCAGTCTGAGCGTTAGCAA GCAAAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGA TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTG AATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCA TGCCTGTTCGAGCGTCATTTCAGCCCCTCAAGCCCGGCTTGTGTGATGGAC GACCGTCCGGCGCCCCCGTTTTTGGGGGGTGCGGGACGCGCCCGAAAAGCA GTGGCCAGGCCGCGATTCCGGCTTCCTAGGCGAATGGGCAACAAACCAGC GCCTCCAGGACCGGCCGCCCTGGCCTCAAAATCTGTTTTATACTTATCAGG TTGACCTCGGATCAGGTAGAATCCCCGAATTCCT	636 bp

Results of alignment of the ribosomal samples of *Trichophyton interdigitale*, showed presence of no variations of nucleic acid comparing with most similar referring reference nucleic acid sequences (GenBank acc. no. KM822820.1, OR083657.1, and MT633048.1) as in figure 4.

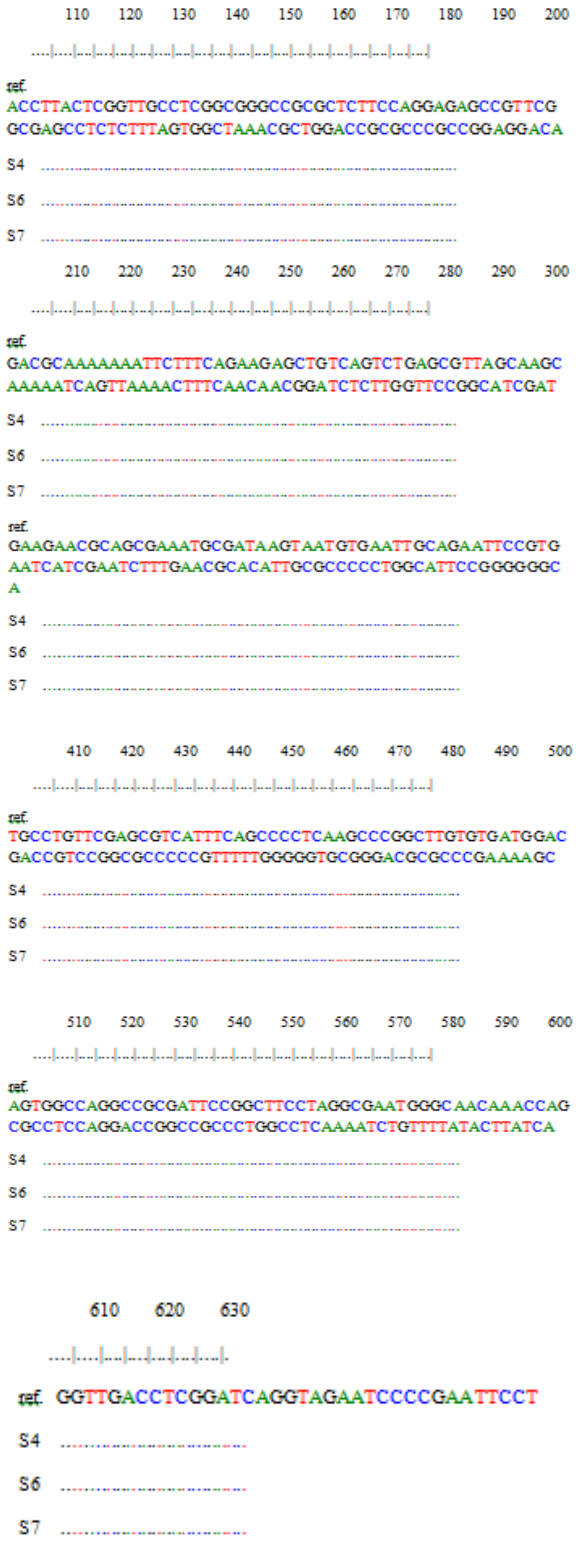

Fig. (4). sequences alignment of nucleic acid of fungal samples with their corresponding reference sequences of the fungal ribosomal genomic sequences. Symbol "ref" represents NCBI referring to sequence GenBank acc. no.

KM822820.1), and letter "S#" refers to the sample number.

Studied samples were uploaded to the NCBI web server, and the analyzed sequences were given individual accession numbers. Deposited sequences received the GenBank accession number OR453229, OR453230, and OR453231 to represent the samples of *Trichophyton interdigitale*. Based on nucleic acid sequences found in the amplified ribosomal amplicons, a comprehensive phylogenetic tree was created in the present study for genus which offers a phylogenetic understanding of actual distances between our investigated samples and most related reference strains of the amplified fungal samples.Phylogenetic tree include the amplified samples with other relative nucleic acid sequences of their relative sequences. Cladograms were generated for explain two different representations of the incorporated fungal sequences, a rectangular cladogram. Within the clade of *Trichophyton interdigitale*, the S4, S6, and S7 samples were positioned beside one strain isolated from India (GenBank KM822820.1).

The phylogenetic tree of Trichophyton sp.

This comprehensive tree had a total of 37 aligned nucleic acid sequences. The most intriguing finding in our Trichophyton isolates is the separation of our samples into three separate phylogenetic clades within the Trichophyton genus.This sort of diversity was reflected via the observed phylogenetic effects of the observed nucleic acid sequence due to their ability to cause apparent differences in their positioning in the generated clade. Thus, these variations have constituted significant discrimination within the same species of Trichophyton as in Figure (5).

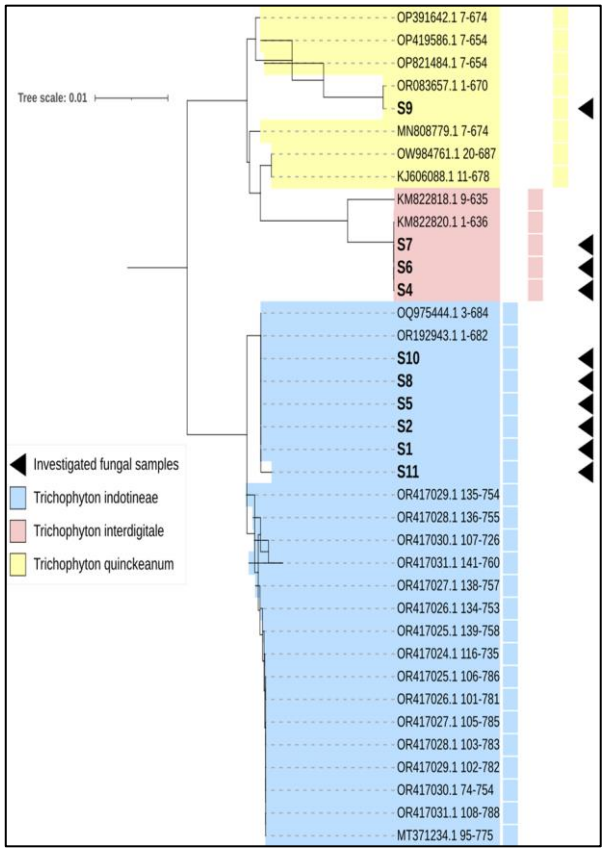

Fig (5) Comprehensive cladogram phylogenetic tree of Trichophyton species' ribosomal sequences.The triangle in black represents the Trichophyton samples that were examined.All of the numbers corresponded to the referred species' GenBank accession numbers.The number at the top of the tree indicates the scale range among the diverse tree-categorised organisms. The code for the samples under investigation is denoted by the letter "S#".

[12] reported that they sequenced four *T. mentagrophytes* genomes and discovered that *T. interdigitale & T. mentagrophytes* are the same phylogenetic species. According to [13], *T. interdigitale* isolates are only anthropophilic, while zoophilic *T. interdigitale* isolates are *T. mentagrophytes.T. interdigitale* and *T. mentagrophytes* were identified as species complexes which might be affected by epigenetic change throughout human & animal body localization based on the overall average of intraspecies and interspecies pairwise

distances of combination[14,15]. They also have a common ancestor, and *T. interdigitale* species are descended from *T. mentagrophytes* species. This study also confirmed that the ITS1 region is appropriate for supplying target genes for molecular recognition *Trichophyton interdigitale*. ITS1 region's nucleotide composition variation is effectively utilized for sample recognition. For DNA-based pathogenic *Trichophyton interdigitale* identification and discrimination, a variety of targets can be utilized.

Conclusion

We accurately identified the genetic polymorphisms within these isolates using specific PCR amplification and Sanger sequencing. Our results revealed distinct identities for each isolate, with six isolates identified as *Trichophyton interdigitale* (S4, S6, S7). This study enhances our understanding of the biological diversity of fungal isolates in the region and provides valuable insights into their genetic variations.

Ethical Approval

The specimens of this study took the patient's approval for adult patients and the consent of the irrigation for young people as the law and directives of the human rights organizations with adequate information in an ethical manner.

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