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Original Research Article

Molecular Characterization of *Chrysosporium indicum* Associated with Dermatophytic Infections

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Abstract: Keratinophilic fungi are molds with similar morphology and physiological characteristics that generate the keratinase enzyme, which degrades keratin materials in or on the soil. The study included isolation of *Chrysosporium indicum* which represents keratinophilic isolate associated with dermatophytic infections. The genetic variant of the ribosomal sequences was investigated in this research for the pattern of biological diversity of isolate collected from province of Babylon. The findings revealed the precise identification of amplified sample was *Chrysosporium indicum*. Two adjacent variants were located in sample (480T>C and 481G>A). Additionally, phylogenetic analysis verified their position among the appropriate clades.

Keywords: *Chrysosporium indicum*, phylogeny, Keratinophilic fungi.

INTRODUCTION

Chrysosporium spp. are commonly found in soil or sediments of freshwater, in addition to skin, feathers, hair of birds, reptiles, and mammals [1]. It is occasionally isolated from human infections. This genus adapts to hot weather areas, is constant and dominant throughout northern Argentina and is cosmopolitan as regards its distribution [2]. They are distinguished by whitish to pale colonies, conidia that are either sessile or borne on a short stalk emerging from the fertile hyphae, and are frequently subglobose, pyriform, or claviform, with rhexolytic release. The order Onygenales are classified into four families based on conidial types ascospore wall features, and enzymatic capabilities: Gymnoascaceae, Onygenaceae, Myxotrichaceae and Arthrodermataceae. Many Chrysosporium species have already been reported, and their classifications have been researched [3]. The genus Chrysosporium (obsolete synonym: Glenosporella) is a member of the kingdom Fungi, subphylum Pezizomycotina, class Eurotiomycetes, order Onygenales, and mitosporic Onygenales group. The teleomorphs of Chrysosporium spp. belong to the families Onygenaceae, order Onygenales, and genera Aphanoascus, Nannizziopsis, and Uncinocarpus [4]. The identification of Chrysosporium species has benefited from the application of molecular systematics due to the advancement of molecular techniques. A few new species have been described using ITS phylogeny and morphology [5]. This study aimed to evaluate the diversity of Keratinophilic fungus isolated from humans by means of molecular analysis of internal transcribed spacer (ITS) region sequences.

MATERIALS AND METHODS

DNA Extraction

Utilizing the EasyPure® Genomic DNA Kit (TrannsGenBiotech-China) and according to the manufacturer's instructions, genomic DNA was extracted from isolates. Primers was used to amplify the ITS region as in the Table (1) under the conditions as showen in Table (2) [6].

Table 1: Primers utilized in amplified of ITS region

Primer	Sequence	Reference
ITS1	50-TCCGTAGGTGAACCTGCG-30	Gurung *et al.*,2018
ITS4	50-TCCTCCGCTTATTGATATGC-30	

Table 2: Thermocycler of PCR product

Stage	Time	Temperature	Cycles
initial denaturation	2 min	95 C	1
Annealing	30 s	60 C	35
Extension	60 s	72 C	
Denaturation	30 s	95 C	
Extension	7 min	72 C	1

Sequencing

The amplified product of PCR had been sequenced utilizing an ABI prism 3730 DNA analyzer (Applied Biosystems, Foster City, USA). The sequence had been verified to reference ITS sequence from GenBank at the (NCBI) utilizing (BLAST). The nucleotide sequence was submitted to GenBank and assigned accession number GenBank acc. MH858234.1.

Phylogenetic analysis

Kimura's two parameter model was used to calculate evolutionary distance matrices utilizing the neighbor-joining procedure [7]. Molecular Evolutionary Genetic Analysis (MEGA 6) software was used to evaluate phylogenetic relationships [8]. A multi-replication bootstrap technique was utilized to find the support for the clade.

RESULTS AND DISCUSSION

Concerning the ribosomal amplicons of S12, sequence similarity between the sequenced sample and the desired reference target sequence was found to be 99% utilizing the NCBI BLASTn engine. exact positioning and additional characteristics of the obtained PCR fragment was identified via comparison of the sequence of nucleic acid related to studied sample with retrieved nucleic acid sequence (GenBank acc. MH858234.1). Entire targeted locus' length was determined utilizing server of NCBI, and its beginning and ending positions were verified within the most homologous target of *Chrysosporium indicum* (Fig 1).

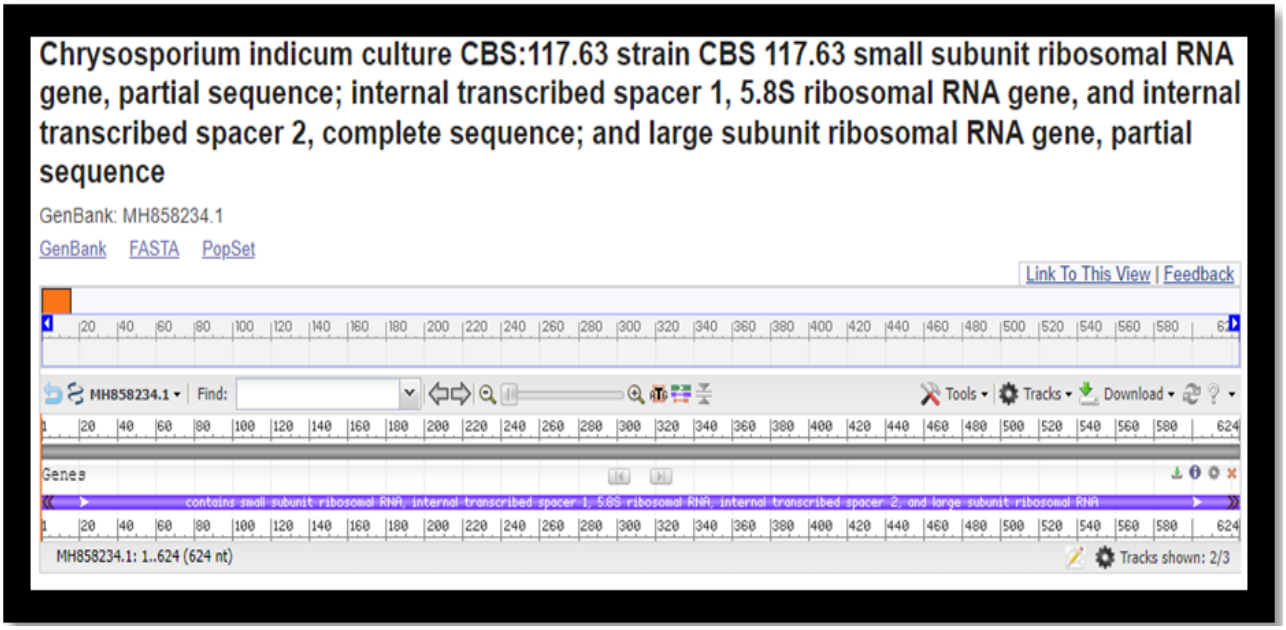

Figure 1: Specific position of the amplicon of retrieved PCR is partially included the various fungal genomic ribosomal regions (MH858234.1)

The features of the ribosomal amplicons' sequence had been highlighted immediately following placing them within the amplified fungal sequences' genomic sequences, and the overall length of the amplified amplicons was also ascertained (Table 3).

Table 3: PCR amplicons position and length that used to partiall amplify the fungal genomic sequences' ITS1, 5.8S, and ITS2 ribosomal sequences (GenBank acc. no. MH858234.1)

Amplicon	Reference locus sequences (5′ - 3′)	length
Ribosomal sequences of *Chrysosporium indicum*	CGTAACAAGGTTTCCGTAGGTGAACCTGCGGAA GGATCATTACAGTGTTCTGGAGGCCGGTCCGCC CCGTCCCTCACCGGGCGGGGTGGCCTGCGCCCC CTCCACACGTGTCTACCACACTCTGTTGCCTTGG TGGGCCTGCCCCCGTGGCTGCCGGGGAACGCCG CCGCGCGTCCCGGGCCCGTGCCCACCGATGGAA AACCCTGAACCCTCTGTGAATCTAGTGTGTCTGA GCGATAGAGCAAATGAAACAAAACTTTCAACAA TGGATCTCTTGGTTCTGGCATCGATGAAGAACGC AGCGAAATGCGATAAGTAATGTGAATTGCAGAA TTCCGTGAATCATCGAATCTTTGAACGCACATTGC GCCCTCTGGTATTCCGGGGGGCATGCCTGTTCGA GCGTCATTGCAACCCCTCAAGCTCGGCTTGTGTGT TGGGCCACCGTCCCCGCGTGTGTGGACGGGCCTC AAAGGCAGTGGCGGCGCCGAGATACCGGTGTCT GAGTGTATGGGCTTCCGTCAGCACTCGTAGGACC CGATCGGCCCCGGTCGTGACTATTTTCATCTTCTA GTTTTGACCTCGGATCAGGTAGGAATACCCGCTG AACTTAAGCATATCAAA	624 bp

When comparing the examined S12 nucleic acid sequence to the most similar referring reference nucleic acid sequences, the alignment results of the Chrysosporium indicum ribosomal samples showed the presence of two nucleic acid variants, indicated by two nucleic acid substitutions (GenBank acc. no. MH858234.1) (Fig 2).

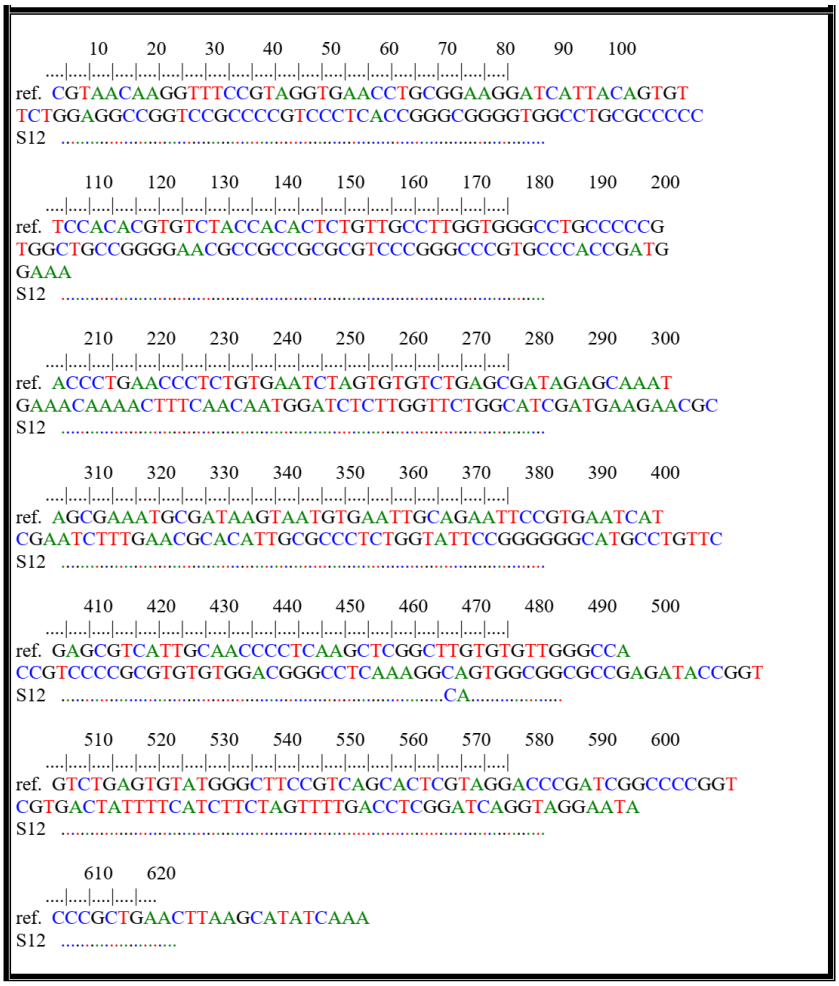

Figure 2: Fungal sample's nucleic acid sequence alignment with matching reference sequences of fungus's ribosomal genomic sequences. The NCBI referencing sequence (GenBank accession no. MH858234.1) is denoted by the symbol "ref," and the sample number is denoted by the letter "S#."

The results showed identification of 480T>C and 481G>A nucleic acid substitutions in the investigated S12 sample. The sequencing chromatogram of the examined sample and its thorough annotations were validated and recorded in order to verify these changes. Additionally, the positions of these variants in the PCR amplicons were displayed in the chromatograms of these variants. These variations were numbered in accordance with their positions in the amplified PCR products, and their existence was verified in their original chromatograms (Fig 3).

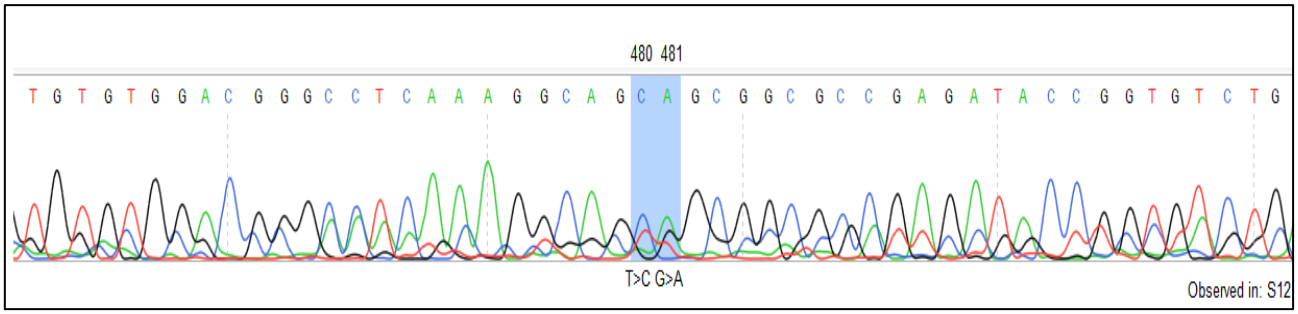

Figure 3: The chromatogram of fungal sequences. The symbol ">" refers to the nucleic acid substitutions, while the symbol "del" refers to deletion mutation of nucleotides in investigated sample in the study

The investigated sample was deposited in the NCBI web server, and unique accession numbers were obtained for the analyzed sequences. The deposited sequences received the GenBank accession number OR453234 to represent the sample of *Chrysosporium indicum*.

In the current study, an comprehensive phylogenetic tree was created for this genus according to nucleic acid sequence detected in amplified ribosomal amplicon. This allowed for phylogenetic determination of the true distances between our sample under investigation and the most similar reference strain of the fungus sample that has been amplified. The amplified sample was included in this phylogenetic tree together with additional relative nucleic acid sequences of their relative sequence.

The phylogenetic tree of *Chrysosporium indicum*

Total number of aligned nucleic acid sequences in this comprehensive tree was twenty-two. The one of the most interesting finding from our investigation of *Chrysosporium indicum* isolate is associated with the position of the S12 into one distinct phylogenetic clade within the genus of *Chrysosporium indicum*. Within the clade of *Chrysosporium indicum*, it was found that the investigated S12 sample was positioned in the vicinity of variable strains isolated from variable positions worldwide. Accordingly, the multiple sources of this investigated sample are confirmed. In addition to these phylogenetic groups of *Chrysosporium indicum* sequences, *Aphanoascus terreus* was placed in a similar tree in order to evaluate the outgroup sequences the phylogenetic pattern and the extent of the identified nucleic acid variations among variable *Aphanoascus terreus* sequences. Distinct phylogenetic distances were observed between both clades, which indicate the clear phylogenetic ability of the utilized ribosomal sequences to discriminate among the investigated fungal samples.

The ribosomal sequences-based comprehensive tree has provided an inclusive tool for the high ability of such genetic fragments to efficiently identify phylogenetic positioning using the ITS1-ITS2 fragment. This provides a further indication of the capability of the currently utilized rRNA sequences to describe the investigated fungal sample (Fig 4).

The most complicated medium for microbial inhabitants, including fungi, is thought to be soil. Certain soil fungus can lead to long-term issues and are linked to diseases in both humans and animals. While some fungi grow naturally in the soil, most of the fungi are supported by the soil's unique fungal flora and differ in its chemical composition. Because they contain a wide range of metabolic diseases, fungi have a great degree of adaptability to environmental conditions and lifestyles. Under various environmental circumstances, the microbiome's diversity and composition, longevity, compatibility, and fungal resistance in the soil all vary significantly. The advent of various techniques has considerably facilitated fungal diversity, allowing thousands of DNA samples to be sequenced in order to identify isolates [9]. Chrysosporium sp. large genus of the saprophytic life mode, and it is extensively dispersed and capable of residing on a variety of substrates in a broad range of humidity and temperature conditions [10]. According to [3], chysposporium is frequently found in soils, the air, and the surface bodies of animals. Chrysosporium's ability to manufacture distinct enzymes and secondary metabolites is linked to its ability to respond to a variety of environmental conditions [11]. The fungus has been identified as an emerging pathogen for the past 20 years due to its strong activity in using keratinous tissues. Certain kinds of reptiles may die from a cutaneous Chrysosporium infection [12]. According to [13], species of Chrysosporium have been isolated from skin and nail scrap samples, particularly from feet and a rare subcutaneous

infection. Protease activity is a strain-dependent characteristic, which could account for Chrysosporim sp.'s modest proteolytic activity [14].

Numerous keratinophilic fungi cause different types of skin infections; yet, there is a paucity of epidemiological information regarding fungal diseases affecting human skin. Understanding the prevalence and range of human etiological agents, animal mycosis, and other potentially harmful fungus on human healthy hair and nails is crucial for comprehending the epidemiological cycle of these fungi [15].

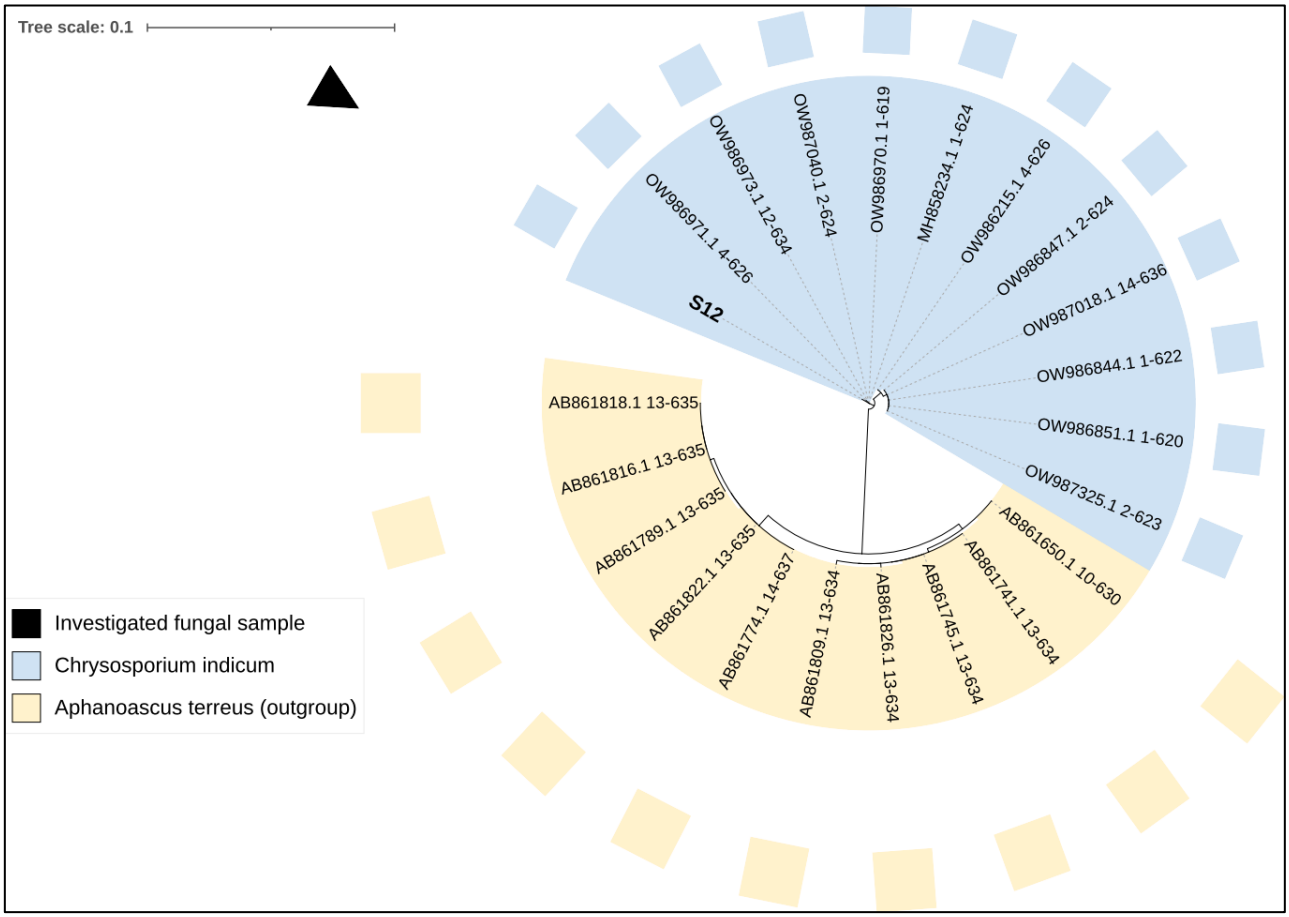

Figure 4: The whole ribosomal sequencing phylogenetic tree of *Chrysosporium indicum*. The sample of *Chrysosporium indicum* that was analyzed is indicated by the black triangle. Every number that was mentioned was the GenBank accession number for the corresponding species. The number at the top of the tree represents the extent of size variation among all organisms classified in the comprehensive tree. The code of the sample under investigation is indicated by the letter "S#."

CONCLUSION

Under appropriate conditions, some reservoirs may contribute to the spread of surface infections like ringworm or tinea through regular human contact. The necessity to control fungal appearances is important due to the high prevalence of keratinophilic fungi. C.indicum can be responsible of degradation of human hair and it was discovered to be the most promising isolate for protein release.

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