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Identification of Genetic Mutations in a Number of Yeasts *Candida Tropicalis* Isolated from Smoking Patients in Thi-Qar Governorate

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***Abstract*—Patients from the Specialized Heart Center in Thi-Qar Governorate with various diseases. A number of fungal species, including *Candida tropicalis*, were isolated by two samples from this isolate. Yeasts were purified and their DNA was extracted and then the DNA was transferred for the purpose of sending it to the Korean company in order to identify genetic mutations. For these species, a number of important genetic mutations were identified and recorded in the Gen Bank, as these isolated species contained genetic mutations that were compared with species isolated in other countries of the world that were officially registered in the Gen Bank. These fungi are considered the most important types of yeasts. They belong to the genus *Candida* and create a range of health problems for most people due to the fungal toxins they carry or the significant lung pathologies they cause. They can also be lethal or cause a number of serious ailments in most individuals.**

***Keywords*—genetic mutations, smokers, yeasts, Candida**

I. INTRODUCTION

Candida tropicalis infection is one of the most common fungal inflammation cases for human subjects, which become a critical issue in many pathological cases worldwide. The biological diversity of the ribosomal sequences of these fungal organisms may be correlated with the pathogenic status of the infected persons from which they were isolated. Accordingly, the present study was conducted to identify the pattern of the genetic variation of the investigated ribosomal sequences from human-infecting *Candida tropicalis* sequences. Based on the genetic variants of the investigated ribosomal RNA sequences, the pattern of the genetic diversity of these infections was assessed in two fungi samples (assigned S1 and S2) in the Middle Euphrates region in Iraq.

II. METHODS

A. Samples collection:

Twenty samples were gathered from the adult attendees the heart center in Dhi-Qar who suffer from different throat infections due to smoking. The study included a number of male patients who smoked and whose ages ranged from 25 to 45 years. Samples were taken from the lower part of the larynx using 20 samples by a specialist doctor and after determining the fungal type. Samples were collected through a Smear from the larynx's lower region and taken to the laboratory for the purpose of completing the isolation of fungi from them [1].

B. Culture media:

Saprouid medium was used for the purpose of isolating and diagnosing fungi from the collected samples. Prepare the medium according to the manufacturer's instructions

3-Isolation and identification of fungi: Isolation of a group of fungal species and yeasts such *C. tropicalis* after culturing samples on some fungal culture media such as Asperoid agar medium, and then those fungi and yeasts were purified.

C. Sequencing Methods

A specific PCR fragment that includes the coding regions for the transcribed 1 and 5.8S inner spacer as well as the 2 and 18S rRNA transcribed inner spacer was selected within this study. The enlarged sections were presented directly to the Sanger-Sequence experiments for the purpose of evaluating the pattern of genetic polymorphisms in these collected fungal samples. Then, a comprehensive and specific genetic tree was drawn for all possible role assessments, the observed variants, and the rest of the evolutionary distributions.

D. Nucleic acids sequencing in PCR Amplicons

The generated PCR amplicons were professionally sequenced in each direction (forward and reverse), according to the sequencing company's (Macrogen Inc. Geumchen, Seoul, South Korea) instruction manuals. To confirm that the annotation and variations are not the result of PCR or sequencing artifacts, additional analysis was only performed on clear chromatographs acquired from ABI (Applied Biosystem) sequence files. The virtual locations and other information of the obtained PCR fragments were determined by comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences. [2]

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E. Interpretation to data of sequencing

Using Bio Edit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA), the sequencing results for the PCR products of the targeted samples were edited, aligned, and assessed together with the corresponding sequences in the reference database [3]. Each sequenced sample's detected differences were assigned numbers in both their corresponding location in the reference genome's PCR amplicons. The detected nucleic acids were assigned numbers in both their respective places in the reference genome and in PCR amplicons. SnapGene Viewer version 4.0.4 (https://www.snapgene.com) annotated each variation found in the fungus sequences. The annotated sequences of the investigated fungal samples [4], were deposited in NCBI, and a unique GenBank acc. no. was obtained for each one.[5]

F. Creation of an extensive phylogenetic tree

According to Hashim et al. (2020)'s neighbor-joining methodology, a special comprehensive tree was built for this investigation. Using the NCBI-BLASTn service, the detected variations were compared to their nearby homologous reference sequences [6]. Then, using the iTOL suit, a complete inclusive tree was constructed using the neighbor-joining approach, incorporating the observed variation [7]. The comprehensive tree's sequences for each included species were colored in an appropriate color.[8]

III. RESULTS AND DISCUSSION

Sequencing reactions accurately identified the examined materials and revealed that all investigated samples were entirely homologous to C. (GenBank acc. no. MG720231.1). No nucleic acid variants were observed compared with the referring sequences of the C.tropicalis. It was inferred to the tree that investigated nucleic acid sequences showed a tight positioning with many C. tropicalis isolates deposited from various Asian geographical positions Additionally, the distances between neighbors in this tree's phylogenetic tree showed a significant biological variety of C. tropicalis sequences.

Within the targeted ribosomal loci, two fungal. In the current investigation, samples were used. To amplify these *C.tropicalis* ribosomal sequences, these samples were examined. Since the ribosomal sequences may have the potential to adapt to varying genetic variety, as was shown in several fungal situations, they may thus be employed for these fungal characterizations [6].

After executing NCBI blastn for these PCR amplicons, the sequencing reactions confirmed the precise identification. The NCBI BLASTn engine revealed 100% sequence similarity between the sequenced samples and one kind of fungus reference target sequence for the examined amplicons (GenBank accession MG720231.1) [11]. The precise locations and other information of the obtained PCR fragments were determined by comparing the observed nucleic acid sequences of these studied samples with the retrieved nucleic acid sequences. (Fig. 1). Sequencing reactions showed the presence of one species within Candida sequences, namely *C. tropicalis* (Fig. 1).[9, 15]

Fig. 1. The positioning of the retrieved rRNA amplicons that covered the internal transcribed spacer 1, 5.8S, internal transcribed spacer 2, and 18S rRNA within Candida tropicalis genomic sequences (GenBank acc. MG720231.1).

The specifics of its sequences were highlighted, the length of the amplified amplicons was also determined, and the sequences of the forward and reverse primers were identified after situating the targeted ribosomal amplicons inside the genomic sequences of *Candida*. (Table 1).

Table 1.The position and length of PCR amplicons which are used to amplify a portion of ribosomal sequences within two samples of *Candida tropicalis* genomic sequences (GenBank acc. MG720231.1).

Amplicon	Reference locus sequences (5′ - 3′)	length
rRNA sequences	*GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACTG ATTTGCTTAATTGCACCACATGTGTTTTTTATTGAACAAATTTCTTTGGTGGCGGGAG CAATCCTACCGCCAGAGGTTATAACTAAACCAAACTTTTTATTTACAGTCAAACTTGA TTTATTATTACAATAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATG AAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAATCATCGAA TCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCA TTTCTCCCTCAAACCCCCGGGTTTGGTGTTGAGCAATACGCTAGGTTTGTTTGAAAGA ATTTAACGTGGAAACTTATTTTAAGCGACTTAGGTTTATCCAAAAACGCTTATTTTGC TAGTGGCCACCACAATTTATTTCATAACTTTGACCTCAAATCAGGTAGGACTACCCGC TGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAAC**	558 bp

* Forward primer placed in the forward direction
** Reverse primer placed in the reverse complement direction

In contrast to the most comparable referencing reference nucleic acid sequences of *C.tropicalis*, the amplified samples' alignment findings showed no evidence of nucleic acid alterations in the investigated samples. (Fig. 2). Sequencing results detected an entire homology with the corresponding reference sequences (GenBank acc. MG720231.1).

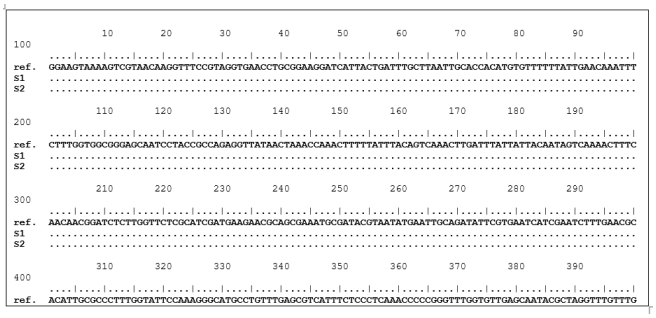

Fig.(2) Nucleic acid alignment of two samples with the rRNA amplicons of *C. tropicalis'* matching reference sequences. The letter "S" followed by a number designates the sample number, while the symbol "ref" stands for the NCBI referencing sequence.[13]

The sequencing chromatograms of the examined samples, as well as their in-depth annotations, were checked and recorded in order to support the found homology with the fungus reference sequences. According to where each sequence was found in the PCR amplicons, their chromatograms were shown.(Fig.3).

Both of the studied rRNA sequences were assigned unique accession numbers when they were uploaded to the

NCBI website.specifically, GenBank acc. ON875337 to ON875338, which were deposited to represent the C. tropicalis samples of S1 and S2 respectively.

A comprehensive phylogenetic tree was generated in the present study according to nucleic acid sequences analyzed in the amplified ribosomal amplicons. This phylogenetic tree included additional related nucleic acid sequences of C. tropicalis along with S1 and S2 samples. Our examined samples were included in this tree together with other relevant sequences to form one main clade of included sequences in the cladogram. One species of C. tropicalis sequences served as a representation for these sequences. Along with the predominant lineage of *C. tropicalis*, three outgroups were also incorporated to assess the pattern of actual phylogenetic distributions among the investigated samples and the extent of the observed sequences in inducing any possible phylogenetic distribution within the major clade in which they were incorporated. Each identified group was respectively incorporated within one phylogenetic clade without being interacted with the others[14]. This observation indicated the high homology between the four indicated organisms and their corresponding species within each separate clade of the tree, and indicate the excellent capacity of the used rRNA to separate the studied sequences into one major group and three minor outgroups without including any obviously similar sequences from different species. In this extensive tree, there were forty-four aligned nucleic acid sequences in total. A circular (Fig. 4A) and a rectangular (Fig. 4B) cladogram were created to explain two dissimilar interpretations of the included C. tropicalis. Within the investigated Candida samples, the investigated samples were clustered into four distinct phylogenetic clades in both of the constructed cladograms. These clades included *C. tropicalis* (the major clade), *C. albicans*, *C. glabrata*, *and C. parapsilosis* sequences, with separated phylogenetic distances between them. The correlation between our examined fungal isolates was the most intriguing finding. a unique phylogenetic positioning without being interacted with the neighbour species. Within the major clade of *C. tropicalis*, a total of twenty-two sequences of the same species were incorporated, including twenty referring sequences of *C. tropicalis* alongside the investigated S1 and S2 samples. All incorporated sequences exerted variable-associated phylogenetic distributions within this clade. Both of the investigated samples were wild-type samples and no nucleic acid variations were detected in anyone of them. However, both of them were positioned in the vicinity of several C. tropicalis isolates deposited from various Asian places, such as Malaysia Viet Nam (GenBank acc. no MT102792.1), India (GenBank acc. no MK356075.1), and Malaysia (GenBank acc. no MH718818.1). As a result of demonstrating the real neighbor-joining-based placement in such studied sequences, the most recent observations of this complete tree have corroborated sequencing reactions. It is interesting to note that the examined S1 and S2 samples' Asian origins could not be disregarded. Notably, the use of the ITS1, 5.3 S, ITS2, and 18S rRNA sequences in this work has provided additional evidence for the presence of precise identification of this yeast. The three out groups of *C. albicans*, *C. glabrata*, *and C. parapsilosis* that were included in this study and placed in separate phylogenetic locations apart from the main clade of C. tropicalis make this point clear. This complete tree is based on rRNA this pattern of phylogenetic distribution to discriminate between these species without being confused with any cross-species phylogenetic similarity. (Chen, S. Metal 2017.Alfayyadh,et al.2022)

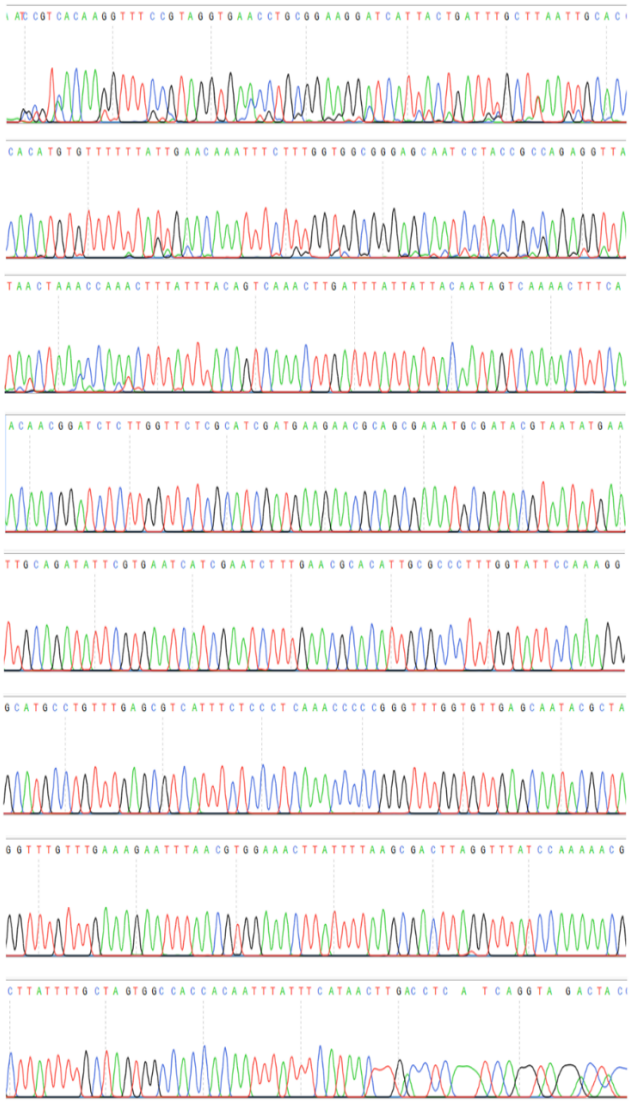

Fig. (3). The chromatogram shows the DNA sequences from two samples of *Candida tropicalis*. The analyzed samples in this study that have this variation have the letter "S" as their code.

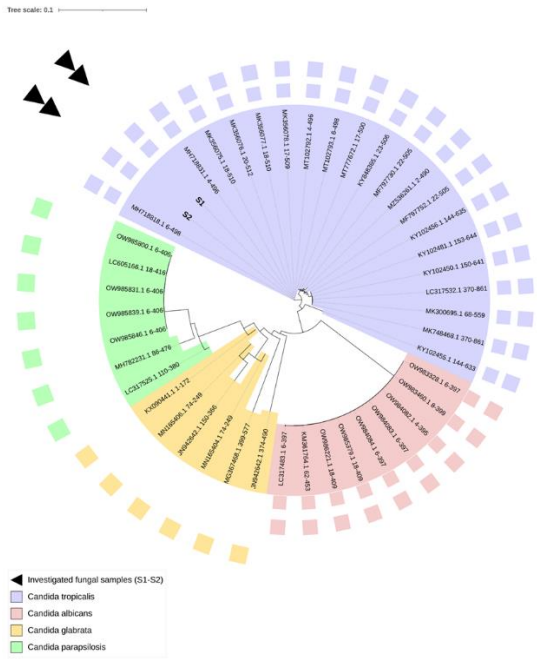

Fig. (4A.) A comprehensive circular cladogram phylogenetic tree constructed using the genetic sequences of two samples of *Candida tropicalis*'s rRNA fragment. The studied fungus varieties are shown by the triangle in black. All of the numbers were the corresponding GenBank entry numbers for the referenced species.

The complete tree classified creatures' degree of scale range is shown by the number "0.1" at the top of the tree. The code for the samples under investigation is denoted by the letter "S#".

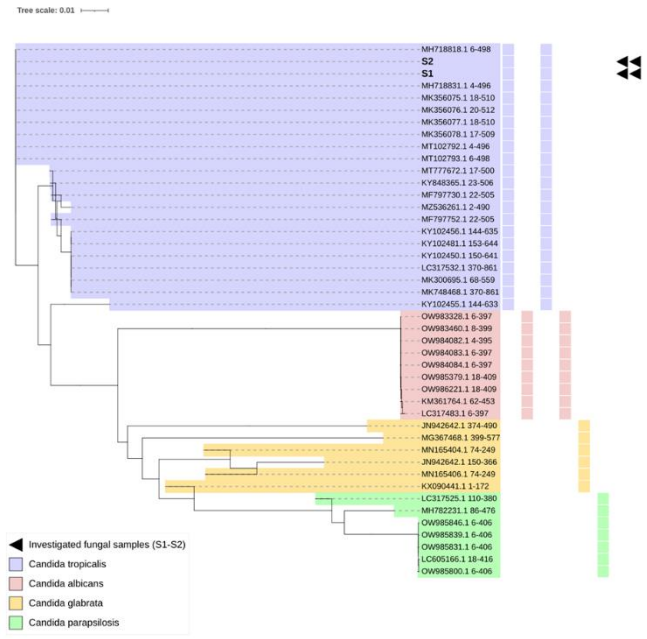

Fig. 4B. A comprehensive Rectangular cladogram phylogenetic tree constructed using the genetic sequences of two samples of *C. tropicalis*' rRNA fragment. The studied fungus varieties are shown by the triangle in black. All of the numbers were the corresponding GenBank entry numbers for the referenced species.

The complete tree classified creatures' degree of scale range is shown by the number "0.01" at the top of the tree. The code for the samples under investigation is denoted by the letter "S#".

IV. CONCLUSION

The ability of the utilized ribosomal fragments to detect and discriminate between *C. tropicalis* sequences in infected patients. Moreover, this work suggests possible employment for these ribosomal amplicons to discriminate between the phylogenetic diversity among the other implemented tools. These amplicons can efficiently be employed to a larger variety of *Candida tropicalis* sequences' biological diversity. To learn more, you may also look into these encouraging tools within these identified species of *C. tropicalis* sequences in various clinical infections.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

[1] I. H. Al-ffayyadh, I. H. Khudhair, and A. S. Jaber, "Isolation and Diagnosis of Fungus Fungi from Blood and Skin Samples in Dhi-Qar Province," *Indian Journal of Public Health Research & Development,* vol. 10, 2019.

[2] J. Bünger, G. Westphal, A. Mönnich, B. Hinnendahl, E. Hallier, and M. Müller, "Cytotoxicity of occupationally and environmentally relevant mycotoxins," *Toxicology,* vol. 202, pp. 199-211, 2004.

[3] C. L. Medrano-Díaz, A. Vega-González, E. Ruiz-Baca, A. Moreno, and M. Cuéllar-Cruz, "Moonlighting proteins induce protection in a mouse model against Candida species," *Microbial pathogenesis,* vol. 124, pp. 21-29, 2018.

[4] R. Samy and D. Aljaza, "Morphology and molecular identification of Candida Species isolated from oral pediatric," University of Thi-Qar Journal of Science, vol. 10, 2023.

[5] M. Tscherner, T. Schwarzmüller, and K. Kuchler, "Pathogenesis and antifungal drug resistance of the human fungal pathogen Candida glabrata," *Pharmaceuticals,* vol. 4, pp. 169-186, 2011.

[6] Z. Zhang, S. Schwartz, L. Wagner, and W. Miller, "A greedy algorithm for aligning DNA sequences," *Journal of Computational biology,* vol. 7, pp. 203-214, 2000.

[7] I. Letunic and P. Bork, "Interactive Tree Of Life (iTOL) v4: recent updates and new developments," *Nucleic acids research,* vol. 47, pp. W256-W259, 2019.

[8] K. V. Clemons, M. Espiritu, R. Parmar, and D. A. Stevens, "Assessment of the paradoxical effect of caspofungin in therapy of candidiasis," *Antimicrobial agents and chemotherapy,* vol. 50, pp. 1293-1297, 2006.

[9] T. J. White, T. Bruns, S. Lee, and J. Taylor, "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics," *PCR*

protocols: a guide to methods and applications, vol. 18, pp. 315-322, 1990.
[10] H. O. Hashim, M. K. Mohammed, M. J. Mousa, H. H. Abdulameer, A. T. Alhassnawi, S. A. Hassan, *et al.*, "Infection with different strains of SARS-CoV-2 in patients with COVID-19," *Archives of Biological Sciences*, vol. 72, pp. 575-585, 2020.
[11] C. Zaugg, M. Borg-von Zepelin, U. Reichard, D. Sanglard, and M. Monod, "Secreted aspartic proteinase family of *Candida tropicalis*," *Infection and immunity*, vol. 69, pp. 405-412, 2001.
[12] D. Larone, "Medically important fungi: a guide to identification," 2002.
[13] S. Nadem, "Awareness and approach in a sample of Community Pharmacists' towards Patient Counseling in Thi-Qar/Iraq and providing the appropriate solutions for the development of counseling," University of Thi-Qar Journal of Science, vol. 10, 2023.
[14] I. H. Al Fayyadh and M. H. al-Yasiri, "Quantitative and Qualitative Determination of Gliotoxin and Acetaldehyde Toxins in Yeasts Isolated from some Respiratory Patients and Study of Yeast Resistance to some Antibiotics," *Indian Journal of Forensic Medicine & Toxicology*, vol. 16, pp. 199-207, 2022.
[15] I. H. A. Fayyadh, "Isolation and Diagnosis of Fungi from Samples of Spices in the City of Nasiriya," *University of Thi-Qar Journal of Science*, vol. 7, pp. 7-9, 2020.