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Sequencing of Ribosome-Based Prints in Three Isolates of Candida Dublinensis

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Abstract

10 sputum samples were collected from patients attending the Respiratory Center in Thi-Qar. A number of fungal species, including Candida dublinensis, were isolated by three samples from this isolate. The isolates were purified and the nucleic acid belonging to these fungal yeasts was extracted, then the nucleic acid was transmigrated and then sent to the Korean company for the purpose of identifying genetic mutations. For these species, a number of genetic mutations were identified and recorded in the Gen Bank, as these isolated species contained genetic mutations that were compared with species isolated from other countries around the world and officially registered in the Gen Bank. These yeasts are among the most important fungal species that it belongs to the fungal Candida, which may cause health problems in patients because it contains fungal toxins that can be fatal or cause serious diseases in most patients.

Keywords: *Sputum, Candida Dublinensis, Genetic Mutations, Fungi, Gen Bank*

INTRODUCTION

Nowadays, infections with fungi and yeasts have gradually developed, which were accompanied by immunocompromised states and various disease states such as respiratory and reproductive infections, blood diseases and cancers that have led to the progressive development of pathogens such as candidiasis (Tscherner et al., 2011; Chen et al., 2017). Fungal yeasts such as Candida are natural flora found on the surface of the body, as well as in the internal organs, respiratory system, and reproductive system. It spreads very quickly to different parts of the body and from there to the brain or heart and bones, especially when most patients present with symptoms of immunodeficiency (Clemons et al., 2006) and infection.

Candida dubliniensis 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 (Bunger J,; et al .2004). . Accordingly, the present study was conducted to identify the pattern of the genetic variation of the investigated ribosomal sequences from human-infecting Candida dubliniensis sequences. Based on the genetic variants of the investigated ribosomal RNA sequences, the pattern of the genetic diversity of these infections was assessed in three fungi samples (assigned S1 – S3) in the Middle Euphrates region in Iraq.(Medrano-Diaz et64 al., 2018)

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METHODS

1.1. Nucleic acids sequencing of PCR amplicons

The resolved PCR amplicons were commercially sequenced from both (forward and reverse) directions, following to instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (Applied Biosystem) sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences, the virtual positions, and other details of the retrieved PCR fragments were identified

1.2. Comprehensive phylogenetic tree construction

A specific comprehensive tree was constructed in this study according to the neighbour-joining protocol described by Hashim et al. (2020). The observed variants were compared with their neighbour homologous reference sequences using the NCBI-BLASTn server (Zhang et al. 2001). Then, a full inclusive tree, including the observed variant, was built by the neighbour-joining method and visualized as a circular cladogram using the iTOL suit (Letunic and Bork, 2019 https://itol.embl.de/). The sequences of each incorporated species in the comprehensive tree were colored in an appropriate color

1.3 Cultural media

In this study, a group of media was used, Culture media that included Sabouraud Dextrose-Agar (SDA), BROTH MEDIUM RPMI and Nutrient broth and agar, Brain Heart infusion agar and Broth .were used and used according to the manufacturer's instruction.

2. DNA extraction:

Blocks of different origins were used for the purpose of DNA extraction of the included yeasts Genomic DNA extraction kit, AccuPower PCR PreMix and Taq DNA polymerase Bioneer/ South Korea.

1.4. Primer:

The primers were designed during this study using NCBI Gene-Bank and Primer 2 online and were provided by (Bioneer Corporation, Korea) as shown in Table (3).

Table 1: Primer sequences used for yeast gene amplification (White .T.J, Bruns. T.D.; et al . 1990)

No	Primer Sequence (5 to 3)	Primer Concentration (pmol/ul)	Product size
1	F:GGAAGKARAAGTCGTAACAAG R:GTTTCTTTTCCTCCGCTTAR	17 pmol/ul 17 pmol/ul	625
2	F:GGAAGKARAAGTCGTAACAAG R:GTTTCTTTTCCTCCGCTTAR	17 pmol/ul 17 pmol/ul	625

*F: forward, *R: reverse, A: adenine, C: cytosine, G: guanine, T: thymine

1.5. Sample collection: (10) sputum samples were collected from patients attending the Respiratory Center and in Dhi Qar Governorate. The samples were collected by using a cotton swab. The samples were grown on pre-prepared media for the purpose of identifying and identifying the types of fungi and yeast in the dish.

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3. Amplification and ITS sequencing

The fungus-specific universal primers ITS1F (5-TCCGTAGGTGAACCTGCGG-3) and ITS4R (5-GCATATCAATAAGCGGAGGA-3) were used to amplify the ITS1 and ITS2 regions as well as 5.8S region. The amplification was carried out according to amplification conversional and by using Thermo cycler (35) and PCR conditions

RESULTS

Within the targeted ribosomal loci, eleven fungal samples were included in the present study. These samples were screened to amplify these ribosomal sequences of Candida dubliniensis. Thus, the variation of the ribosomal sequences can be used for these fungal characterizations due to its possible ability to adapt to variable genetic diversity as was seen in different fungal cases.

The sequencing reactions indicated the exact identity after performing NCBI blastn for these PCR amplicons. Concerning the investigated amplicons, the NCBI BLASTn engine showed about 99% to 100% sequence similarities between the sequenced samples and one type of fungal reference target sequence **(GenBank acc. MH545916.1).** By comparing the observed nucleic acid sequences of these investigated samples with the retrieved nucleic acid sequences, the accurate positions and other details of the retrieved PCR fragments were identified (Fig. 1). Sequencing reactions showed the presence of one species within Candida sequences, namely Candida dubliniensis (Fig. 1).

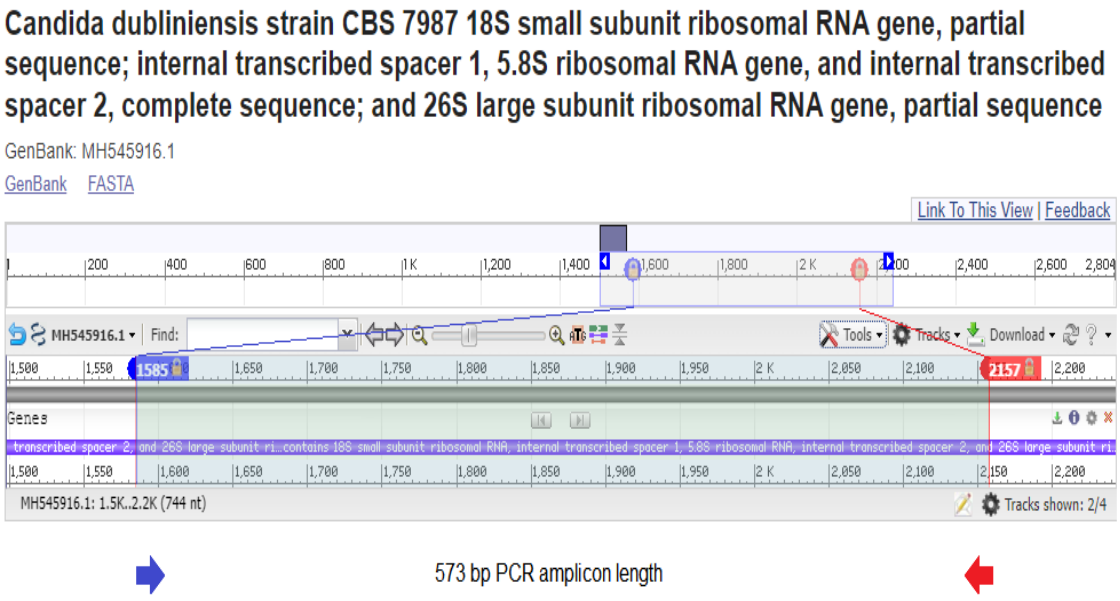

Fig 1: The positioning of the retrieved rRNA amplicons that covered the internal transcribed spacer 1, 5.8S, internal transcribed spacer 2, and 18S rRNA within Candida dubliniensis genomic sequences (GenBank acc. MH545916.1).

After positioning the targeted ribosomal amplicons within the genomic sequences of Candida, the details of its sequences were highlighted, the total length of the amplified amplicons was also determined, and the sequences of the forward and reverse primers were located (Table 1).

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Table 2: The position and length of PCR amplicons that are used to amplify a portion of ribosomal sequences within four species of Candida dubliniensis genomic sequences (GenBank acc. MH545916.1).

Amplicon	Reference locus sequences (5′ - 3′)
rRNA sequences	*GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT ACTGATTTGCTTAATTGCACCACATGTGTTTTGTTCTGG

The alignment results of the amplified samples revealed the presence of four nucleic acid mutations in variable positions of the analyzed samples in comparison with the most similar referring reference nucleic acid sequences of Candida dubliniensis (Fig. 2). Sequencing results detected the presence of three substitutions three nucleic acid substitutions (89C>T, 117C>T, and 136C>T) detected in the S2 sample and one nucleic acid substitution (185A>T). Whereas the other investigated samples (S1, S3, and S2) showed an entire homology with the corresponding reference sequences (GenBank acc. MH545916.1).

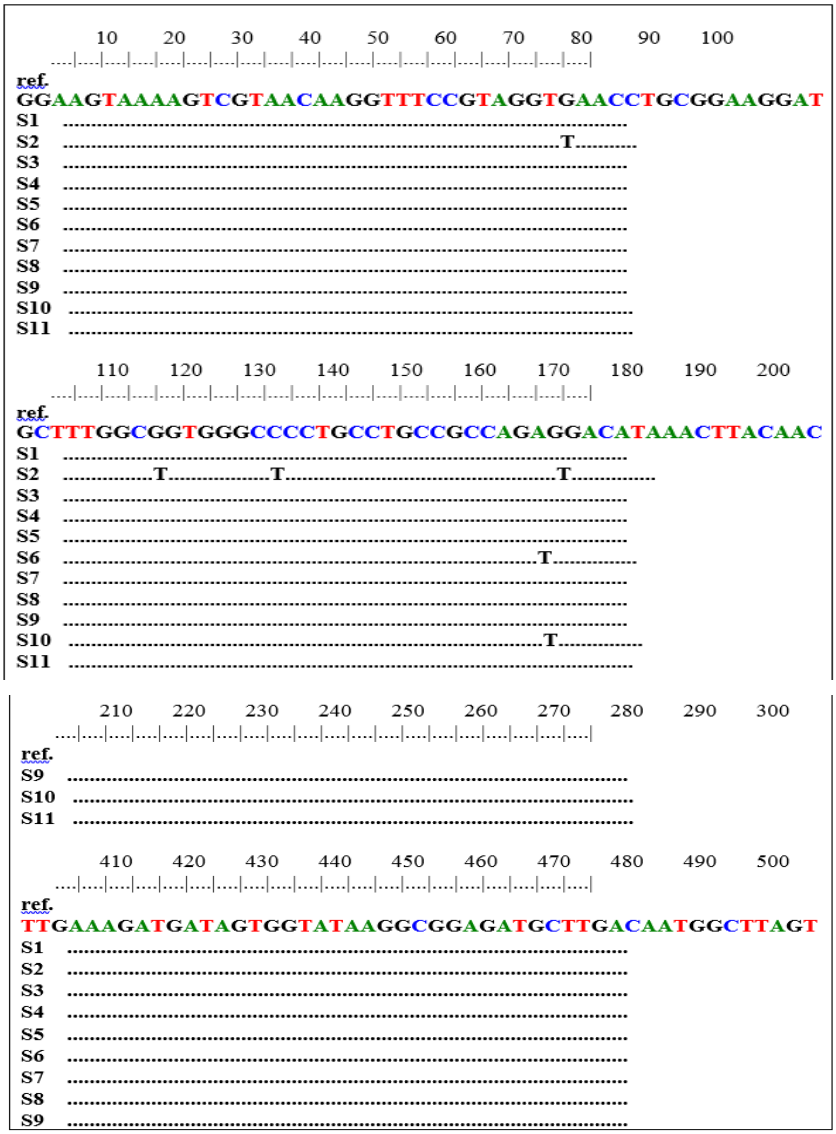

Fig 2: Nucleic acid sequences alignment of eleven samples with their corresponding reference sequences of the rRNA amplicons of Candida dubliniensis. The symbol "ref" refers to the NCBI

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To confirm the observed variations, the sequencing chromatograms of the investigated samples, as well as their detailed annotations, were verified and documented. The chromatograms of the investigated sequences were shown according to their positions in the PCR amplicons (Fig. 3).

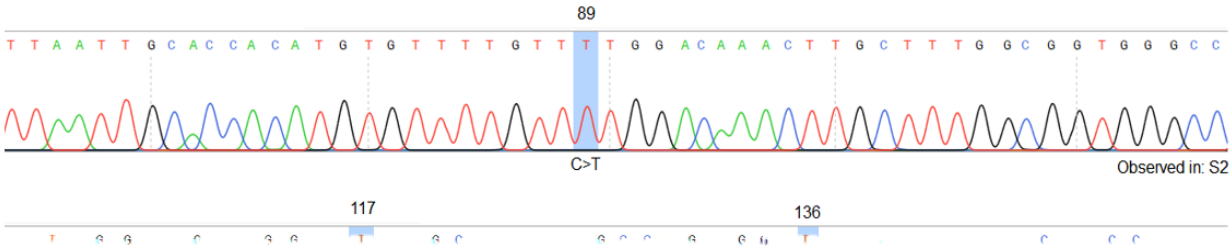

Fig 3: The chromatogram of four species of Candida dubliniensis DNA sequences. The letter "S" refers to the code of the investigated samples having this variant in this study.

To summarize all the results obtained from the sequenced ribosomal fragments, the exact positions of the observed variations are described in (Table 3). All the investigated rRNA sequences were deposited in the NCBI web server, and unique accession numbers were obtained for all analyzed sequences, namely GenBank acc. ON875326 to ON875336 which were deposited to represent the Candida dubliniensis samples of S1 to S3 respectively.

Table 3: The pattern of the observed mutation in the rRNA amplicons of eleven species of Candida dubliniensis in comparison with the NCBI referring sequences (GenBank acc. MH545916.1).

Sample	Native	Allele	Position in the PCR product	Type of variation	Variant summary
S2	C	T	89	Substitution	89C>T
S2	C	T	117	Substitution	117C>T

A comprehensive phylogenetic tree was generated in the present study according to nucleic acid variations observed in the amplified ribosomal amplicons. This phylogenetic tree contained S1 to S3 samples alongside other relative nucleic acid sequences of Candida dubliniensis sequences. Within this tree, our investigated samples were incorporated alongside other relative sequences to constitute one major clade of incorporated sequences within the cladogram. These sequences were represented by one species of Candida dubliniensis sequences. In addition to the main clade of Candida dubliniensis, two outgroups were also incorporated to assess the pattern of actual phylogenetic distributions among the investigated samples and the extent of the observed variations in inducing any possible phylogenetic alterations within the major clade in which they were incorporated.

Two types of cladograms were generated to explain two divergent representations of the incorporated Candida dubliniensis, a circular (Fig. 4A), and a rectangular cladogram. In both cases of the constructed cladograms, the investigated samples were clustered into three distinct phylogenetic clades within the investigated Candida samples. The most interesting observation in our investigated fungal isolates was correlated with the ability of the utilized rRNA-based amplicons to categorize the Candida dubliniensis sequences into a unique phylogenetic positioning without being interacted with the neighbour species.

Within the major clade of Candida dubliniensis, a total of three sequences of the same species were incorporated, including twenty referring sequences of Candida dubliniensis alongside the investigated S1 – S3 samples. All incorporated sequences exerted closely-associated phylogenetic distributions within this clade. This observation indicated a slight phylogenetic role of the detected nucleic acid substitutions in altering the phylogenetic distributions in the investigated samples. As a consequence of the observed variants, two closely-related phylogenetic groups were found for our investigated

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samples within this clade., S1, S3, and S1 were suited near various isolates of Candida dubliniensis deposited from several Asian and South American sources, such as South Korea (GenBank acc. no AF405231.1), Kuwait (GenBank acc. no LT716020.1), and Brazil (GenBank acc. no KC905078.1). However, these eight samples were all wild-type samples and no nucleic acid variations were detected in anyone of them. In contrast to the phylogenetic clade occupied by S3, S2, and S1, it was found that S3, S1, and S2 exhibited a slight tilt from that observed from the wild-type counterpart. This was due to the presence of the nucleic acid substitutions that cause these samples to take these slightly altered positions. Within these three samples, S2 samples were taken more altered position due to the detection of three nucleic acid substitutions (89C>T, 117C>T, and 136C>T) only in this sample. This sort of positioning indicated a tiny effect of the observed variants in inducing such deviation within the generated tree, which was only a minor tilt within the same Candida dubliniensis organism. However, all three (S1, S3, and S2) samples tended to be positioned in the vicinity of Candida dubliniensis isolate deposited from Qatar (GenBank acc. no KF959847.1).

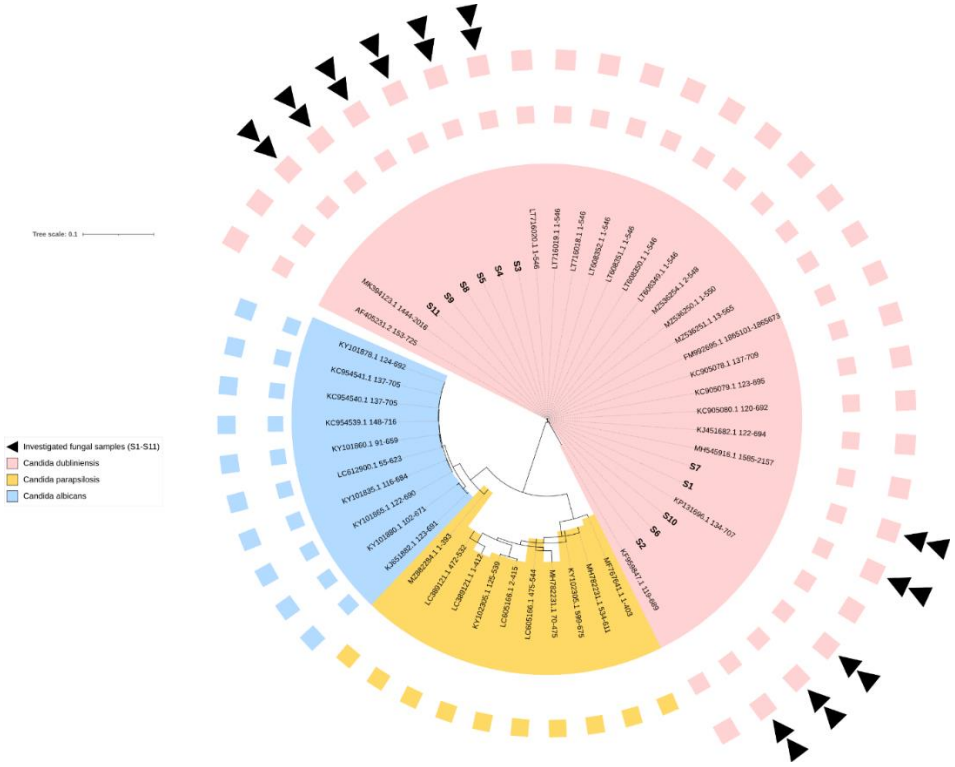

Fig 4A: A comprehensive circular cladogram phylogenetic tree of genetic variants of the rRNA fragment of three species of Candida dubliniensis samples. The black-colored triangle refers to the analyzed fungi variants.

DISCUSSION

Confirmatory detection of DNA sequences for locally isolated yeast isolates using local bases and alignment analysis (NCBI-Blast):

It is clear from the results obtained from the genetic sequence of the yeast samples that were isolated from the sputum of patients who were referred to hospitals affiliated with respiratory diseases in Thi-Qar Governorate, which were mentioned in the results, that these results agreed with what the researcher (JM Garcı´a Martı´, 2010), who identified pathogenic yeasts by nucleotide sequencing using PCR primers. With what is in the GenBank at 99-100%. The output of the PCR for yeast genes

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in this study was that all those samples were identical and consistent with what was recorded in NCBI-GenBank,

CONCLUSIONS

This work showed the ability of the utilized ribosomal fragments to detect and discriminate between Candida dubliniensis 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 detect the biological diversity of a wider spectrum of Candida dubliniensis sequences. These promising tools can also be explored to discover further details within these identified species of Candida dubliniensis sequences in various clinical infections

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