Original Article

Access this article online
Quick Response Code:

Website:
www.carcinogenesis.com
DOI:
10.4103/jcar.jcar_22_02_03

Molecular Diagnosis for Candida spp. In Hospitalized Patients in the City of Karbala

Suhad khalid AL-sgheer [1], Ban Taha Mohammed [1], Ali Tareq Abdul Hasan [2]

Abstract

The research was conducted at the College of Education for Pure Science, University of Karbala, from May 1, 2022, to June 1, 2023. The primary objective of the study was to isolate and molecularly diagnose certain species of the Candida genus obtained from hospitals in the Karbala governorate. To accomplish this, a molecular analysis was conducted on 13 isolates using the Internal Transcribed Spacer (ITS) region by employing the ITS1 and ITS4 primers. By comparing the nucleotide sequences of the studied isolates with globally registered isolates at the National Center for Biotechnology Information (NCBI), the results indicated that 10 strains obtained in this study were previously unrecorded in Iraq. These strains exhibited mutations in certain nitrogenous bases. Furthermore, the molecular diagnosis confirmed the identification of a species, C. ciferri, which was recorded for the first time in Iraq at the molecular level. The molecular diagnosis of Candida isolates indicated that they belong to the following types: C. albicans 6 isolates, C. tropicalis 2 isolates, C. parapsilosis 2 isolates, C. glabrata 1 isolate, C. dubiniensis 1 isolate, and C. ciferri 1 isolate. After completing the analysis of the results of the molecular study, the above diagnosed species were registered in NCBI under identifiable accession numbers: OP293043, OP293044, OP293045, OP293046, OP293047, OP293048, OP2 93049, OP293050, OP293051, OP293052, OP293053, OP293054 and OP325504 respectively.

Keywords:

Molecular diagnosis of fungi, Candida spp.

Introduction

Fungi are widely distributed in various environments and play a significant role as living organisms, they can be classified into molds and yeasts, with yeasts being single-celled organisms that reproduce through budding [1]. Yeasts encompass different types, some of which have beneficial effects on humans, while others, like the Candida, occasionally are known to cause diseases.

Among the various types of yeasts, Candida is particularly prevalent due to its possession of multiple virulence factors, making it capable of causing internal infections in the respiratory, digestive, urinary, and genital tracts. In some cases, it can even enter the bloodstream [2, 3]. Although the Candida is typically present in the normal flora of the human body, it can lead to fungal infections in humans and adversely impact their well-being [4, 5]. In healthy individuals, the immune system is generally capable of clearing most infections caused by Candida species, however, individuals with immunodeficiency or chronic conditions such as asthma, diabetes, organ or tissue transplants, and other ailments are more susceptible to fungal infections [6, 7].

Mycoses fungal infections may be chronic due to their slow growth and are the main cause of life-threatening infections for people with weak immunity [8]. Candida is the fourth most common fungal cause in many developed countries. The Candida species possesses virulence factors such as biofilm formation, adherence and the production of enzymes that cause tissue damage [9] ,and has the ability to produce proteinase and phospholipase [10, 11] showed that catalytic enzymes play an important role in the growth of Candida , as these

[1]University of Kerbala / College of Education for pure Science/ Iraq. [2]University of Kerbala / College of Medicine /Iraq.

Address for correspondence:
Suhad khalid AL-sgheer,
University of Kerbala / College of Education for pure Science/ Iraq.
E-mail:
suhad_khalid@karbala.edu.iq

Submitted: 16-Jan-2023
Revised: 08-Aug-2023
Accepted: 23-Sep-2023
Published: 11-Oct-2023

How to cite this article: AL-sgheer S K, Mohammed B T, Hasan A T A. Molecular Diagnosis for Candida spp. in Hospitalized Patients in the City of Karbala. J Carcinog 2023;22(2):21-33

enzymes facilitate adhesion and penetration of tissues and thus invasion of the host. Changing the phenotype and secretion of toxins are also considered virulence factors [12, 13].

Molecular diagnostics, as opposed to traditional diagnostic and biochemical tests employed in laboratory diagnosis of fungi, demonstrated remarkable precision, efficiency, and specificity in distinguishing between various species and subspecies of fungi [14]. The Polymerase Chain Reaction (PCR) technique is utilized for the diagnosis of fungi, even in cases where the fungal colony has lost its distinctive features, is newly formed, or is no longer viable [15].

DNA sequencing is a valuable and significant technique for understanding the evolutionary relationships among fungal species, it has emerged as an ideal approach for species identification and diagnosis when applied to various fungal strains [16]. Currently, there is a growing inclination to integrate genetic diagnosis with phenotypic diagnosis, maximizing the benefits of both approaches [17].

The discovery of a wide range of molecular mechanisms over the past few decades has provided valuable insights into the origins of drug-resistant fungal isolates and has paved the way for the development of contemporary approaches to treat *Candida* and minimize its impact on human health and the associated economic consequences, among the various diagnostic methods available [18], DNA sequencing stands out as one of the most effective techniques [19].

Therefore, the aimed of the study to diagnosing some types of *Candida* spp. Molecularly, by knowing the sequence of the nitrogenous bases of the ITS gene and drawing the genetic relationship between the isolated species [20].

Materials and methods

1- One hundred fifty samples (70 mouth, 45 vaginas, 25 diaper areas and 10 blood) were collected for men, women and children of different age groups from 1 day to 70 years during the period from 03/17/2022 to 10/15/2022 from different places in Karbala Governorate (Obstetrics and Gynecology Teaching Hospital, Karbala Children's Teaching Hospital, Al-Hussein Teaching Hospital, Kidney Dialysis Unit and Oncology Center) and the Public Health Laboratory. All these samples are samples taken from people suffering from chronic diseases, except for children.

2- Preparation of the culture medium (Sabouraud dextrose agar (SDA)): The preparation of this medium followed the manufacturer's instructions. The antibiotic Chloramphenicol was added to the medium at a concentration of 250 mg per liter [21].

3- The isolates were incubated at 37 °C for 48 hours, then examined phenotypically in terms of color and size.

Molecular identification of *Candida* spp.

1- DNA extraction from Candida yeast were grown and activated on SDB culture medium, and DNA was extracted and purified using the extraction kit: Wizard Genomic DNA Purification Kit prepared by \USA Promega company and according to the steps listed for DNA extraction as follows:

1. Take 2 ml of the liquid medium after growing the yeast in it, then centrifuge 3,000-16,0001 cycles for two minutes.
2. Take the precipitate and put it in EDTA (50 ml).
3. Add 7.5 µL of 20 mg/ml lyticase enzyme and mix gently.
4. The tubes were incubated for 30-60 minutes at a temperature of 37°C.
5. Then add 300 µl of Nuclei Lysis Solution The protein precipitation and DNA processing steps were applied as follows:

1- 100 microliters of Protein Precipitation Solution were added, mixed with a Vortex device, then placed on ice for 5 minutes.
2- The samples were placed in a centrifuge at a rate of 13000-16000 cycles for 3 minutes.
3- Transfer the upper liquid (the upper layer) to a clean tube containing isopropanol alcohol in a volume of 300 microliters at room temperature.
4- The samples were mixed with reversible stirring, then placed in a centrifuge at a rate of 13000-16000 cycles for 2 minutes.
5- The top layer was removed and 300 microliters of ethanol (70%) was added to the remainder of the precipitate at room temperature.
6- The samples were introduced into a centrifuge at a rate of 13000-16000 cycles for 2 minutes.
7- The ethanol was left to evaporate and dry in the air.
8- 50 microliters of DNA Rehydration solution were added and placed at 65 °C for 1 hour.
9- RNase Solution was added for 15 minutes

2- Determination of the concentration and purity of the DNA for this purpose, a nanodrop device was used by placing a small drop of 0.7 microliters of DNA extract on the sensitive lens in the device after calibrating it with a similar drop of elution buffer to filter it, then the DNA concentration and purity values were recorded at a wavelength of 260/280.

3- DNA electrophoresis on agarose gel was performed to confirm the presence of DNA following the extraction process or to detect the amplified products of the polymerase chain reaction. A standard DNA sample was included to facilitate size determination of the bands on the agarose gel. The agarose gel was prepareds. This involved dissolving 1.5 grams of agarose in 100 ml of a TBE solution, followed by heating the mixture until it reached boiling point. After cooling to a temperature

range of 45-50 °C, Red safe dye was added to enable visualization of the nucleic acids. A gel support plate was prepared by placing a comb in position, and then the gel mixture was carefully poured onto it. The gel was left undisturbed for approximately 30 minutes to solidify. Subsequently, the comb was gently removed from the agarose gel, and the gel was positioned on a stand within the horizontal electrophoresis unit, represented by a tank filled with TBE solution.

4- For sample preparation, a mixture was created by combining 3 microliters of loading solution (Intron/Korea) with 5 microliters of the nucleic acid extract obtained using the procedure outlined in section 3-4-6-1. The resulting mixture was subjected to electrophoresis under an electric current of 70 volts for a duration of 1-2 hours. The process continued until the dye migrated to the opposite end of the gel. Subsequently, the gel was examined using a 336 nm UV light source for analysis.

5- To prepare the reaction, the primers were diluted by adding distilled water to achieve a concentration of 100 picomoles per microliter, following the instructions provided by the supplier as per the attached leaflet. The required concentration was then prepared by taking 10 microliters of the original solution and adjusting the volume to 100 microliters by adding distilled water. In this study, the ITS1 primer was utilized, along with the forward and reverse primer ITS4 obtained from Inc IDT (Integrated DNA Technologies company, Canada.). Please refer to Table 1 for more details.

Table (1) ITS primers used in the study according to [22]

Primer	Sequence	Tm (°C)	Product size
Forward ITS1	5'- TCCGTAGGTGAACCTGCGG -3'	62	550 base pair
Reverse ITS4	5'TCCTCCGCTTATTGATATGC -3'	58	

6- Polymerase Chain Reaction (PCR). The PCR technique was used to amplify the ITS region using the ITS1 and ITS4 primers. A PCR amplification was performed with a total volume of 25 µL containing 7 µL of DNA, 5 µL of Taq PCR PreMix, 1 µL of each primer (µM (10), then distilled water was added to the tube for a total volume of 25 µl (Table 2), the reaction conditions were carried out according to the steps in Table (3).

Table (2) Components of the Maxime PCR PreMix kit (i-Taq)

Material	Volume
i-Taq DNA Polymerase	5U/µl
DNTPs(G,C,A and T)	2.5Mm
Reaction buffer (10X)	1X
Gel loading buffer	1X

Table 3: Sample preparation steps for ITS region amplification by PCR, according to [22]

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	1 µl (10 picomols/µl)
Reverse primer	1 µl (10 picomols/µl)
DNA	7µl
D.D.W	Up to 25 µl

Table (4) Phases of the thermal cycles of the polymerase chain reaction (PCR) technology, according to [22]

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95 C	3 min.	1 cycle
2-	Denaturation	95 C	45sec	
3-	Annealing	54 C	1 min	35 cycle
4-	Extension-1	72 C	1 min	
5-	Final Extension	72 C	7 min.	1 cycle
6-	Storage	4° C		

7- Detection of PCR products After completing the polymerase chain reaction, the amplification results were electrophoresed on a 1.5% agarose gel plate using the DNA leddar molecular marker (1000plus) and with the previous migration steps of the extracted DNA sample that were carried out before as in paragraph 3-, then the separated bundles of DNA were determined. On the agarose gel plate after being examined with ultraviolet light (302 nm) and photographed with the digital camera to be studied.

8- Studying the sequence of bases in the amplified DNA segment. To determine the phylogenetic tree analysis of the sex under study, the nucleotide sequence of the DNA sequencer was studied by determining the sequence of the ITS region resulting from the polymerase chain reaction and using the prefix ITS1 and ITS4. After conducting the PCR examination, the product of the PCR reaction was sent to the Macrogen corporation in Korea. To perform the DNA sequencing using a device (genetic analyzer), the NCBI- database program and the Mega-X program were also used to analyze the results related to the yeast nucleotide sequences under study and to draw the evolutionary tree Phylogenetic analysis.

Results and discussion

Molecular diagnosis of clinical isolates of *Candida* spp.

DNA extraction from Candida spp

After examination and phenotypic diagnosis of Candida yeast isolates, 13 isolates were elected for accurate molecular diagnosis. The DNA of these isolates was extracted in order to complete the molecular study and diagnosis by identifying the sequences of the ITS region. The results revealed the possibility of extracting DNA from pure cultures of Candida yeast isolates and According to the extraction kit prepared by Promega \ USA and listed as Wizard® Genomic DNA Purification Kit, the presence of DNA in the isolates selected for

molecular study was confirmed by electrophoresis on an agarose gel at a concentration of 1.5% at 70 volts for one hour; The appearance of bands on the agarose gel indicates the presence of DNA in all isolates, as shown in Figure (1).

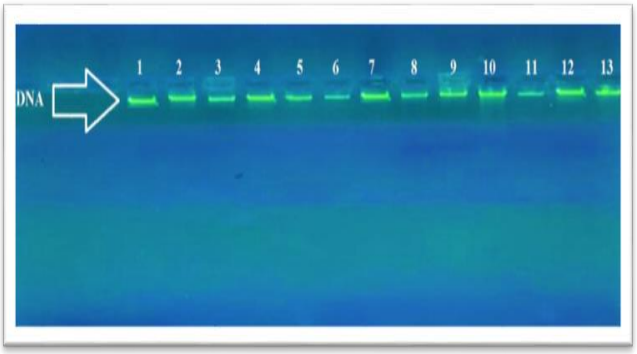


Figure (1): Electrophoresis of DNA extracted from Candida spp. on a 1.5% agarose gel at 70 volts for one hour

Measuring the concentration and purity of DNA

Table (5) indicates the measurement of DNA concentration and purity in the Nanodrop device for all isolates of *Candida* spp. The purity ranged between 1.58 - 2.0, and the concentration of the samples ranged between 17.61 - 81 ng/ml.

Table (5) Measurement of DNA concentration and purity of Candida spp. in the Nanodrop device

260/280 purity	Nucleic acid conc. (ng/ml)	sample ID
1.72	81	1
1.7	77.2	2
1.53	22.62	3
1.79	43	4
1.8	33	5
2.0	29.7	6
1.58	66.11	7
1.71	81	8
1.94	39.53	9
1.9	80.31	10
1.79	17.61	11
1.8	20.17	12
2.0	36.3	13

The results of DNA extraction based on the extraction kit prepared by Promega \ USA and listed as Wizard® Genomic DNA Purification Kit showed that the results were good, and this was confirmed by obtaining a good concentration and purity of the DNA that was used in the subsequent steps of the molecular study, as mentioned by [23].

The top priority of the molecular study is to provide an efficient method for extracting a good and pure amount of DNA. Various methods are available to isolate DNA from fungi, but it takes a long time, and its quality and quantity are not satisfactory [24]. The main obstacle during the extraction of DNA from a culture is Fungi break cell walls containing tough chitin, as it resists the traditional DNA isolation method. As mentioned by In fungi and yeast, it is crucial to perform adequate and precise DNA isolation before any further analysis. This is because the DNA extraction process is often accompanied by numerous interfering substances, including salts, proteins, polysaccharides, and other unknown compounds. For PCR-based analyses in particular, it is essential to swiftly isolate relatively pure genomic DNA of substantial size, as it serves as a prerequisite for accurate results

The electrophoresis of PCR amplification products was conducted to analyze the results of amplifying the ITS region between rDNA genes using the ITS1 and ITS4 forward and reverse primers. A total of 12 DNA samples extracted from 13 Candida yeast isolates were subjected to this analysis. The replication was performed through the polymerase chain reaction (PCR) using the specific primers, followed by electrophoresis of the replicated products.

The results revealed the presence of bands in all samples, appearing at approximately 570 base pairs (bp), when compared to the DNA ladder (which included a band at 1000 bp). This observation indicates the successful binding of the primers and the occurrence of replication in all analyzed samples, as demonstrated in Figure (2).

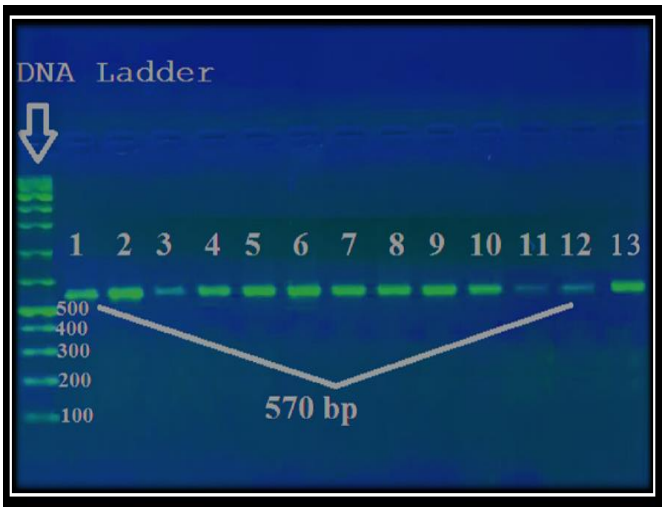


Figure (2): Electrophoresis of PCR amplification products using primers ITS4 - ITS1 for 13 isolates of Candida yeast on 1.5% agarose gel at 70 V for 1.5 hours and the appearance of bands at approximately 570 bp compared to DNA ladder (1000 plus).

The results of the PCR duplication results confirmed the doubling of the target region, according to the primers used. It was confirmed that the specific region was duplicated through the appearance of bands during electrophoresis on the agarose gel. This was confirmed by studies that the polymerase chain reaction (PCR) is a fast and specific method with high sensitivity in detecting a fraction target DNA and offers microbiology an alternative to traditional procedures, especially in the diagnosis of fungi [23, 25]. [26] also reported that molecular

identification techniques based on total extraction of fungal DNA provide a unique code for the identification and diagnosis of different fungal isolates even at the species level. Fungi are diagnosed using molecular techniques such as polymerase chain reaction (PCR) by amplifying rDNA genes with universal primers for fungal species [27, 28].

Identification of yeast isolates spp

Candida under study based on the sequence of nitrogenous bases of the ITS region: The results of the nitrogen bases sequence analysis, after comparing them with the isolates and species registered in NCBI, proved that all the isolates are isolates belonging to the genus *Candida*, and this result confirms the results of the isolation and initial diagnosis of the yeast under study. The nitrogen bases registration document for all *Candida* yeast isolates on the NCBI site clarified the classification of the genus under study as follows: Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomyces's; Saccharomycetales; Debaryomycetaceae; Lodderomyces clade; *Candida*. The molecular study, and through the results of comparison with the globally registered isolates, showed the accurate diagnosis at the species level of the 13 isolates of Candida that they belong to the following species: C. albicans 6 isolates, C. tropicalis 2 isolates, C. parapsilosis 2 isolates, C. glabrata 1 isolate, C. dubiniensis 1 isolate, and C. ciferri 1 isolate. The results of the comparison with isolates and strains registered globally in NCBI showed that the eight strains obtained in this study are strains registered for the first time in Iraq through the presence of mutations in some nitrogenous bases, as shown in Table (6).

Also, the study, through molecular diagnosis, confirmed the registration of a species recorded for the first time in Iraq at the molecular level, which is *C. ciferri*.

Table (6) the global isolates and their accession numbers in NCBI, which were compared with them through the BLAST program, showing the type and location of the heterogeneity, in addition to the percentage of congruence with the isolates under study.

Gene: ITS internal transcribed spacer						
No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Transition	412	A\G	ID: MK805514.1	Candida albicans	99%
2	Transition	41	C\T	ID: MF797769.1	Candida albicans	99%
	Transvertion	210	C\G			
	Transvertion	214	T\G			
3	Transvertion	100	T\G	ID: ON845633.1	Candida albicans	98.80%
	Transvertion	155	T\A			
	Transvertion	262	T\A			
	Transvertion	424	G\C			
	Transition	439	A\G			
4	-	-	-	ID: MN263159.1	Candida albicans	100%
5	Transition	114	C\T	ID: OP031644.1	Candida albicans	99%
	Transvertion	160	A\C			
6	-	-	-	ID: KY101889.1	Candida albicans	99%
7	Transition	364	T\A	ID: OP077322.1	Candida tropicalis	99%
	Transition	378	C\G			
8	-	-	-	ID: AF268095.1	Candida tropicalis	99%
9	Transvertion	11	C\A	ID: OP042388.1	Candida parapsilosis	98.53%
	Transvertion	36	T\A			
	Transition	75	A\G			
	Transvertion	141	A\C			
	Transvertion	218	G\T			
	Transition	400	C\T			
10	Transvertion	93	T\G	ID: ON954678.1	Candida parapsilosis	99%
	Transvertion	97	C\G			
	Transition	280	T\A			
11	-	-	-	ID: GU199447.1	Candida glabrata	100%
12	Transvertion	396	C\G	ID: KY412696.1	Candida dubiniensis	99.70%
13	Transition	79	C\T	ID: KY065365.1	Candida sp.	99%

Results of comparison (alignment) of each isolate under study with isolates registered in NCBI The results of the sequence of nitrogenous bases obtained from the Macrogen company in Korea for the isolates under study in the BLAST program were analyzed and through comparison with the isolates registered in NCBI, the following results were recorded:

1- Isolate No. 1 (*C. albicans* strain suhad 1). The results of the molecular analysis showed that this strain was diagnosed for the first time in Iraq, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 1 showed that it was 99% identical to the isolate *C. albicans* registered in China under ID: MK805514.1.

The results of the alignment shown in Figure (3) also showed that there was a variation at site 412, as the guanine nitrogenous base was recorded in the sequences of the isolate under study instead of the adenine

nitrogenous base (G\A), and this variation was of the type of Transition (equivalent substitution).

Candida albicans strain TBS611 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MK805514.1 Length: 532 Number of Matches: 1

Range 1: 143 to 478 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
616 bits(333)	9e-172	335/336(99%)	0/336(0%)	Plus/Plus

```
Query  1    ATCAACTTGTCACACCAGATTATTACTTAATAGTCAAAACTTTCAACAACGGATCTCTTG  60
Sbjct  143  ............................................................  202

Query  61   GTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTC  120
Sbjct  203  ............................................................  262

Query  121  GTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTG  180
Sbjct  263  ............................................................  322

Query  181  TTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGTGTTGAGCAATACGACTTGGGTT  240
Sbjct  323  ............................................................  382

Query  241  TGCTTGAAAGACGGTAGTGGTAAGGCGGGGTCGCTTTGACAATGGCTTAGGTCTAACCAA  300
Sbjct  383  ............................A...............................  442

Query  301  AAACATTGCTTGCGGCGGTAACGTCCACCACGTATA  336
Sbjct  443  ....................................  478
```

Figure (3): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 1 under study and the isolate with the highest matching C. albicans registered in China under ID: MK805514.1, with a match rate of 99%

2- Isolate No. 2 (C. albicans strain suhad 2). The results of the molecular analysis showed that this strain was diagnosed for the first time in Iraq, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 2 showed that it was 99% identical to the isolate C. albicans registered in Brazil under ID: MF797769.1. The results of the alignment shown in Figure (4) also showed that there was a discrepancy at position 41, as the nitrogenous base thymine was recorded instead of cytosine (T\C). \C) and with thymine (G\T) respectively and the covariance was of non-equivalent substitution type Transvertion.

Candida albicans isolate S250 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MF797769.1 Length: 529 Number of Matches: 1

Range 1: 14 to 492 GenBank Graphics ▼ Next Match ▲ Previous Match

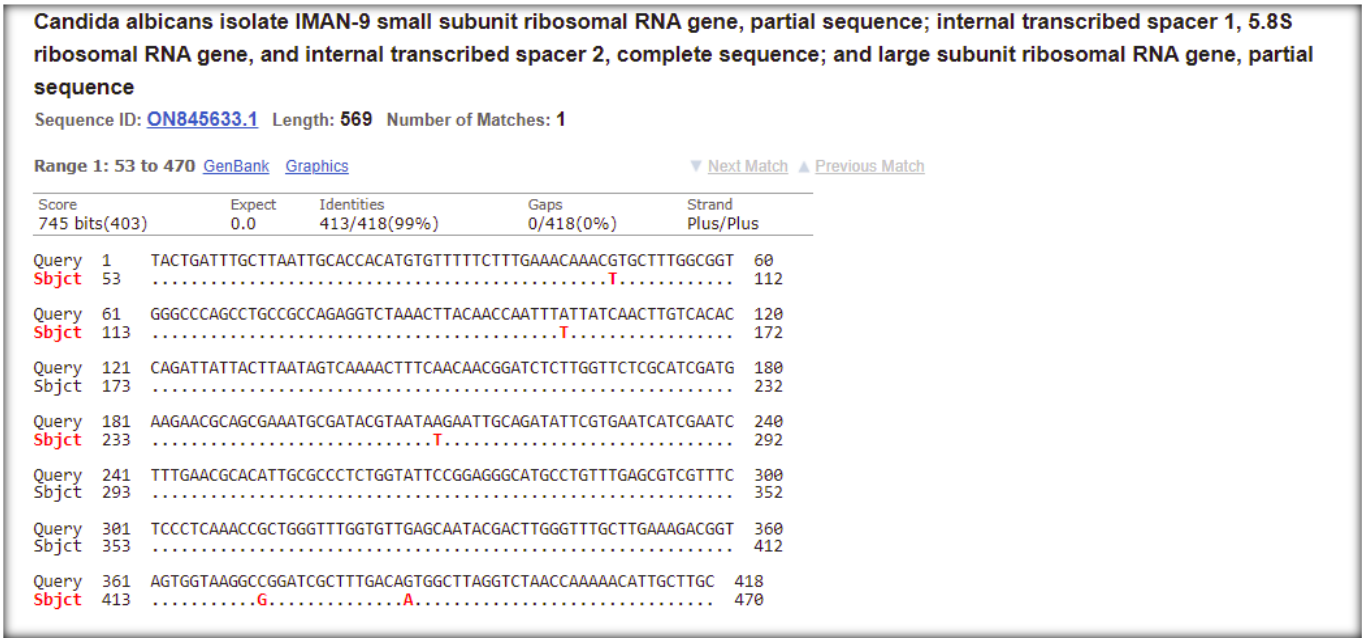
Score	Expect	Identities	Gaps	Strand
869 bits(470)	0.0	476/479(99%)	0/479(0%)	Plus/Plus

```
Query  1    GTCGTAACAAGGTTTCCGTAGGTGAACTTGCGGAAGGATCATTACTGATTTGCTTAATTG  60
Sbjct  14   ..........................C.............................  73

Query  61   CACCACATGTGTTTTTCTTTGAAACAAACTTGCTTTGGCGGTGGGCCCAGCCTGCCGCCA  120
Sbjct  74   ............................................................  133

Query  121  GAGGTCTAAACTTACAACCAATTTTTTATCAACTTGTCACACCAGATTATTACTTAATAG  180
Sbjct  134  ............................................................  193

Query  181  TCAAAACTTTCAACAAGGGAGCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGC  240
Sbjct  194  ..............C...T.....................................  253

Query  241  GATACGTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGC  300
Sbjct  254  ............................................................  313

Query  301  CCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCCTCAAACCGCTGGGT  360
Sbjct  314  ............................................................  373

Query  361  TTGGTGTTGAGCAATACGACTTGGGTTTGCTTGAAAGACGGTAGTGGTAAGGCGGGATCG  420
Sbjct  374  ............................................................  433

Query  421  CTTTGACAATGGCTTAGGTCTAACCAAAAACATTGCTTGCGGCGGTAACGTCCACCACG  479
Sbjct  434  ............................................................  492
```

Figure (4): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 2 under study and the isolate with the highest matching C. albicans registered in Brazil under ID: MF797769.1, with a match rate of 99%

3- Isolate No. 3 (C. albicans strain suhad 3). The results of the molecular analysis showed that this strain was diagnosed for the first time in Iraq, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 3 showed that it was identical by 98.80% with the isolate C. albicans registered in Iraq under ID: ON845633.1. The results of the alignment shown in Figure (5) also showed that there were five variations at position 100, 155 and 262, as the nitrogenous bases G, adenine A and adenine A were recorded instead of the base thymine T in the sequences under study, respectively, and the variations were of the

type of Transvertion; There was also a discrepancy at position 424, as base C replaced G in the type of Transvertion, and there was a discrepancy at 439, as base G replaced A in place of Transition type



Candida albicans isolate IMAN-9 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: ON845633.1 Length: 569 Number of Matches: 1

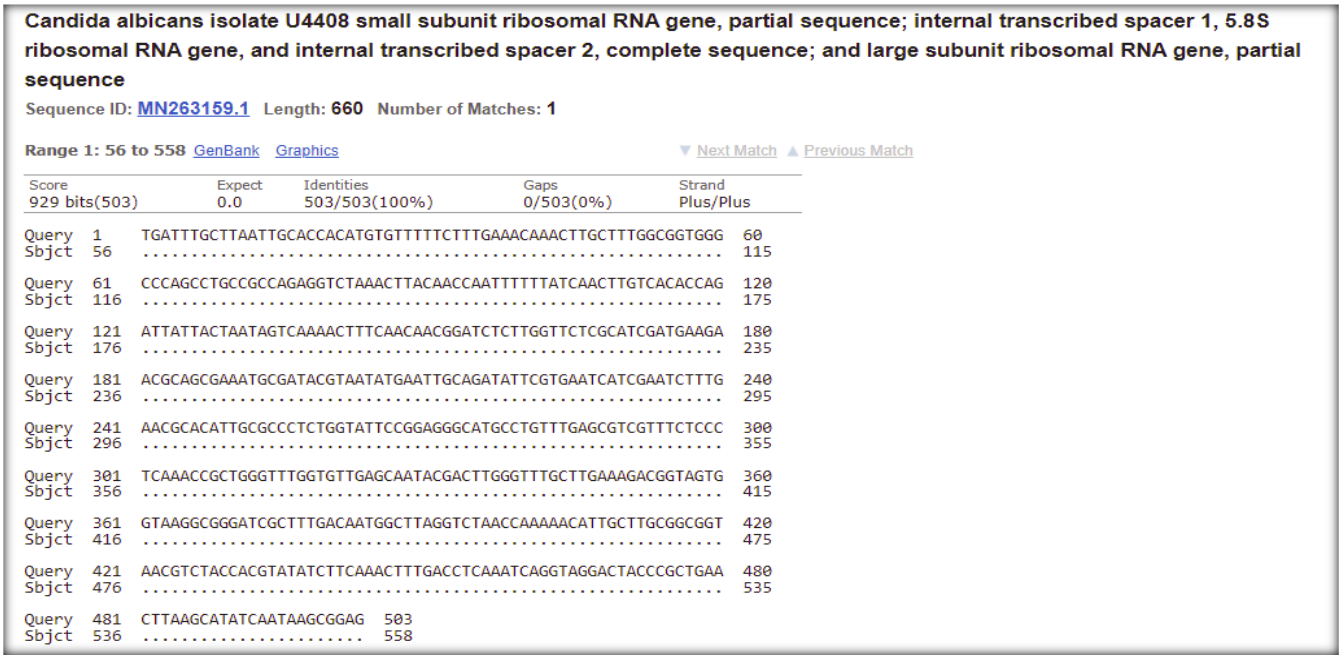
Range 1: 53 to 470 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
745 bits(403)	0.0	413/418(99%)	0/418(0%)	Plus/Plus

```
Query  1    TACTGATTTGCTTAATTGCACCACATGTGTTTTTCTTTGAAACAAACGTGCTTTGGCGGT  60
Sbjct  53   ..............................................T.............  112

Query  61   GGGCCCAGCCTGCCGCCAGAGGTCTAAACTTACAACCAATTTATTATCAACTTGTCACAC  120
Sbjct  113  ..........................................T..................  172

Query  121  CAGATTATTACTTAATAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATG  180
Sbjct  173  ............................................................  232

Query  181  AAGAACGCAGCGAAATGCGATACGTAATAAGAATTGCAGATATTCGTGAATCATCGAATC  240
Sbjct  233  ............................T...............................  292

Query  241  TTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTC  300
Sbjct  293  ............................................................  352

Query  301  TCCCTCAAACCGCTGGGTTTGGTGTTGAGCAATACGACTTGGGTTTGCTTGAAAGACGGT  360
Sbjct  353  ............................................................  412

Query  361  AGTGGTAAGGCCGGATCGCTTTGACAGTGGCTTAGGTCTAACCAAAAACATTGCTTGC  418
Sbjct  413  ...........G..............A.............................  470
```

Figure (5): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 3 under study and the isolate with the highest matching C. albicans registered in Iraq under ID: ON845633.1, with a match rate of 98.80%

4- Isolate No. 4 (C. albicans strain suhad 4). The results of the molecular analysis showed that the results of the comparison of the nitrogenous base sequences of isolate No. 4 were 100% identical to the isolate C. albicans registered in Vietnam under an accession ID: MN263159.1, as shown in Figure (6).



Candida albicans isolate U4408 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MN263159.1 Length: 660 Number of Matches: 1

Range 1: 56 to 558 GenBank Graphics ▼ Next Match ▲ Previous Match

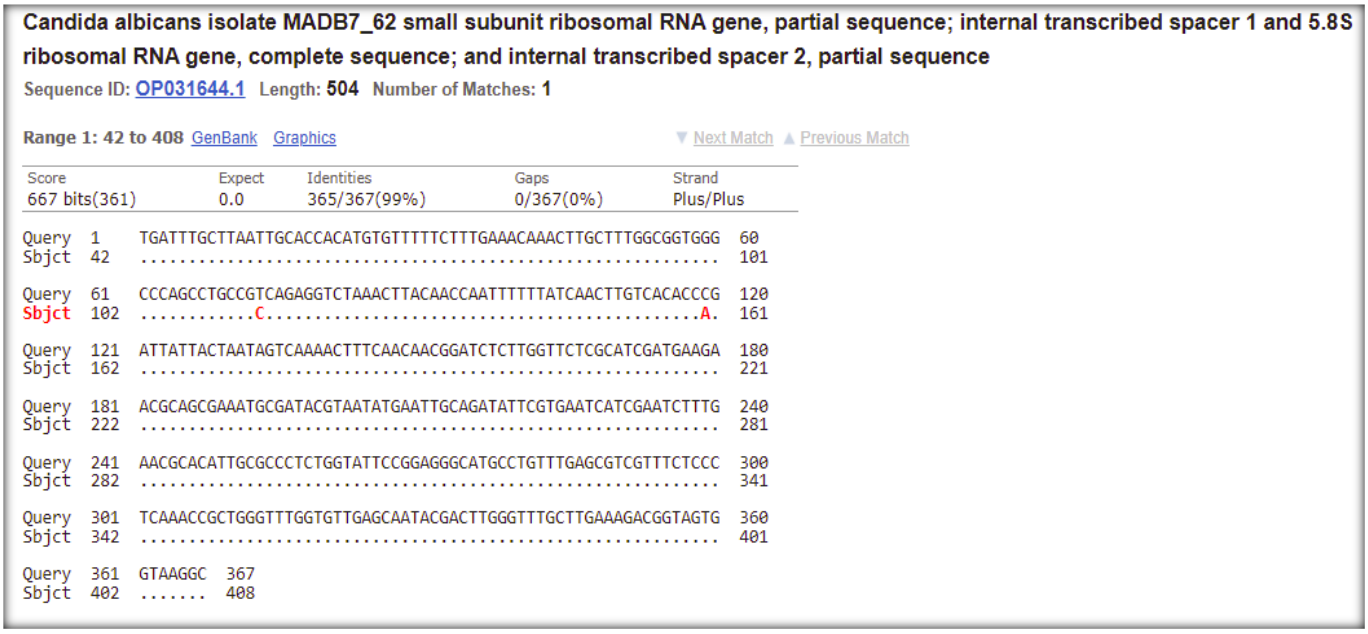
Score	Expect	Identities	Gaps	Strand
929 bits(503)	0.0	503/503(100%)	0/503(0%)	Plus/Plus

```
Query  1    TGATTTGCTTAATTGCACCACATGTGTTTTTCTTTGAAACAAACTTGCTTTGGCGGTGGG  60
Sbjct  56   ............................................................  115

Query  61   CCCAGCCTGCCGCCAGAGGTCTAAACTTACAACCAATTTTTTATCAACTTGTCACACCAG  120
Sbjct  116  ............................................................  175

Query  121  ATTATTACTAATAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  180
Sbjct  176  ............................................................  235

Query  181  ACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTG  240
Sbjct  236  ............................................................  295

Query  241  AACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCC  300
Sbjct  296  ............................................................  355

Query  301  TCAAACCGCTGGGTTTGGTGTTGAGCAATACGACTTGGGTTTGCTTGAAAGACGGTAGTG  360
Sbjct  356  ............................................................  415

Query  361  GTAAGGCGGGATCGCTTTGACAATGGCTTAGGTCTAACCAAAAACATTGCTTGCGGCGGT  420
Sbjct  416  ............................................................  475

Query  421  AACGTCTACCACGTATATCTTCAAACTTTGACCTCAAATCAGGTAGGACTACCCGCTGAA  480
Sbjct  476  ............................................................  535

Query  481  CTTAAGCATATCAATAAGCGGAG  503
Sbjct  536  .......................  558
```

Figure (6): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 4 under study and the isolate with the highest matching C. albicans registered in Vietnam under ID: MN263159.1, with a match rate of 100%

5- Isolate No. 5 (C. albicans strain suhad 5). The results of the molecular analysis showed that this strain was diagnosed for the first time in Iraq, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 5 showed that it was 99% identical to the isolate C. albicans registered in Iraq under ID: OP031644.1. The results of the alignment shown in Figure (4-10) also showed that there was a discrepancy at position 114, as the nitrogenous base thymine was recorded instead of cytosine (T\C). (C\A) and the type of variation was Transvertion. The strain is closely related to an existing isolate, but there is a specific

variation at position 114, involving a transversion from thymine to cytosine and from cytosine to adenine.



Candida albicans isolate MADB7_62 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: OP031644.1 Length: 504 Number of Matches: 1

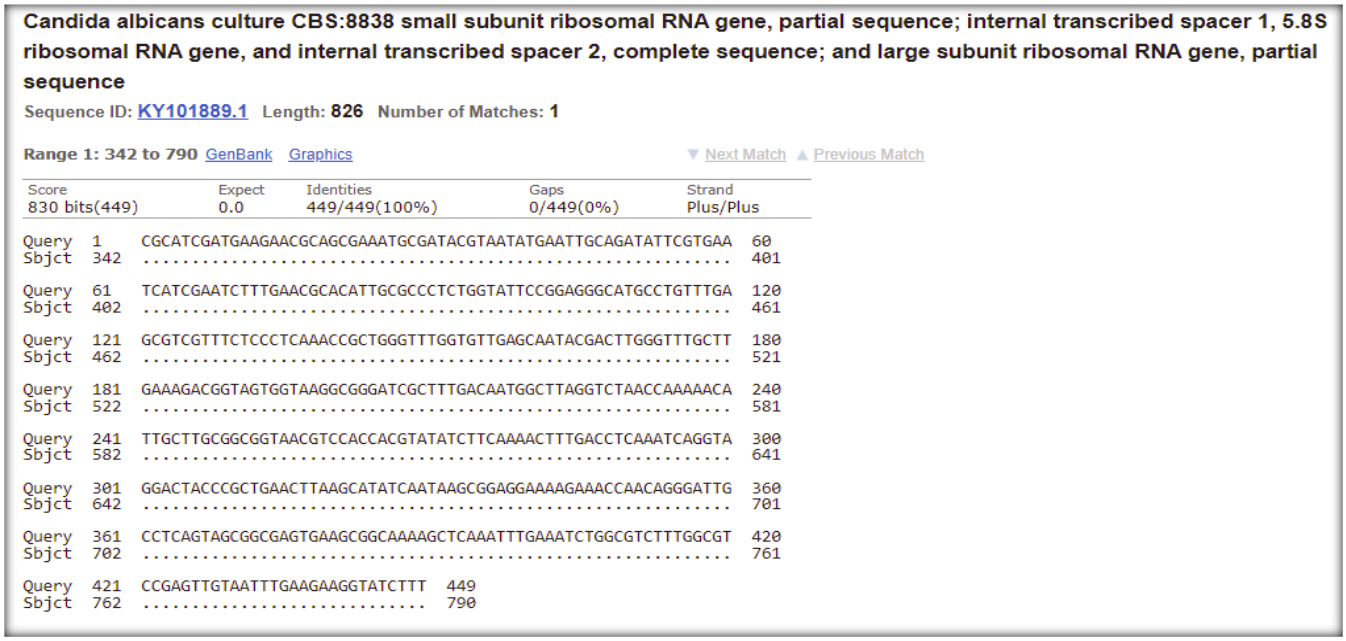
Range 1: 42 to 408 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
667 bits(361)	0.0	365/367(99%)	0/367(0%)	Plus/Plus

```
Query  1    TGATTTGCTTAATTGCACCACATGTGTTTTTCTTTGAAACAAACTTGCTTTGGCGGTGGG  60
Sbjct  42   ............................................................  101

Query  61   CCCAGCCTGCCGTCAGAGGTCTAAACTTACAACCAATTTTTTATCAACTTGTCACACCCG  120
Sbjct  102  ............C...........................................A.  161

Query  121  ATTATTACTAATAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  180
Sbjct  162  ............................................................  221

Query  181  ACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTG  240
Sbjct  222  ............................................................  281

Query  241  AACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGCGTCGTTTCTCCC  300
Sbjct  282  ............................................................  341

Query  301  TCAAACCGCTGGGTTTGGTGTTGAGCAATACGACTTGGGTTTGCTTGAAAGACGGTAGTG  360
Sbjct  342  ............................................................  401

Query  361  GTAAGGC  367
Sbjct  402  .......  408
```

Figure (7): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 5 under study and the isolate with the highest matching C. albicans registered in Iraq under ID: OP031644.1, with a match rate of 99%.

6- Isolate No. 6 (C. albicans strain suhad 6). The results of the molecular analysis showed that the results of the comparison of the sequences of the nitrogenous bases of isolate No. 6 were 100% identical to the isolate C. albicans registered in the Netherlands under the accession identification number ID: KY101889.1, as shown in Figure (8). This high degree of identity suggests a close relationship between the two isolates, which could have implications for understanding the genetic similarities and potential epidemiological connections between strains from different geographical locations.



Candida albicans culture CBS:8838 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: KY101889.1 Length: 826 Number of Matches: 1

Range 1: 342 to 790 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
830 bits(449)	0.0	449/449(100%)	0/449(0%)	Plus/Plus

```
Query  1    CGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAA  60
Sbjct  342  ............................................................  401

Query  61   TCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGA  120
Sbjct  402  ............................................................  461

Query  121  GCGTCGTTTCTCCCTCAAACCGCTGGGTTTGGTGTTGAGCAATACGACTTGGGTTTGCTT  180
Sbjct  462  ............................................................  521

Query  181  GAAAGACGGTAGTGGTAAGGCGGGATCGCTTTGACAATGGCTTAGGTCTAACCAAAAACA  240
Sbjct  522  ............................................................  581

Query  241  TTGCTTGCGGCGGTAACGTCCACCACGTATATCTTCAAAACTTTGACCTCAAATCAGGTA  300
Sbjct  582  ............................................................  641

Query  301  GGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTG  360
Sbjct  642  ............................................................  701

Query  361  CCTCAGTAGCGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGGCGTCTTTGGCGT  420
Sbjct  702  ............................................................  761

Query  421  CCGAGTTGTAATTTGAAGAAGGTATCTTT  449
Sbjct  762  ............................  790
```

Figure (8): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 6 under study and the isolate with the highest matching C. albicans registered in the Netherlands under ID: KY101889.1, with a match rate of 100%.

7- Isolate No. 7 (C. tropicalis strain suhad 7). The results of the molecular analysis showed that this strain was diagnosed for the first time in Iraq, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 7 showed that it was 99% identical to the isolate C. tropicalis registered in India under ID: OP077322.1. The results of the alignment shown in Figure (9) also showed that there is a discrepancy at site 364, as the nitrogenous base adenine was recorded instead of thymine (A\T);

There was also a variation in the position 378, as the base of the two guanines was replaced by the cytosine (G/C), and each of the variants was of the type of Transvertion [29].

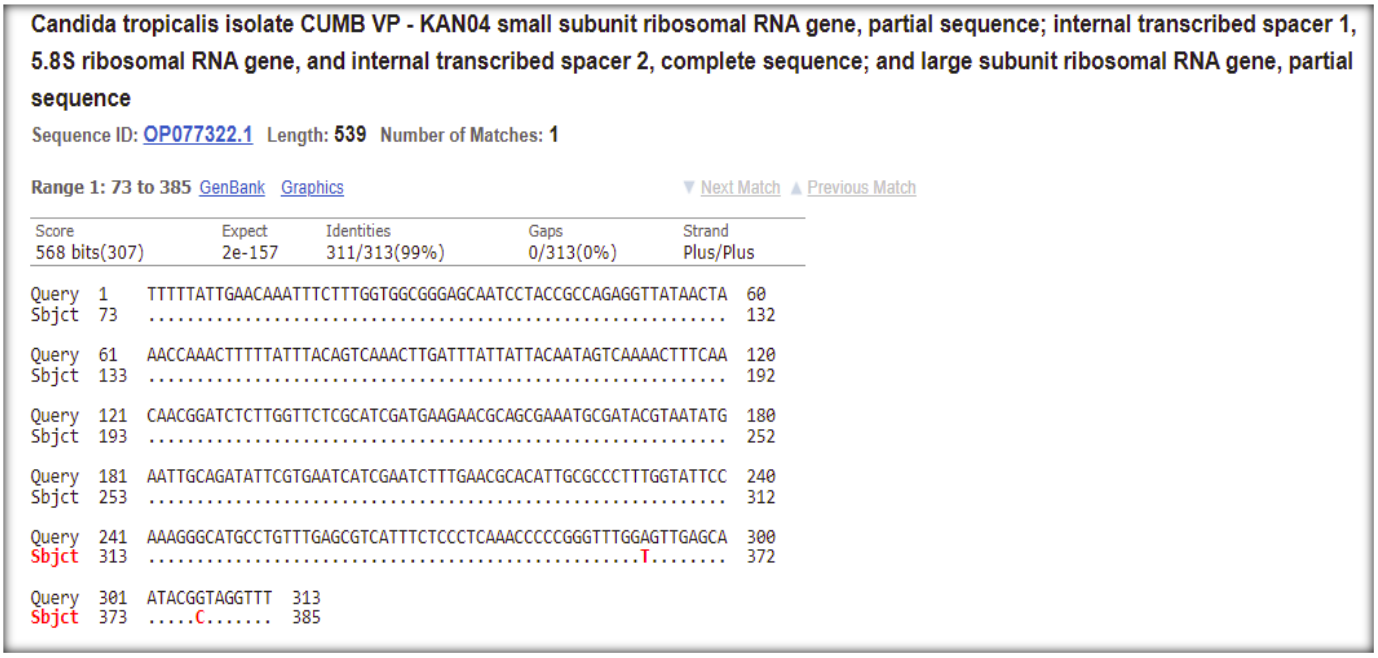


Figure (9) Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 7 under study and the highest matching isolate C. tropicalis registered in India under accession ID: OP077322.1, with a match rate of 99%.

8 - Isolate No. 8 (C. tropicalis strain suhad 8). The results of the molecular analysis showed that the results of the comparison of the sequences of the nitrogenous bases of isolate No. 8 were 100% identical to the isolate C. tropicalis registered in Argentina under the accession identification number ID: AF268095.1, as shown in Figure (10). This result indicates a complete match in the genetic makeup of isolate No. 8 with the C. tropicalis isolate from Argentina. Such findings are crucial for understanding the relationships between different isolates and can contribute to insights into the genetic diversity, evolution, or transmission patterns of the microorganism

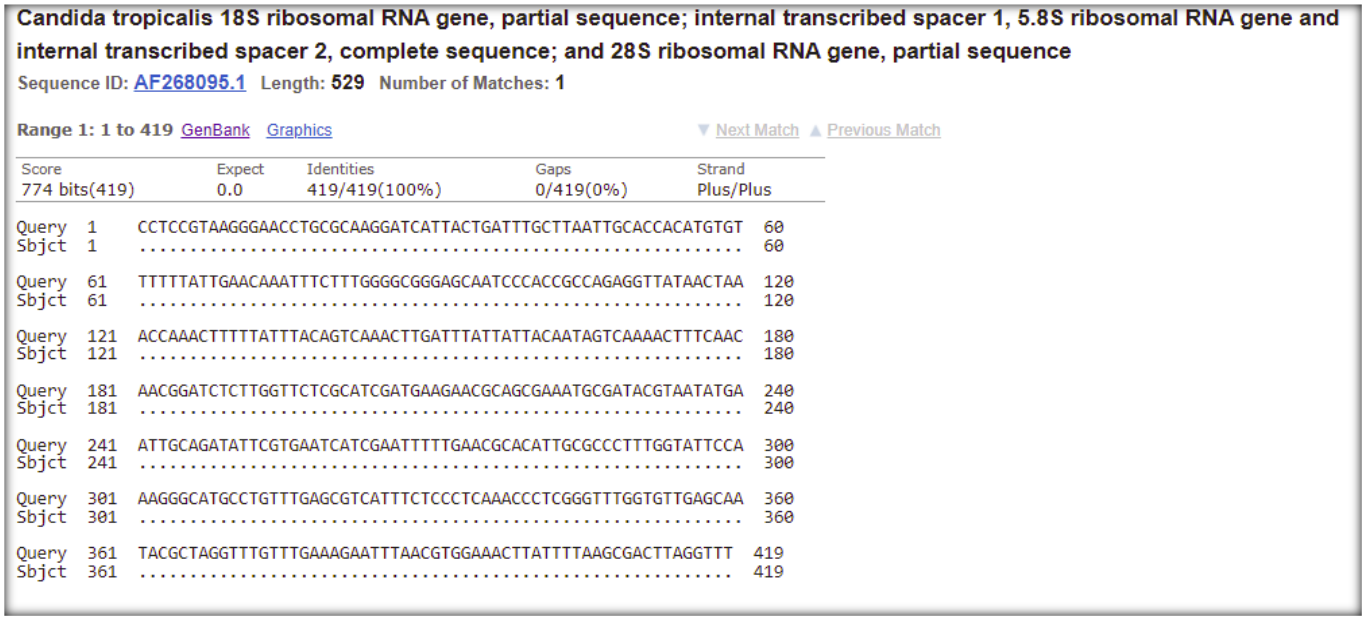
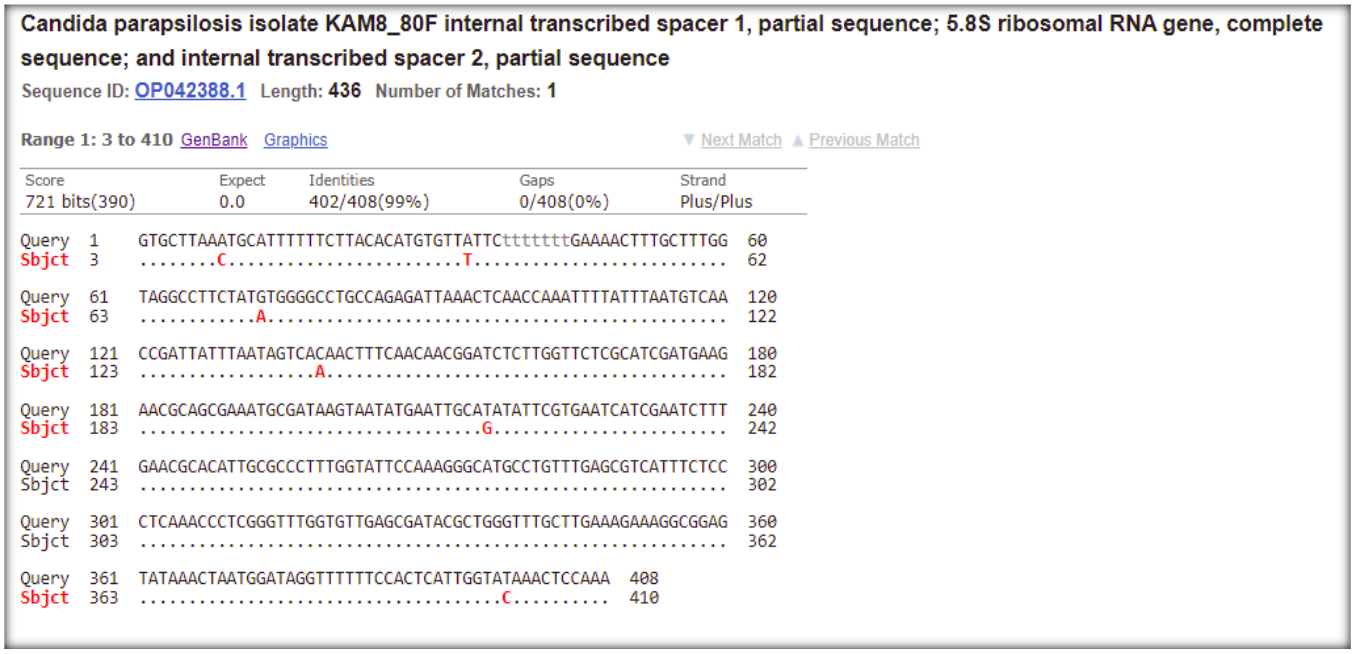


Figure (10) Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 8 under study and the most identical isolate C. tropicalis registered in Argentina under ID: AF268095.1, with a match rate of 100%

9- Isolate No. 9 (C. parapsilosis strain suhad 9). The results of the molecular analysis showed that this strain was diagnosed for the first time in Iraq, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 9 showed that it was identical by 98.53% with the isolate C. parapsilosis registered in Iraq under

the accession ID: OP042388.1. The results of the alignment shown in Figure (11) also showed that there are six variations at locations 11, 36, 141 and 218, all of which were of the Transvertion type, while the variation was of the Transition type.

The high similarity with the registered isolate suggests a close relationship, while the identified variations at specific locations, including Transversion and Transition types, contribute to understanding the genetic diversity within this strain.



Candida parapsilosis isolate KAM8_80F internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: OP042388.1 Length: 436 Number of Matches: 1

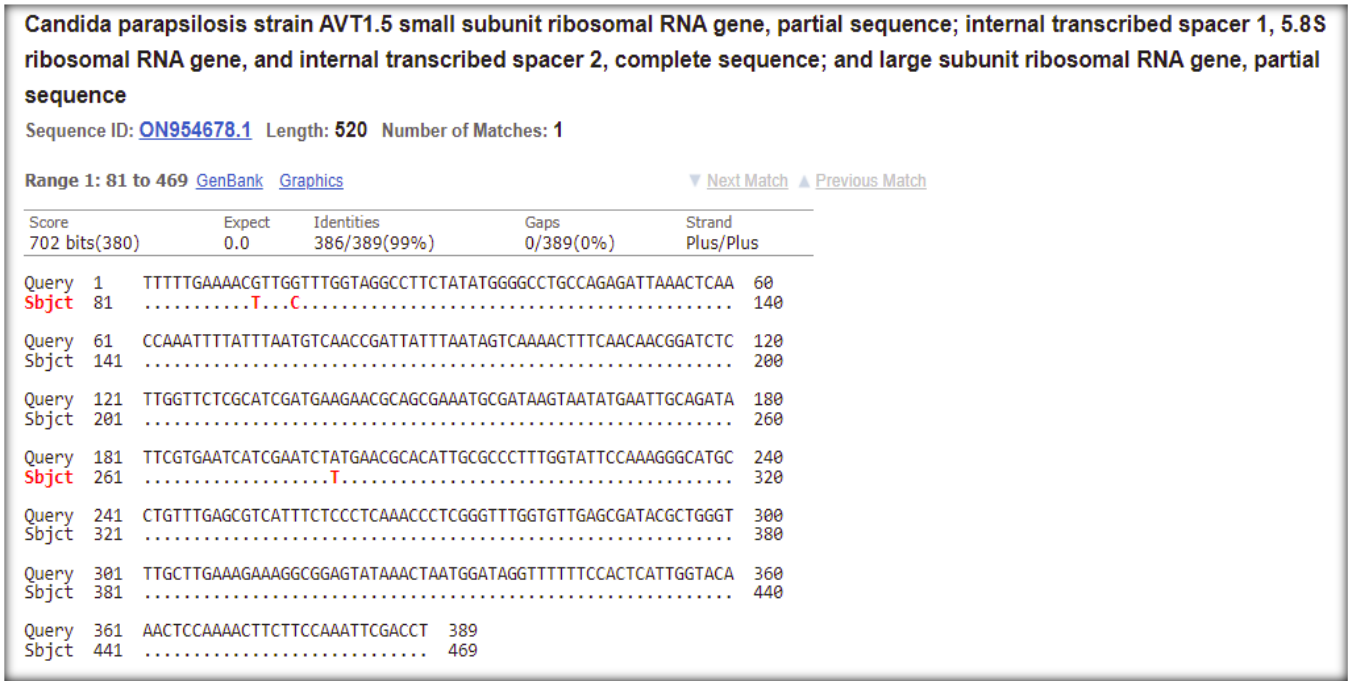
Range 1: 3 to 410 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
721 bits(390)	0.0	402/408(99%)	0/408(0%)	Plus/Plus

```
Query  1    GTGCTTAAATGCATTTTTTCTTACACATGTGTTATTCttttttttGAAAACTTTGCTTTGG  60
Sbjct  3    ........C.........................T.............................  62

Query  61   TAGGCCTTCTATGTGGGGCCTGCCAGAGATTAAACTCAACCAAATTTTATTTAATGTCAA  120
Sbjct  63   ............A.................................................  122

Query  121  CCGATTATTTAATAGTCACAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAG  180
Sbjct  123  ..................A.....................................  182

Query  181  AACGCAGCGAAATGCGATAAGTAATATGAATTGCATATATTCGTGAATCATCGAATCTTT  240
Sbjct  183  .....................................G.....................  242

Query  241  GAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCATTTCTCC  300
Sbjct  243  ............................................................  302

Query  301  CTCAAACCCTCGGGTTTGGTGTTGAGCGATACGCTGGGTTTGCTTGAAAGAAAGGCGGAG  360
Sbjct  303  ............................................................  362

Query  361  TATAAACTAATGGATAGGTTTTTTCCACTCATTGGTATAAACTCCAAA  408
Sbjct  363  .....................................C..........  410
```

Figure (11): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 9 under study and the isolate with the highest matching C. parapsilosis registered in Iraq under ID: OP042388.1, with a match rate of 98.53%

10 - Isolate No. 10 (C. parapsilosis strain suhad 10). The results of the molecular analysis showed that this strain was diagnosed for the first time in Iraq, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 10 showed that it was 99% identical to the isolate C. parapsilosis registered in India under the accession ID: ON954678.1. The results of the alignment shown in Figure (13) also showed that there were three variations at locations 93, 97 and 280, and all of them were of the type of Transvertion.



Candida parapsilosis strain AVT1.5 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: ON954678.1 Length: 520 Number of Matches: 1

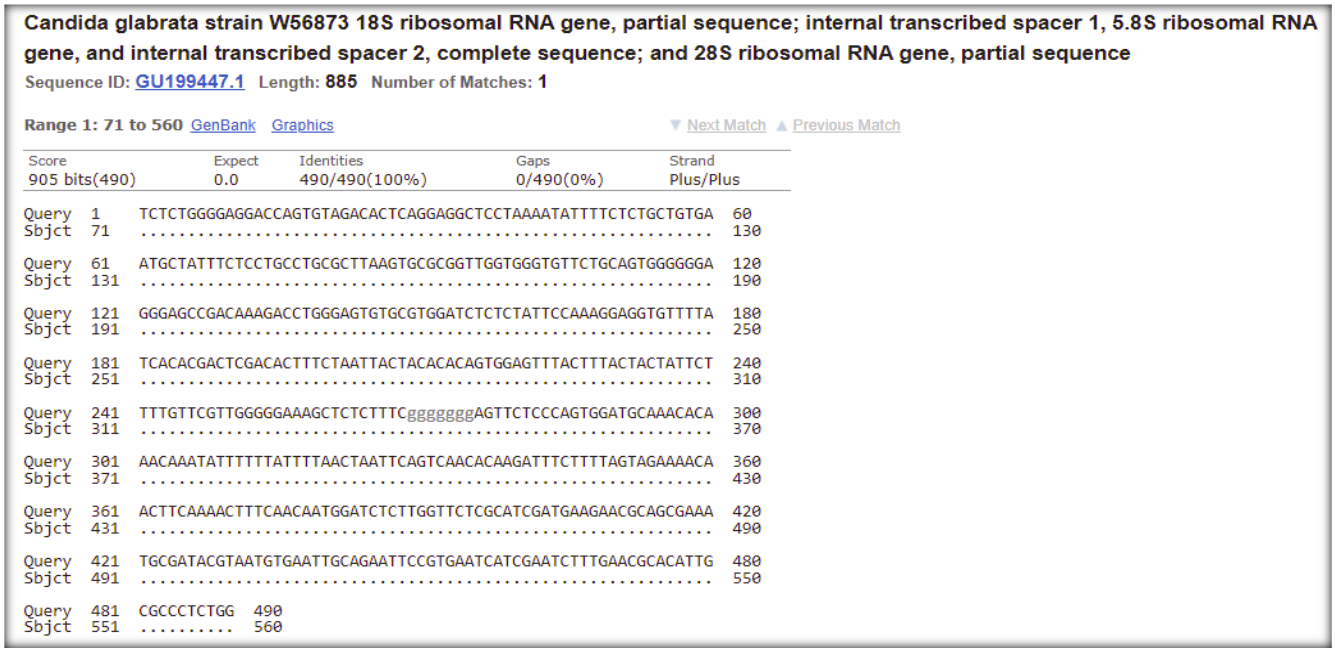
Range 1: 81 to 469 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
702 bits(380)	0.0	386/389(99%)	0/389(0%)	Plus/Plus

```
Query  1    TTTTTGAAAACGTTGGTTTGGTAGGCCTTCTATATGGGGCCTGCCAGAGATTAAACTCAA  60
Sbjct  81   ..........T...C.....................................  140

Query  61   CCAAATTTTATTTAATGTCAACCGATTATTTAATAGTCAAAACTTTCAACAACGGATCTC  120
Sbjct  141  ............................................................  200

Query  121  TTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATA  180
Sbjct  201  ............................................................  260

Query  181  TTCGTGAATCATCGAATCTATGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGC  240
Sbjct  261  ..................T.....................................  320

Query  241  CTGTTTGAGCGTCATTTCTCCCTCAAACCCTCGGGTTTGGTGTTGAGCGATACGCTGGGT  300
Sbjct  321  ............................................................  380

Query  301  TTGCTTGAAAGAAAGGCGGAGTATAAACTAATGGATAGGTTTTTTCCACTCATTGGTACA  360
Sbjct  381  ............................................................  440

Query  361  AACTCCAAAACTTCTTCCAAATTCGACCT  389
Sbjct  441  ............................  469
```

Figure (12): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 10 under study and the isolate that matches C. parapsilosis, registered in India under accession ID: ON954678.1, with a match rate of 99%

11 - Isolate No. 11 (C. glabrata strain suhad 11). The results of the molecular analysis showed that the results of the comparison of the nitrogenous base sequences of isolate No. 11 were 100% identical to the isolate C. glabrata registered in Iran under ID: GU199447.1, as shown in Figure (13).



Candida glabrata strain W56873 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: GU199447.1 Length: 885 Number of Matches: 1

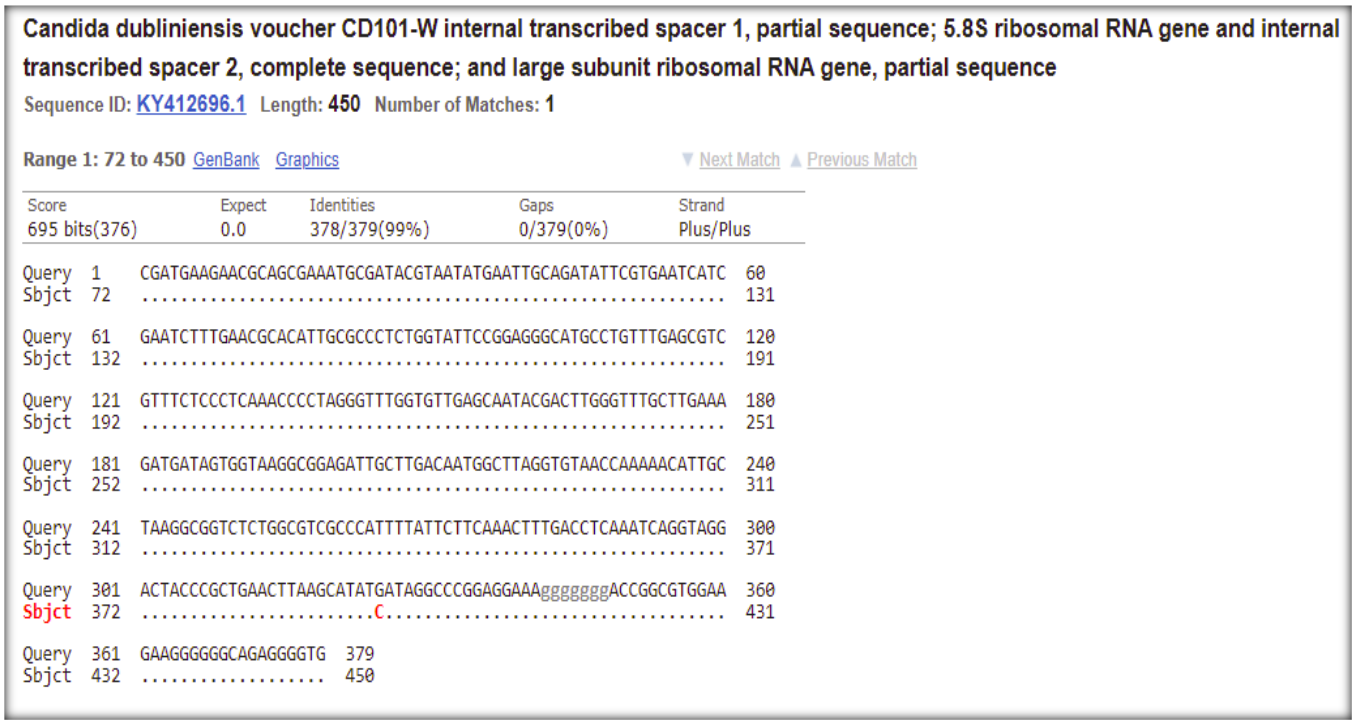
Range 1: 71 to 560 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
905 bits(490)	0.0	490/490(100%)	0/490(0%)	Plus/Plus

```
Query  1    TCTCTGGGGAGGACCAGTGTAGACACTCAGGAGGCTCCTAAAATATTTTCTCTGCTGTGA  60
Sbjct  71   ............................................................  130

Query  61   ATGCTATTTCTCCTGCCTGCGCTTAAGTGCGCGGTTGGTGGGTGTTCTGCAGTGGGGGGA  120
Sbjct  131  ............................................................  190

Query  121  GGGAGCCGACAAAGACCTGGGAGTGTGCGTGGATCTCTCTATTCCAAAGGAGGTGTTTTA  180
Sbjct  191  ............................................................  250

Query  181  TCACACGACTCGACACTTTCTAATTACTACACACAGTGGAGTTTACTTTACTACTATTCT  240
Sbjct  251  ............................................................  310

Query  241  TTTGTTCGTTGGGGGAAAGCTCTCTTTCgggggggAGTTCTCCCAGTGGATGCAAACACA  300
Sbjct  311  ............................................................  370

Query  301  AACAAATATTTTTTATTTTAACTAATTCAGTCAACACAAGATTTCTTTTAGTAGAAAACA  360
Sbjct  371  ............................................................  430

Query  361  ACTTCAAAACTTTCAACAATGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAA  420
Sbjct  431  ............................................................  490

Query  421  TGCGATACGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTG  480
Sbjct  491  ............................................................  550

Query  481  CGCCCTCTGG  490
Sbjct  551  ..........  560
```

Figure (13): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 11 under study and the highest matching isolate C. glabrata registered in Iran under ID: GU199447.1 with a 100% match rate

12- Isolate No. 12 (C. dubiniensis strain suhad 12). The results of the molecular analysis showed that this strain was diagnosed for the first time in Iraq, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 12 showed that it was 99.70% identical to the isolate C [30]. dubiniensis registered in Iran under the accession ID: KY412696.1. The results of the alignment shown in Figure (14) also showed that there was only one variation at the site at 396, and it was a type of Transvertion, as the nitrogenous base replaced the two guanines in the sequences under study instead of Cytosine (G\C).



Candida dubliniensis voucher CD101-W internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: KY412696.1 Length: 450 Number of Matches: 1

Range 1: 72 to 450 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
695 bits(376)	0.0	378/379(99%)	0/379(0%)	Plus/Plus

```
Query  1    CGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTGCAGATATTCGTGAATCATC  60
Sbjct  72   ............................................................  131

Query  61   GAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGCGTC  120
Sbjct  132  ............................................................  191

Query  121  GTTTCTCCCTCAAACCCCTAGGGTTTGGTGTTGAGCAATACGACTTGGGTTTGCTTGAAA  180
Sbjct  192  ............................................................  251

Query  181  GATGATAGTGGTAAGGCGGAGATTGCTTGACAATGGCTTAGGTGTAACCAAAAACATTGC  240
Sbjct  252  ............................................................  311

Query  241  TAAGGCGGTCTCTGGCGTCGCCCATTTTATTCTTCAAACTTTGACCTCAAATCAGGTAGG  300
Sbjct  312  ............................................................  371

Query  301  ACTACCCGCTGAACTTAAGCATATGATAGGCCCGGAGGAAAgggggggACCGGCGTGGAA  360
Sbjct  372  .....................C..............................  431

Query  361  GAAGGGGGGCAGAGGGGTG  379
Sbjct  432  ..................  450
```

Figure (14): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 12 under study and the highest matching isolate C. dubiniensis registered in Iran under ID: KY412696.1 with a match rate of 99.70%

13- Isolate No. 13 (C. ciferrii strain suhad 13). The results of the molecular analysis showed that this type of yeast was recorded for the first time in Iraq at the molecular level, as the results of the comparison of the sequence of the nitrogenous bases of isolate No. 13 showed that it was 99% identical to the isolate registered in India under an accession identification number ID: KY065365.1. The alignment shown in Figure (15) indicates that there is a discrepancy at position 79, and the discrepancy is of the Transition type [31].

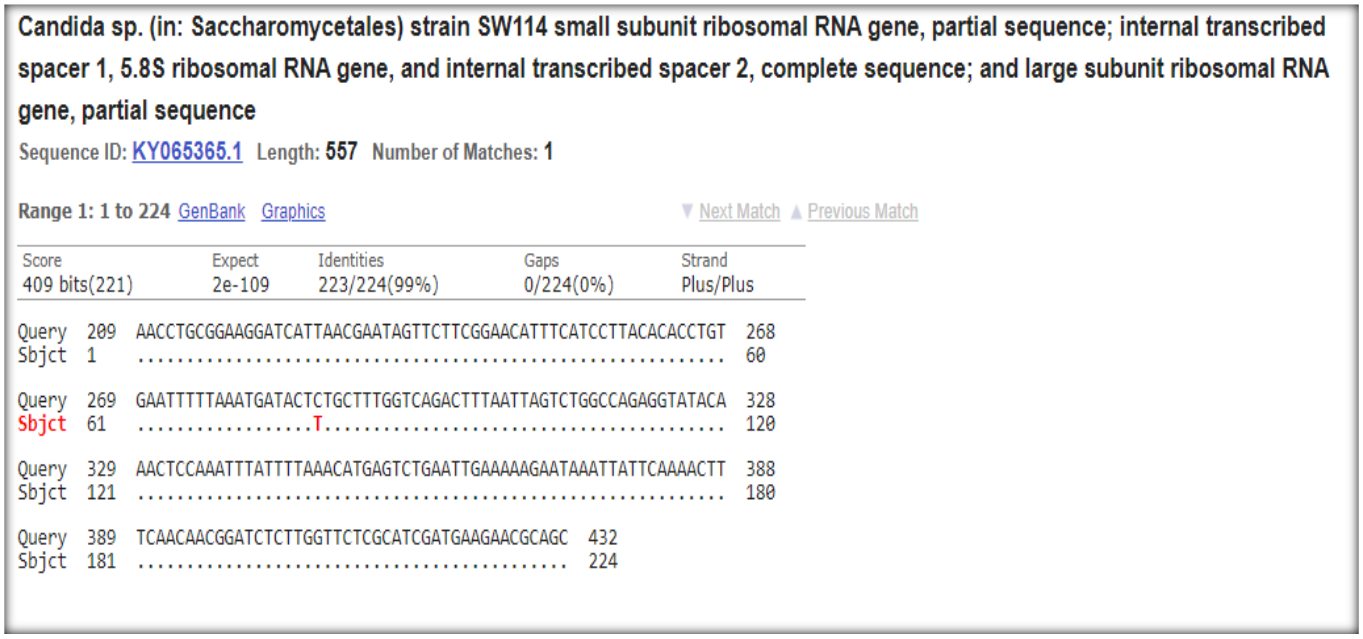


Figure (15): Alignment of the sequence of nitrogenous bases in the BLAST program between isolate No. 13 under study and the highest isolate matching Candida sp. Registered in India under ID: KY065365.1 with a 99% match rate

Initially, the DNA sequences were subjected to verification in order to confirm their nucleotide sequence. Subsequently, a comparison was made between these sequences and other universal strains. This comparison was performed using the nucleotide-online NCBI-BLAST-Query software, which facilitated accurate results and enabled comparison with international strains, yielding a high similarity ranging between 98-99%. To further analyze the data, the MEGA software was utilized. MEGA is a specialized application designed for comparative analysis of gene sequences that are homologous, facilitating the examination of evolutionary relationships and patterns of DNA and protein evolution [32]. Alongside statistical data analysis tools, MEGA offers several features, including the ability to retrieve and display phylogenetic trees by incorporating data from internet resources and data repositories [28, 33].

Conclusions

Molecular diagnosis had a major role in differentiating between strains and species, as 6 different strains of *C. albicans* were diagnosed through the presence of several distinct mutations between each strain. New strains of Candida species were registered for the first time in Iraq, which were confirmed by molecular diagnosis, as C. ciferri was recorded for the first time in Iraq. All isolates were registered in NCBI under identifiable accession numbers: OP293043, OP293044, OP293045, OP293046, OP293047, OP293048, OP293049, OP293050, OP293051, OP293052, OP293053, OP29. 3054 and OP325504.

References

1. P. Andreo-Martinez, N. Garcia-Martinez, J. Quesada-Medina, E. P. Sanchez-Samper, and A. E. Martinez-Gonzalez, "Candida spp. in the gut microbiota of people with autism: a systematic review," *Revista de Neurologia,* vol. 68, no. 1, pp. 1-6, 2019.
2. M. Monod, O. Bontems, C. Zaugg, B. Léchenne, M. Fratti, and R. Panizzon, "Fast and reliable PCR/sequencing/RFLP assay for identification of fungi in onychomycoses," *Journal of medical microbiology,* vol. 55, no. 9, pp. 1211-1216, 2006.
3. V. Mohandas and M. Ballal, "Distribution of Candida species in different clinical samples and their virulence: biofilm formation, proteinase and phospholipase production: a study on hospitalized patients in southern India," *Journal of global infectious diseases,* vol. 3, no. 1, p. 4, 2011.
4. F. Mohammadi, M. R. Javaheri, S. Nekoeian, and P. Dehghan, "Identification of Candida species in the oral cavity of diabetic patients," *Current medical mycology,* vol. 2, no. 2, p. 1, 2016.
5. C. J. Mena *et al.*, "Microscopic and PCR-based detection of microsporidia spores in human stool samples," *Revista Argentina de Microbiología,* vol. 53, no. 2, pp. 124-128, 2021.
6. A. M. AL-Yasiry, "Dental health of osteopenia diabetes mellitus male patients," *Med J Babylon,* vol. 15, no. 2, p. 118, 2018.
7. D. W. Denning, M. Kneale, J. D. Sobel, and R. Rautemaa-Richardson, "Global burden of recurrent vulvovaginal candidiasis: a systematic review," *The Lancet infectious diseases,* vol. 18, no. 11, pp. e339-e347, 2018.
8. S. Antinori, L. Milazzo, S. Sollima, M. Galli, and M. Corbellino, "Candidemia and invasive candidiasis in adults: A narrative review," *European journal of internal medicine,* vol. 34, pp. 21-28,

2016.
9. S. C. Deorukhkar, S. Saini, and S. Mathew, "Non-albicans Candida infection: an emerging threat," *Interdisciplinary perspectives on infectious diseases,* vol. 2014, 2014.
10. K. F. D. Dota, M. E. L. Consolaro, T. I. E. Svidzinski, and M. L. Bruschi, "Antifungal activity of Brazilian propolis microparticles against yeasts isolated from vulvovaginal candidiasis," *Evidence-Based Complementary and Alternative Medicine,* vol. 2011, 2011.
11. S. G. Edwards, J. O'callaghan, and A. D. Dobson, "PCR-based detection and quantification of mycotoxigenic fungi," *Mycological Research,* vol. 106, no. 9, pp. 1005-1025, 2002.
12. M. A. ZAREI, M. Zarrin, and F. M. BEHESHTI, "Antifungal Susceptibility of Candida species isolated from candiduria," 2013.
13. J. Shendure and E. L. Aiden, "The expanding scope of DNA sequencing," *Nature biotechnology,* vol. 30, no. 11, pp. 1084-1094, 2012.
14. J. Sardi, L. Scorzoni, T. Bernardi, A. Fusco-Almeida, and M. J. S. Mendes Giannini, "Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options," *Journal of medical microbiology,* vol. 62, no. 1, pp. 10-24, 2013.
15. D. Sanglard, "Finding the needle in a haystack: mapping antifungal drug resistance in fungal pathogen by genomic approaches," *PLoS pathogens,* vol. 15, no. 1, p. e1007478, 2019.
16. C. Sachin, K. Ruchi, and S. Santosh, "In vitro evaluation of proteinase, phospholipase and haemolysin activities of Candida species isolated from clinical specimens," *International journal of Medicine and Biomedical research,* vol. 1, no. 2, pp. 153-157, 2012.
17. M. Razzaghi-Abyaneh *et al.*, "Species distribution and antifungal susceptibility of Candida spp. isolated from superficial candidiasis in outpatients in Iran," *Journal de mycologie médicale,* vol. 24, no. 2, pp. e43-e50, 2014.
18. C. L. S. Meira *et al.*, "Sialolithiasis of the submandibular gland associated with stafne bone defect: case report," *Jornal Brasileiro de Patologia e Medicina Laboratorial,* vol. 58, p. e4292022, 2022.
19. P. G. Pappas *et al.*, "Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America," *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America,* vol. 48, no. 5, p. 503, 2009.
20. A. Muñoz-Villena, R. De la Vega Marcos, G. Cremades, and J. González-Hernández, "SPANISH ADAPTATION OF THE CTAI-2D. TOOL FOR ASSESSING THE TRAIT ANXIETY IN ATHLETES," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte,* vol. 22, no. 85, 2022.
21. N. Al-Sharrad, M. A. Al-Kataan, and M. A. Al-Rejaboo, "Isolation and Identification of Fungal Isolates caused Otomycosis from some Hospitals and Clinics in Mosul with Determination of Their Virulence Factors," *Al-Qadisiyah Journal of Pure Science,* vol. 26, no. 4, pp. 210–220-210–220, 2021.
22. Y.-C. Chen *et al.*, "Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts," *Journal of Clinical Microbiology,* vol. 39, no. 11, pp. 4042-4051, 2001.
23. D. N. Fredricks, C. Smith, and A. Meier, "Comparison of six DNA extraction methods for recovery of fungal DNA as assessed by quantitative PCR," *Journal of clinical microbiology,* vol. 43, no. 10, pp. 5122-5128, 2005.
24. B. Emerenini, R. Williams, R. N. G. R. Grimaldo, K. Wurscher, and R. Ijioma, "Mathematical Modeling and Analysis of Influenza In-Host Infection Dynamics," *Letters in Biomathematics,* vol. 8, no. 1, pp. 229–253, 2021, doi: 10.30707/LiB8.1.1647878866.124006.
25. I. Gontia-Mishra, N. Tripathi, and S. Tiwari, "A simple and rapid DNA extraction protocol for filamentous fungi efficient for molecular studies," 2014.
26. M. Jurado, C. Vázquez, S. Marín, V. Sanchis, and M. T. González-Jaén, "PCR-based strategy to detect contamination with mycotoxigenic Fusarium species in maize," *Systematic and applied microbiology,* vol. 29, no. 8, pp. 681-689, 2006.
27. S. Kumar, M. Nei, J. Dudley, and K. Tamura, "MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences," *Briefings in bioinformatics,* vol. 9, no. 4, pp. 299-306, 2008.
28. R. Landeweert *et al.*, "Molecular identification of ectomycorrhizal mycelium in soil horizons," *Applied and Environmental Microbiology,* vol. 69, no. 1, pp. 327-333, 2003.
29. S. Adib, N. A. BASHA, A. TUFAHHA, I. BARAKAT, and C. CAPAPÉ, "First substantiated record of leopard whipray, Himantura leoparda (Myliobatoidei: Dasyatidae) from the Syrian coast (Eastern Mediterranean Sea)," *FishTaxa,* vol. 19, pp. 5-8, 2021.
30. V. P. d. Oliveira, R. d. M. Esmeraldo, C. M. C. d. Oliveira, F. B. Duarte, A. C. Teixeira, and T. V. d. Sandes-Freitas, "Post Transplant Lymphoproliferative Disease Isolated to Kidney Allograft," *Jornal Brasileiro de Patologia e Medicina Laboratorial,* vol. 58, 2022, doi: 10.1900/JBPML.2022.58.446.
31. J. Fernández-Ortega, F. Garavito-Peña, D. Mendoza-Romero, and D. Oliveros, "INDICATORS OF STRENGTH IN YOUNG WOMEN WITH DIFFERENT RELATIVE STRENGTH RATIO," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte,* vol. 22, no. 85, 2022.
32. E. T. J. Mason *et al.*, "Decoding comparable morphologies: Pigmentation validated for identifying southern California Paralabrax larvae," *FishTaxa,* 2022.
33. D. Liu, S. Coloe, R. Baird, and J. Pedersen, "Application of PCR to the identification of dermatophyte fungi," *Journal of medical microbiology,* vol. 49, no. 6, pp. 493-497, 2000.