Original Article

New Record of Different Yeast Isolates from Patients with Ear Infections Using DNA Sequencing in Al-Najaf Governorate, Iraq

Mohammad Mohsien Alrufae[1], Zuhair Hameed Abboud[2], Ban Mousa Hassan[3]

[1]College of Pharmacy, University of Alkafeel, Najaf, Iraq [2]Department of Medical Laboratory Techniques, Al-Zahrawi University College, Karbala, Iraq, [3]College of Education for Pure Sciences, University of Kerbala, Karbala, Iraq

Abstract

Background: *Candida albicans* may cause other chronic fungal infections in the human ear. Otomycosis is a common disease found in the general clinical setting of otolaryngology. **Objectives:** The present study aims to establish a database of fungal species causing ear canal, infections, characterize their genetics, and compare them with standard isolates documented in the National Center for Biotechnology Information global sites. **Materials and Methods:** A total of 120 samples were collected from patients with ear infections at Al-Sadder Medical City in Al-Najaf Province, and incubated at 37°C for 1–2 days for visible. A specific fungal genomic deoxyribonucleic acid (DNA) kit was used for the purification of young *Candida* colonies' DNA. Amplification of genes was carried out according to the experimental protocol, and the polymerase chain reaction products of *Candida* spp were sent to Macrogen Lab, USA, for sequencing. **Results:** The present study included a collection of 120 ear swab samples from patients with different ear infections, 47 (39.2%) samples of otitis externa, 71 (59.2%) samples of otitis media, and 2 (1.6%) from otitis enterna. *C. albicans* and *Candida tropicals* recorded the highest frequency. All 33 isolates were characterized as genotypes using two pairs of primers (ITS5-ITS4 and ITS3-ITS4), only 13/33 (39.5%) were classified as *C. albicans*, 8/33 (24.25%) were classified as *C. tropicalis*. DNA sequencing for five isolates was received online and aligned to the NCBI database using Blast software. Strain no. 3 was found to be the nearest neighbor to *Meyerozyma guilliermondii* strain MK267616.1 with an identity of 93.42%. **Conclusion:** The DNA sequencing method is considered one of the best methods for the diagnosis of mold and yeasts.

Keywords: *Candida*, DNA sequencing, ear infection, PCR

Introduction

Fungal ear infection is an outer and middle ear infection where one or both ears can become inflamed, with dry, itchy skin, and possibly a smelly discharge. It can become chronic but often is easily treated with antifungal medication.[1]

Many types of fungus may cause this infection, but most infections with otomycosis are connected to *Aspergillus* or, less commonly, *Candida*. Candidiasis occurs in some people and it spreads across the bloodstream to various parts of the human body such as the spleen, heart valves, eyes, and kidneys.[2] The *Candida* spp. are normal flora found on the skin, mucous membrane of the oral cavity, upper respiratory tracts, gastrointestinal tracts, and genital tracts of women. There are about 68 species of *Candida*, the most pathogenic is *Candida albicans*.[3-5]

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1352_23

The impaired immune system or lack of it is due to the absorbed metabolic products called mycotoxins formed by *Candida* and released chronically into the bloodstream.[6]

Candida may cause other chronic fungal infections in the human ear called otomycosis is a common disease found in the general clinical setting of otolaryngology and its prevalence with signs and symptoms of otitis externa. It is a pathologic entity, with *Candida* and *Aspergillus* the most common fungal species. However, it is not clear whether the fungi are the true infective agents or mere colonization

Address for correspondence: Dr. Zuhair Hameed Abboud, Department. of Medical Laboratory Techniques, Al-Zahrawi University College, Karbala, Karbala Governorate 56001, Iraq.
E-mail: Zuhair.alharby@uokerbala.edu.iq

Submission: 05-Sep-2023 **Accepted:** 26-Apr-2024 **Published:** 23-Dec-2024

How to cite this article: Alrufae MM, Abboud ZH, Hassan BM. New record of different yeast isolates from patients with ear infections using DNA sequencing in Al-Najaf Governorate, Iraq. Med J Babylon 2024;21:951-60.

species as a result of compromised local host immunity secondary to bacterial infection.[7]

Different predisposing factors include a humid climate, presence of cerumen, instrumentation of the ear, increased use of topical antibiotics/steroid preparations, immunocompromised host, patients who have undergone open cavity mastoidectomy, and those who wear hearing aids with occlusive ear mold.[8,9]

Colonization by *C. albicans* can be found in almost 30%–55% of young adults. *C. albicans* infections remain the top source of fungal infections in immunocompromised people such as in human immunodeficiency virus-compromised patients, where over 90% will develop a case of oropharyngeal candidiasis.[10,11]

Candidiasis is found in a wide variety of diseases ranging from more superficial and milder clinical manifestations, such as esophageal or oropharyngeal candidiasis to serious infections like disseminated candidiasis, whereas the classification of invasive candidiasis includes severe diseases such as candidemia, endocarditis, disseminated infections, infections of the central nervous system, endophthalmitis, and otomycosis.[12]

Epidemiologically, invasive candidiasis in neonates is serious and a common cause of the high mortality rate. *Candida* is the third most frequently seen organism isolated in very low birth weight infants. It may be transmitted vertically from maternal vaginal infection or nosocomially from a hospital-acquired infection.[13] Based on available information in Iraq, there is little data available associated with fungal species isolated from ear infections for this reason, the present study is designed to fill these gaps.

The present study aimed to find a database of fungal species causing infection of the ear canal to provide their genetic characterization and compare them with standard isolates documented in global sites National Center for Biotechnology Information (NCBI).

Materials and Methods

Collection of samples

A total of 120 samples were collected from patients with ear infections, which were obtained from Al-Sadder Medical City in Al-Najaf Province Eye, Nose Throat Consultant, from August 2019 to February 2020. All the above samples were transferred to the laboratory of advanced mycology in the Faculty of Science, University of Kufa, Kufa, Iraq, for diagnosis and study. The samples (including ear swab samples) were taken with sterile cotton swabs. The cotton swabs were aseptically dipped in sterile culture tubes containing sterile Brain Heart Infusion agar with chloramphenicol and were transported as soon as possible to the laboratory for incubation at 37°C for 24 h. The next day, they were subcultured on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA), and CHROMagar *Candida* was performed and incubated at 37°C for 1–2 days for visible growth of *Candida* spp. colonies.[14]

The shape, size, color, edge, and appearance of yeast isolates were studied on PDA and SDA media after 24–48 h, whereas the CHROMagar test was used to help diagnose the *Candida* species based on color.[15]

Preparing the primers

The primers used in this study were received as a solution. The stock solution with TE buffer to get a final working solution of 10 pmol/μL.

Culture and harvest the isolation

To determine the genotypes of the yeast isolates recovered from 120 investigated specimens, 33/120 (27.5%) of the yeast isolates were considered positive, which were used in the genotypic diagnosis. The 16S rDNA amplified deoxyribonucleic acid (DNA) products of ITS region from 33 yeast isolates in the present study, and the cultures were incubated at 30°C for 48 h for early polymerase chain reaction (PCR) analysis.

DNA extraction and purification

A specific EZ-10 spin column fungal genomic DNA mini-preps kit [i-genomic BYF (iNtRON Biotechnology, Korea)], was used for the purification of young yeast colonies' DNA.

Amplification reaction mixture

Amplification of genes was carried out according to the experimental protocol. The PCR reaction mixture was prepared using Premix Bioneer Corporation, Alameda, CA, USA as mentioned in Tables 1 and 2. The tubes were

Table 1: Primers used for *Candida* species detection

Primer	DNA sequences (5′–3′)	Target region
ITS3 F	5′-GCA TCG ATG AAG AAC GCA GC-3′	ITS2
ITS4 R	5′-TCC TCC GCT TAT TGA TAT GC-3′	
ITS5 F	5′-GGA AGT AAA AGT CGT AAC AAG G-3′	ITS1
ITS4 R	5′-TCC TCC GCT TAT TGA TAT GC-3′	

Table 2: The mixture of PCR

No.	Mixture solution	Volume (μL)
1	Master mixture	5 μL
2	DNA template	5 μL
3	Forward primers	2.5 μL
4	Reverse primers	2.5 μL
5	Deionized water (dd water)	5 μL
	Final volume 20 μL	

Table 3: PCR program that applies in the thermocycler

Gene name	Temperature (ºC)/time					Cycles number
	Initial denaturation	Cycling conditions			Final extension	
		Denaturation	Annealing	Extension		
ITS2	95/5 min	94/30 s	56/1 min	72/1 min	72/5 min	30
ITS1	95/5 min	94/30 s	56/1 min	72/1 min	72/5 min	30

mixed with a vortex to dissolve the lyophilized blue pellet and briefly centrifuged.

Primers selection

All primers in this study were primers synthesized by i-genomic BYF, Korea. Optimization of PCR was performed after several tries by using PCR mixture, thus the following mixture according to i-genomic BYF [Table 2].

PCR program

General PCR conditions are listed in Table 3]. Using the amplification of the target gene, the PCR products, and the ladder marker are analyzed by electrophoresis on 1.2% agarose gel. The resolved band indicates the corresponding internal transcribed spacer (ITS)2 and ITS1 genes. The molecular weight identification of the resolved band is based on their correspondence to the ladder bands.

DNA sequencing

The PCR products of Candida species were sent to Macrogen Lab, USA for sequencing, and the data of the sequences were received for every *Candida* spp. The sequencing results were subjected to multiple alignment setup on the BioEdit software (Informer Technologies, Inc., Los Angeles, USA). Finally, the phylogeny tree is commands and the multigene are performed via Mega 6 software (Informer Technologies, Inc., Los Angeles, USA).

Results

Collection of samples

The present study included a collection of 120 ear swab samples from randomly recruited patients with different ear infections, which involved 47 (39.2%) samples of otitis externa, 71 (59.2%) samples of otitis media, and 2 (1.6%) from otitis interna. Out of 120 only 33 (27.5%) were considered positive for growth [Tables 4 and 5].

On the other hand, *C. albicans* and *Candida tropicals* recorded the highest frequency at 19 (57.58%) and 11 (33.33%), respectively. The lowest percentage comprised all *Candida glabrate*, *Candida krusei*, and *Candida kefyr* with 1 (3.03%) [Table 6].

Table 4: Numbers and percentages of samples collected from patients with different ear infection cases in Al-Najaf Governorate

Samples	Samples no.	Sample (%)
Otitis externa	47	39.2
Otitis media	71	59.2
Otitis interna	2	1.6
Total	120	100

Table 5: Numbers and percentages of positive growth samples collected from patients with different ear infection cases in Al-Najaf Governorate

Samples	Samples no.	Sample (%)
Otitis externa	8	24.24
Otitis media	24	72.73
Otitis interna	1	3.03
Total	33	100

Table 6: Numbers and percentages of *Candida* species collected from patients with different ear infection cases in Al-Najaf Governorate

Candida species	Samples no.	Sample (%)
C. albicans	19	57.58%
C. tropicals	11	33.33%
C. krusei	1	3.03%
C. glabrate	1	3.03%
C. kefyr	1	3.03%
Total	33	100%

Molecular identification

The genomic DNAs were used as templates for PCR using amplified DNA products to determine the genotypes of 33 yeast species extracts. The 33 isolates (which are included in the current study) were characterized as genotypes using two pairs of primers (ITS3–ITS4 and ITS5–ITS4) of these isolates.

Genotype and diagnosis of *Candida* isolates

All 33 isolates were characterized as genotypes using two pairs of primers (ITS5–ITS4 and ITS3–ITS4).

Table 7: Molecular weight of bands produced by amplification of ITS1 and ITS2 regions from *Candida* species

Isolates no.	PCR identification			
	(ITS5/ITS4) ITS2 region	Molecular weight	(ITS3/ITS4) ITS1 region	Molecular weight
1	*Candida tropicalis*	Non	*C. tropicalis*	310
2	*C. tropicalis*	310	*C. tropicalis*	310
3	*Pichia guilliermondi*	310	*P. guilliermondi*	310
4	*C. albicans*	310	*C. albicans*	310
5	*C. albicans*	310	*C. albicans*	540
6	*P. guilliermondi*	310	*P. guilliermondi*	310
7	Ni	300	Ni	310
8	*C. kefyr*	310	*C. kefyr*	410
9	*P. guilliermondi*	310	*P. guilliermondi*	310
10	*C. parapsilosis* Prapcilosis	310	*C. parapsilosis*	320
11	*C. albicans*	310	*C. albicans*	Non
12	*C. Krusei*	300	*C. Krusei*	Non
13	*C. albicans*	Non	*C. albicans*	310
14	*C. albicans*	370	*C. albicans*	310
15	*C. albicans*	370	*C. albicans*	310
16	Ni	Non	NI	310
17	*C. albicans*	370	*C. albicans*	310
18	*C. albicans*	505	*C. albicans*	Non
19	*C. glabrata*	505	*C. glabrata*	420
20	*C. tropicalis*	515	*C. tropicalis*	310
21	*C. tropicalis*	505	*C. tropicalis*	310
22	*C. tropicalis*	505	*C. tropicalis*	310
23	*C. tropicalis*	515	*C. tropicalis*	310
24	*C. tropicalis*	505	*C. tropicalis*	310
25	*NI*	940	*NI*	320
26	*C. tropicalis*	515	*C. tropicalis*	370
27	*C. albicans*	505	*C. albicans*	310
28	*C. albicans*	505	*C. albicans*	310
29	*P. guilliermondi*	505	*P. guilliermondi*	370
30	*C. albicans*	505	*C. albicans*	370
31	*C. albicans*	505	*C. albicans*	370
32	*C. albicans*	505	*C. albicans*	Non
33	NI	Non	NI	310

Non: band not detected, NI: non-identify

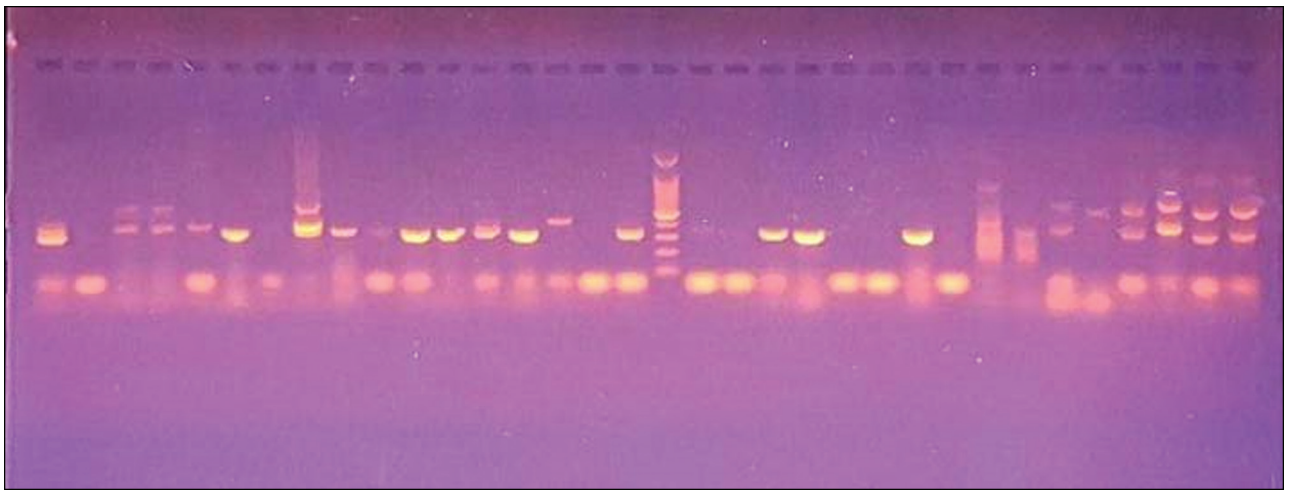


Figure 1: Agarose gel electrophoresis of ITS1 regions PCR product by pair primers (ITS5–ITS4) of different yeast species (1.2 g agarose gel, 80 V for 1 h)

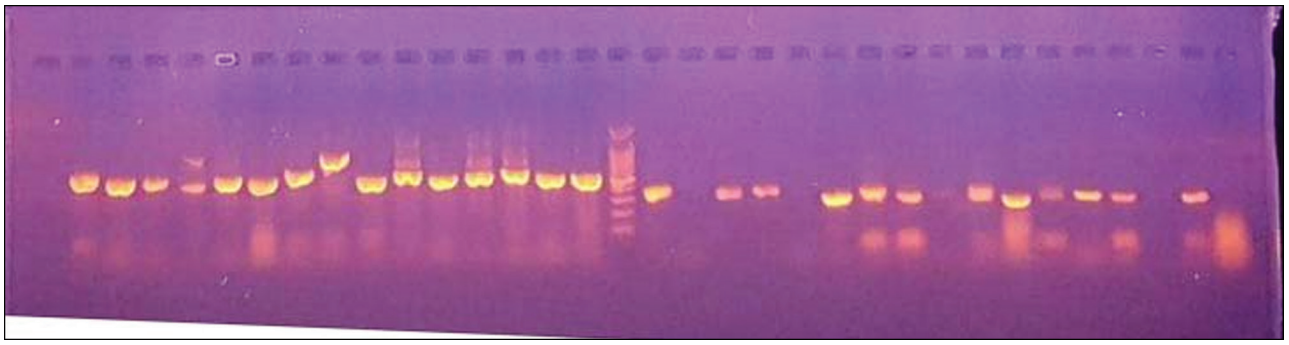
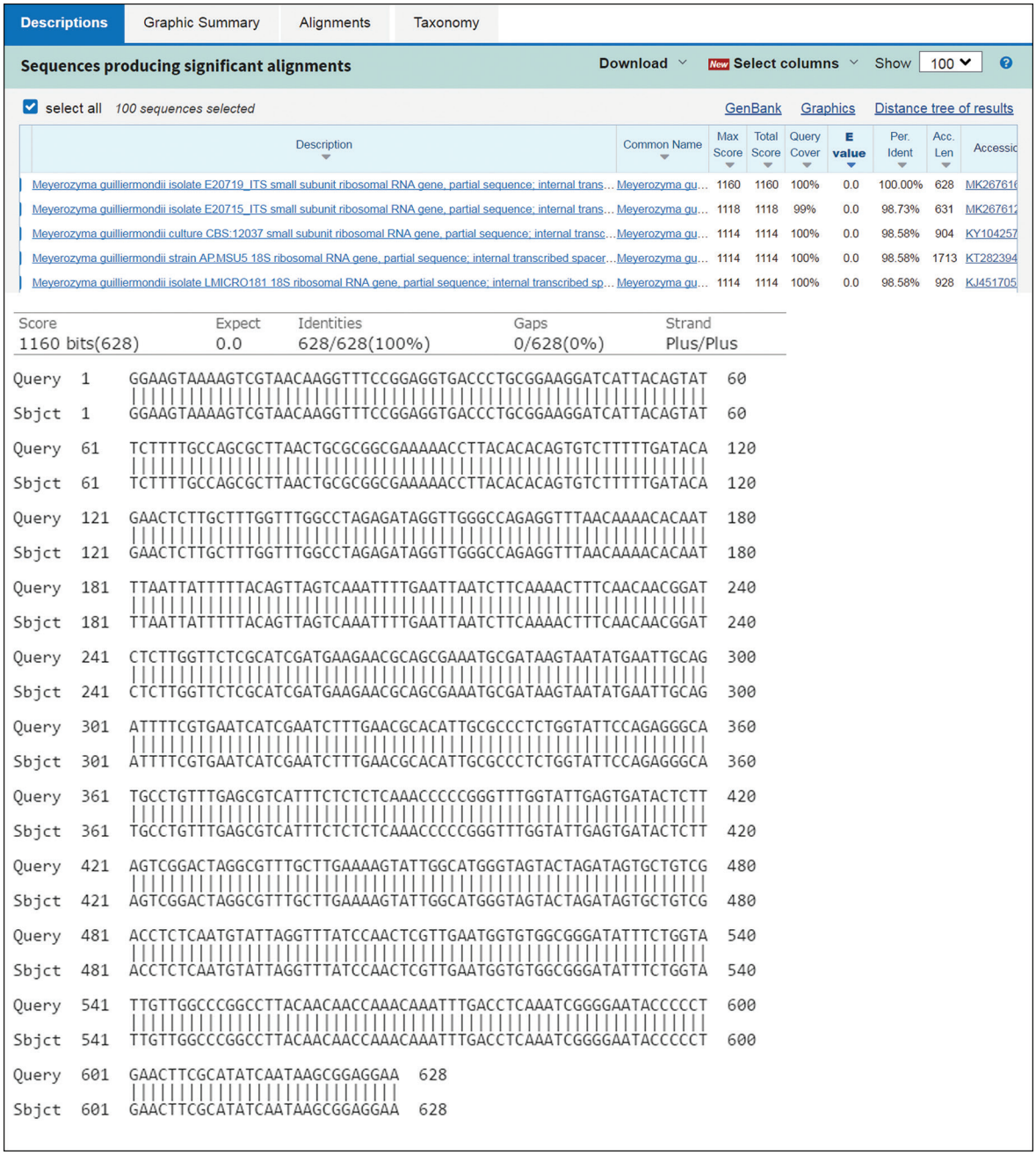


Figure 2: Agarose gel electrophoresis of ITS2 regions PCR product by pair primers (ITS3–ITS4) of different yeast species. (1.2g agarose gel, 80 V for 1 h)



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — New Select columns — Show 100

select all *100 sequences selected* — GenBank — Graphics — Distance tree of results

Description	Common Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accessio
Meyerozyma guilliermondii isolate E20719_ITS small subunit ribosomal RNA gene, partial sequence; internal trans…	Meyerozyma gu…	1160	1160	100%	0.0	100.00%	628	MK267616
Meyerozyma guilliermondii isolate E20715_ITS small subunit ribosomal RNA gene, partial sequence; internal trans…	Meyerozyma gu…	1118	1118	99%	0.0	98.73%	631	MK267612
Meyerozyma guilliermondii culture CBS:12037 small subunit ribosomal RNA gene, partial sequence; internal transc…	Meyerozyma gu…	1114	1114	100%	0.0	98.58%	904	KY104257
Meyerozyma guilliermondii strain AP.MSU5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer…	Meyerozyma gu…	1114	1114	100%	0.0	98.58%	1713	KT282394
Meyerozyma guilliermondii isolate LMICRO181 18S ribosomal RNA gene, partial sequence; internal transcribed sp…	Meyerozyma gu…	1114	1114	100%	0.0	98.58%	928	KJ451705

Score	Expect	Identities	Gaps	Strand
1160 bits(628)	0.0	628/628(100%)	0/628(0%)	Plus/Plus

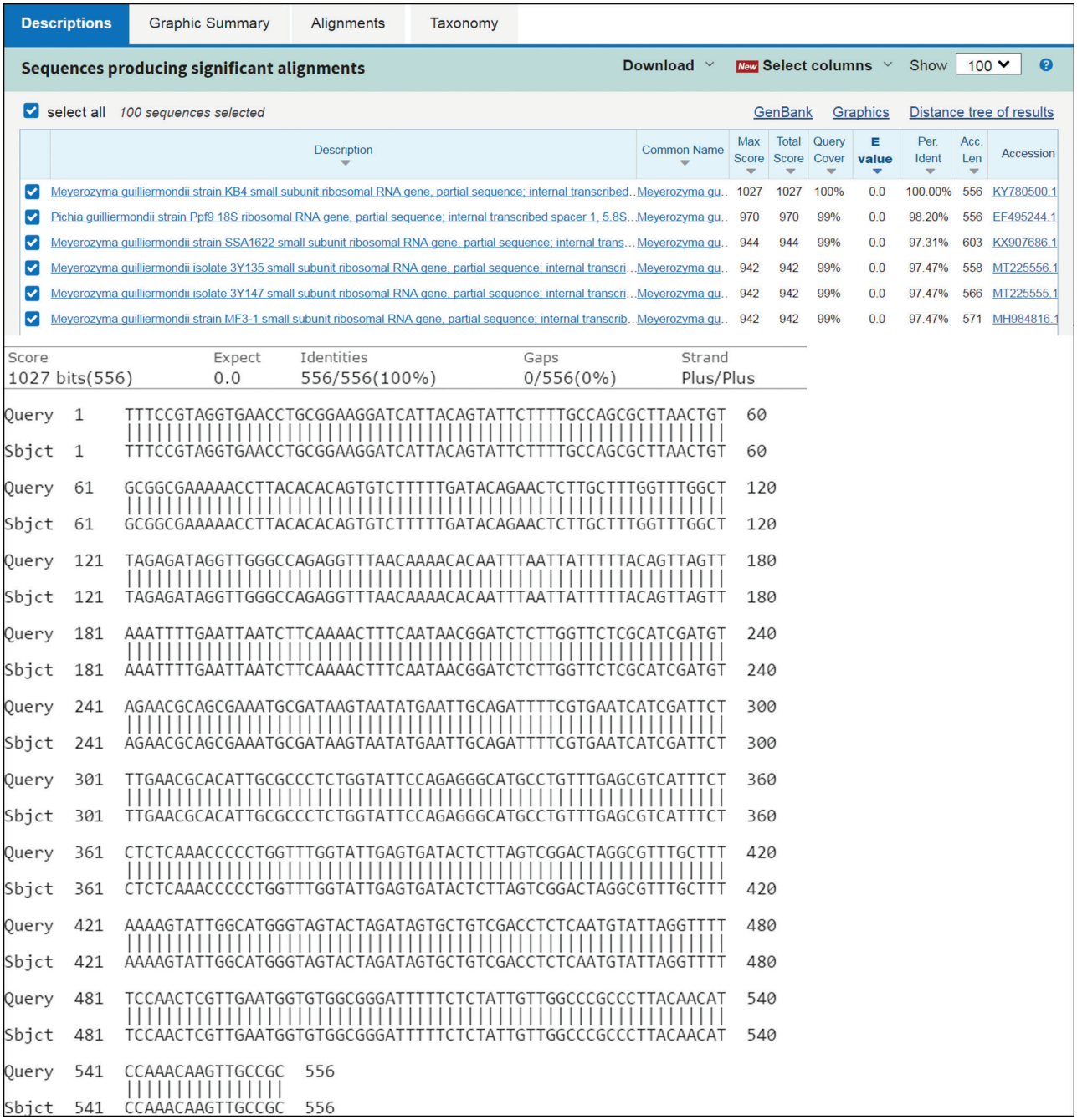
```
Query  1    GGAAGTAAAAGTCGTAACAAGGTTTCCGGAGGTGACCCTGCGGAAGGATCATTACAGTAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GGAAGTAAAAGTCGTAACAAGGTTTCCGGAGGTGACCCTGCGGAAGGATCATTACAGTAT  60

Query  61   TCTTTTGCCAGCGCTTAACTGCGCGGCGAAAAACCTTACACACAGTGTCTTTTTGATACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TCTTTTGCCAGCGCTTAACTGCGCGGCGAAAAACCTTACACACAGTGTCTTTTTGATACA  120

Query  121  GAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAAT  180

Query  181  TTAATTATTTTTACAGTTAGTCAAATTTTGAATTAATCTTCAAAACTTTCAACAACGGAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TTAATTATTTTTACAGTTAGTCAAATTTTGAATTAATCTTCAAAACTTTCAACAACGGAT  240

Query  241  CTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAG  300

Query  301  ATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  ATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCA  360

Query  361  TGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGGGTTTGGTATTGAGTGATACTCTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGGGTTTGGTATTGAGTGATACTCTT  420

Query  421  AGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTAGATAGTGCTGTCG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTAGATAGTGCTGTCG  480

Query  481  ACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGATATTTCTGGTA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  ACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGATATTTCTGGTA  540

Query  541  TTGTTGGCCCGGCCTTACAACAACCAAACAAATTTGACCTCAAATCGGGGAATACCCCCT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  TTGTTGGCCCGGCCTTACAACAACCAAACAAATTTGACCTCAAATCGGGGAATACCCCCT  600

Query  601  GAACTTCGCATATCAATAAGCGGAGGAA  628
            ||||||||||||||||||||||||||||
Sbjct  601  GAACTTCGCATATCAATAAGCGGAGGAA  628
```

Figure 3: Sequences producing significant alignments with strain no. 3 *M. guilliermondii* ITS1-5.8S rRNA gene-ITS2 gene sequences

Of these, only 13/33 (39.5%) isolates were classified as the genotype of, *C. albicans* and 8/33 (24.25%) isolates were classified as the genotype *C. tropicalis*, 4/33 (12%) isolates were classified as the genotypes *Pichia guilliermondi*, 1/33(3%) isolate was classified as genotypes *C.kefyr, C. parapsilosis, C. krusei*, and *C. glabrata*. Finally 4/33 (12%) isolate was classified as the genotype of unknown species of yeast (NI) [Table 7] and [Figures 1 and 2].

Sequences analysis

Sequencing for five isolates (3, 6, 9, 29, and 33) of strains was received online and aligned to the NCBI database using Blast software and multiple aligned to each other using BioEdit software and submitted in FASTA format to NCBI through Sequin software (NCBI, Bethesda, MD, USA). The sequence analysis was conducted to assess the ITS intraspecific variability of the yeast species. The universal primers for fungi are



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — New Select columns — Show 100

select all *100 sequences selected* — GenBank — Graphics — Distance tree of results

Description	Common Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Meyerozyma guilliermondii strain KB4 small subunit ribosomal RNA gene, partial sequence; internal transcribed..	Meyerozyma gu..	1027	1027	100%	0.0	100.00%	556	KY780500.1
Pichia guilliermondii strain Ppf9 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S..	Meyerozyma gu..	970	970	99%	0.0	98.20%	556	EF495244.1
Meyerozyma guilliermondii strain SSA1622 small subunit ribosomal RNA gene, partial sequence; internal trans...	Meyerozyma gu..	944	944	99%	0.0	97.31%	603	KX907686.1
Meyerozyma guilliermondii isolate 3Y135 small subunit ribosomal RNA gene, partial sequence; internal transcri..	Meyerozyma gu..	942	942	99%	0.0	97.47%	558	MT225556.1
Meyerozyma guilliermondii isolate 3Y147 small subunit ribosomal RNA gene, partial sequence; internal transcri..	Meyerozyma gu..	942	942	99%	0.0	97.47%	566	MT225555.1
Meyerozyma guilliermondii strain MF3-1 small subunit ribosomal RNA gene, partial sequence; internal transcrib..	Meyerozyma gu..	942	942	99%	0.0	97.47%	571	MH984816.1

Score	Expect	Identities	Gaps	Strand
1027 bits(556)	0.0	556/556(100%)	0/556(0%)	Plus/Plus

```
Query  1    TTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTTTGCCAGCGCTTAACTGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTTTGCCAGCGCTTAACTGT  60

Query  61   GCGGCGAAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GCGGCGAAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCT  120

Query  121  TAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTT  180

Query  181  AAATTTTGAATTAATCTTCAAAACTTTCAATAACGGATCTCTTGGTTCTCGCATCGATGT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AAATTTTGAATTAATCTTCAAAACTTTCAATAACGGATCTCTTGGTTCTCGCATCGATGT  240

Query  241  AGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGATTCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGATTCT  300

Query  301  TTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  TTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCT  360

Query  361  CTCTCAAACCCCCTGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CTCTCAAACCCCCTGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTT  420

Query  421  AAAAGTATTGGCATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  AAAAGTATTGGCATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTT  480

Query  481  TCCAACTCGTTGAATGGTGTGGCGGGATTTTTCTCTATTGTTGGCCCGCCCTTACAACAT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TCCAACTCGTTGAATGGTGTGGCGGGATTTTTCTCTATTGTTGGCCCGCCCTTACAACAT  540

Query  541  CCAAACAAGTTGCCGC  556
            ||||||||||||||||
Sbjct  541  CCAAACAAGTTGCCGC  556
```

Figure 4: Sequences producing significant alignments with strain no. 6 *M. guilliermondii* ITS1-5.8S rRNA gene-ITS2 sequences

ITS4–ITS5 and ITS3–1TS4 amplified the ITS region for five samples, and in every case, satisfactory sequencing results were obtained. Identification of species, obtained by ITS analysis of two *Meyerozyma* species and one *Pichia* species.

Amplification of ITS2 region by pair primers (ITS3–ITS4)

Pairwise alignment and distance-based phylogeny have been investigated for (strain numbers 3 and 6) ITS1-5.8S rRNAgene-ITS2 gene sequences. Online NCBI BLAST software was used to compare each of the five resulting sequences to the NCBI database. Strain no. 3 was found to be the nearest neighbor to *Meyerozyma guilliermondii* strain MK267616.1|with an identity of 93.42%. These results were completely identical to our PCR identification and completely identical with our CHROMagar identification [Figure 3]. The strain no. 6 was found to be the nearest neighbor to *M. guilliermondii* strain KY780500.1 with an identity of 95.24%. This yeast was considered the first recorded in Iraq, which was isolated from ear infection samples. We were also not able to identify this isolate when using both PCR and CHROMAgar *Candida* identification [Figure 4].

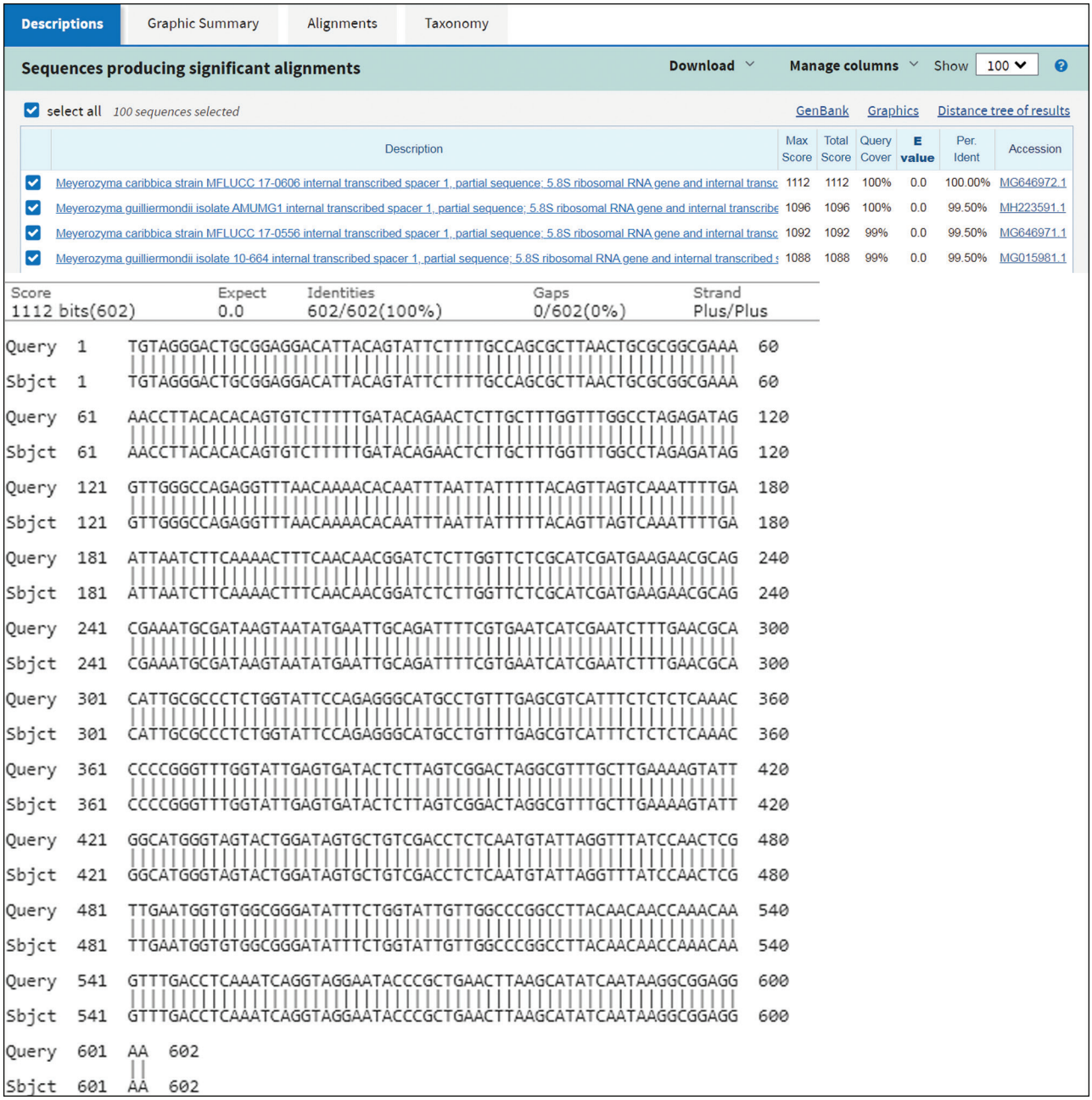


Figure 5: Pairwise alignment of ITS1-5.8S rRNA gene-ITS2 of strain no. 9 with *M. caribbica* strain MG646972.1 using NCBI online BLAST

Amplified of ITS1 region by pair primers (ITS5–ITS4)

The results with the ITS1 region revealed that strain no. 9 was found to be the nearest neighbor to *Meyerozyma caribbica* strain MG646972.1 with an identity of 95.37%. These results were completely not identical to our CHROMAgar identification and completely different from our PCR identification [Figure 5], and strain no. 29 was found to be the nearest neighbor to *Pichia guilliermondii* strain voucher EU663566.1 with an identity of 95.49%. These results were completely identical to our PCR identification and completely identical to our CHROMAgar identification [Figure 6]. The strain no. 33 was found to be nearest to *M. guilliermondii* strain JQ867378.1 with an identity of 98.98%. These results were nonidentical with PCR identification and completely nonidentical with CHROMAgar identification [Figure 7]

Discussion

The phylogenetic analysis of 30 isolates of different yeast species including recombinant DNA analysis of ITS regions were done using past three software. It has been shown to provide taxonomically useful information, to determine whether ITS sequences could accurately identify relationships among diverse yeast taxa. The topologies of the ITS trees are variable in similarity [Figure 8]. In addition, the tree of joining *Candida* spp. basis of ITS region sequences showed closed related intra-isolates groups as in clusters and also revealed differences in some yeasts species, the results showed six isolates of *P. guilliermondii* in cluster 1, and *C. glabrata* two isolates were in cluster 2, whereas *C. albicans* only five isolates were in cluster 3 including (isolates numbers 3, 5, 6, 7, and 8) and the other isolates (1, 2, and 4) were exist in different

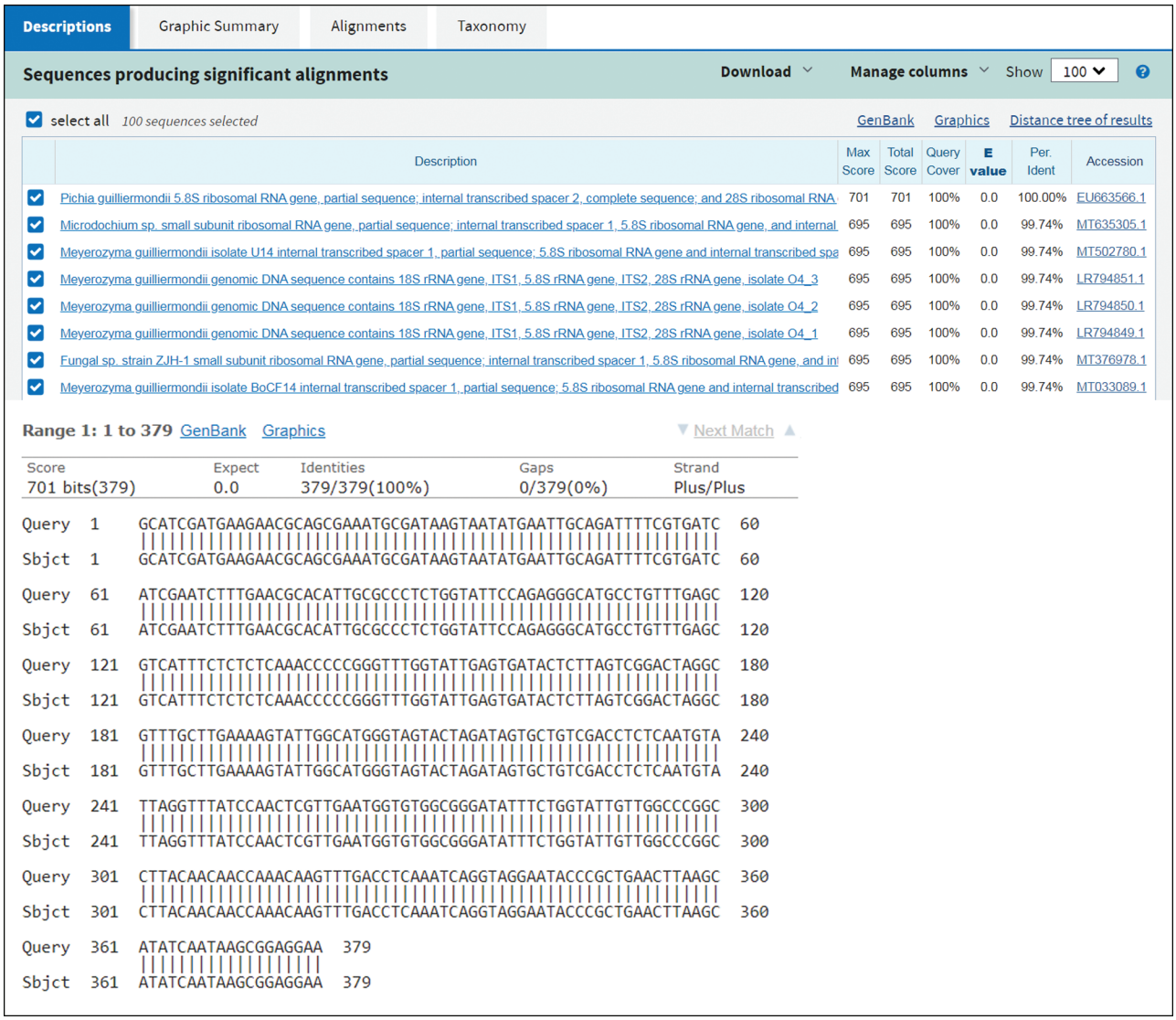


Figure 6: Pairwise alignment of strain no. 29 ITS1-5.8S rRNA gene-ITS2 with *P. guilliermondii* strain EU663566.1 using NCBI online BLAST

locations of phylogenic tree. Concerning *C. tropicalis*, all isolates were clustered in one group 4, and finally, *M. guilliermondi* existed alone.

The isolation of newly identified yeast isolates from ear infection samples using PCR and CHROMagar identification is considered as a first record in Iraq.

This thing is a dangerous indication of the presence of pathological isolates that have not been discovered or have not yet been isolated, which may affect the environmental character of the country.[16,17]

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

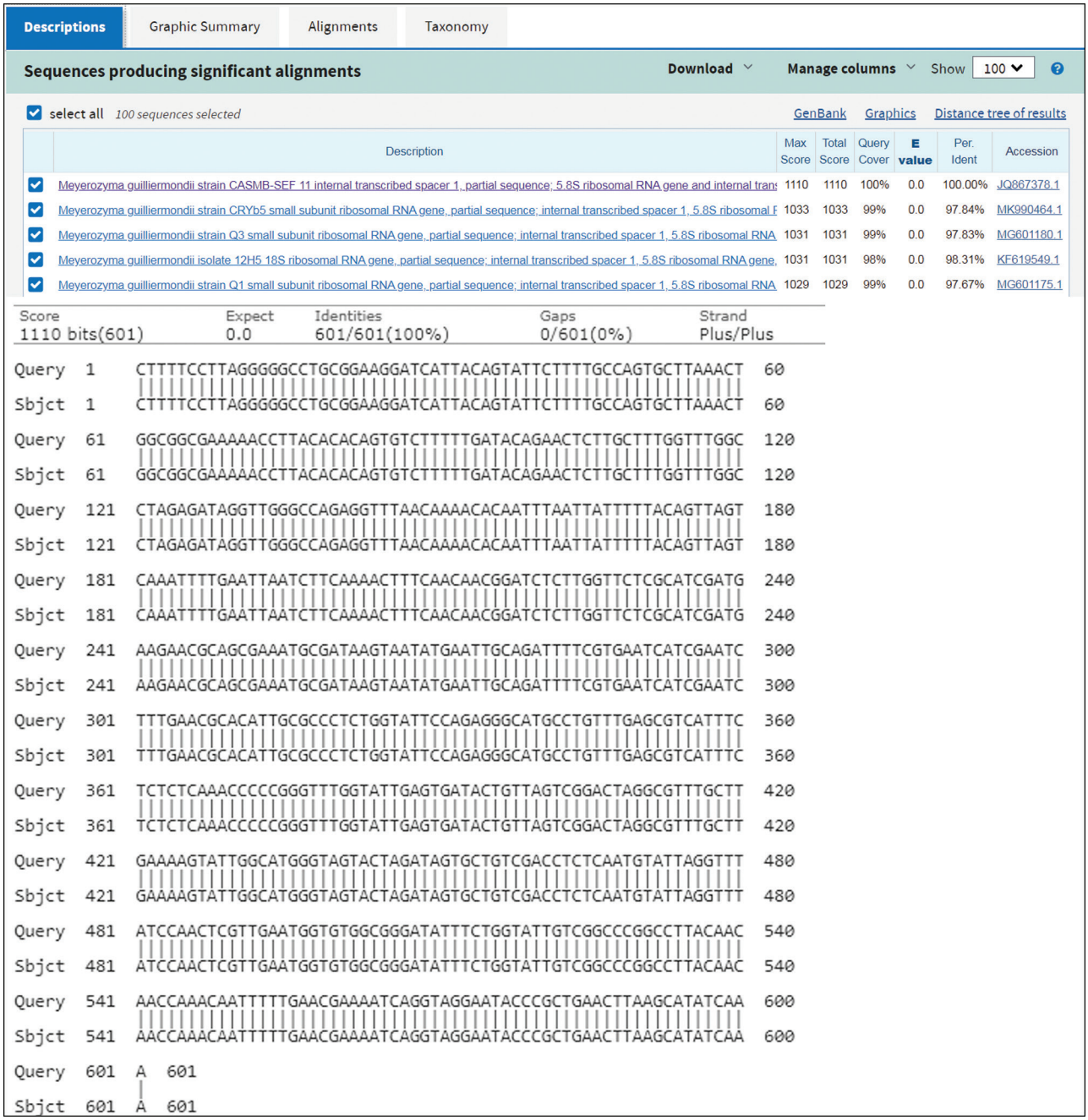


Figure 7: Pairwise alignment of strain no. 33 ITS1-5.8S rRNA gene-ITS2 with *M. guilliermondii* strain JQ867378.1 using NCBI online BLAST

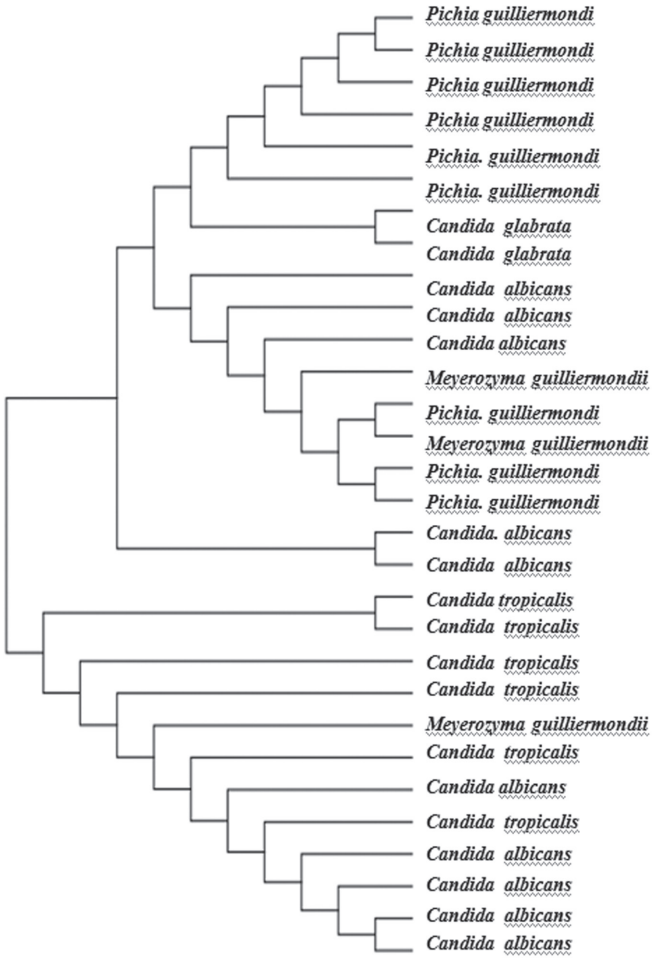


Figure 8: Phylogenic tree for 33 yeasts isolates (18 of *Candida* spp, 9 of *P. guilliermondii,* 3 *M. guilliermondii*, and 3 of nonidentified isolates NI)

References

1. Suleyman G, Alangaden GJ. Fungal infections: Epidemiology, infection control, and prevention. Infect Dis Clin North Am 2016;30:1023-52.
2. Chang A, Neofytos D, Horn D. Candidemia in the 21st century. Future Microbiol 2008;3:463-72.
3. Khatib R, Riederer KM, Ramanathan J, Baran Jr J. Faecal fungal flora in healthy volunteers and inpatients. Mycoses 2001;44:151-6.
4. Soll DR. Candida and virulence: The evolution of phenotypic plasticity. Acta Trop 2002;81:101-10.
5. Segal E, Elad D. Candidiasis. In: Topley and Wilsons Medical Mycology. 10th ed. London, UK: Edward Arnold Publishers; 2005. p. 579-623.
6. Vasquez A. Reducing pain and inflammation naturally, part 6: nutritional and botanical treatments against "Silent Infection" and gastrointestinal dysbiosis, commonly overlooked causes of neuromusculoskeletal inflammation and chronic health problems. J Council Nutr Am Chiropr Assoc 2006;29:5-21.
7. Mugliston T, O'Donoghue G, Laryngol J. Otomycosis a continuing problem. J Laryngol Otol 1985;99:327-33.
8. Meirtusovas SM. Yeasts and fungi isolated at the mycology laboratory of the First Dermatovenerology Clinic of the Medical Faculty Hospital of Comenius University in Bratislava 1995-2000. Epidemiol Mikrobiol Imunol 2003;52:76-80.
9. Simaljakova M. Systemic therapy of psoriasis: methotrexate. Bratisl Lek Listy 2000;112:390-4.
10. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, *et al*. Patient-derived xenograft models: An emerging platform for translational cancer research. Cancer Discov 2014;4:998-1013.
11. de Repentigny L, Lewandowski D, Jolicoeur P. Immunopathogenesis of oropharyngeal candidiasis in human immunodeficiency virus infection. Clin Microbiol Rev 2014;17:729-59.
12. Pappas M, Pfaller PG, Wingard JR. Invasive fungal pathogens: current epidemiological trends. Clin Infect Dis 2006;43:3-14.
13. Ali GY, Algohary EH, Rashed KA, Almoghanum M, Khalifa AA. Prevalence of Candida colonization in preterm newborns and VLBW in neonatal intensive care unit: Role of maternal colonization as a risk factor in transmission of disease. J Matern Fetal Neonatal Med 2011;25:789-95.
14. Loveday HP, Wilson JA, Pratt RJ, Golsorkhi M, Tingle A, Bak A, *et al*. National evidence based guideline for preventing health care associated infections in NHS hospitals in England. J Hosp Infect 2014;86:51-70.
15. Horvath LL, Hospenthal DR, Murray CK, Dooley DPs. Direct isolation of Candida spp. from blood cultures on the chromogenic medium CHROMagar Candida. J Clin Microbiol 2003;41:2629-32.
16. Alzaidi JR, Hussien FH, Al-Charrakh AH. The effect of vaginal bacillus (Lactobacillus acidophilus) towards Candida spp. isolated from women with Candidiasis. New Armen Med J 2021;15:77-83.
17. Al-Janabi, Uday HK. Multi-factors assessment in COVID-19 patients: Fungal infection, ABO blood group, and age factors. Med J Babylon 2023;20:871-74.