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Research Article

New record of ascomycetous yeasts strains from soil in Basrah, Iraq

Najwa Mohammed Jameel Ali Abu-Mejdad[1]*, Adnan I. Al-Badran[1], Abdullah H. Al-Saadoon[2]

ABSTRACT

Background: Yeasts are cosmopolitan in nature. They have been found in soil with widely different texture, chemical composition, humidity, and hydrogen ion concentration at numerous geographic locations, atmospheric condition, whether in uncultivated or cultivated soils. **Materials and Methods:** The dilution plate method for 75 soil samples was collected from 21 area in Basrah and Dhi Qar Provinces, Southern Iraq. Soil samples were collected from both cultivated and uncultivated lands to seek the presence of ascomycetous yeasts. **Results:** The results reported a 1st time record of six species belong to five genera; moreover, test the ability of yeasts on halotolerance and osmotolerance media the finding showed they have variation in tolerance of salt and sugar beside of reported he ability of them in growth at 15°C, 25°C, and 37°C, the results showed different in ability of growth at three temperatures. For in addition for more accurate discrimination between morphological alike species, a molecular study represented by polymerase chain reaction and DNA sequencing of internal transcribed spacer (ITS1-ITS2) region rDNA gene was conducted confirm our findings. **Conclusions:** This first study in Iraq for molecular identification of ascomycetous yeast revealed about biodiversity of yeast in soil.

KEY WORDS: Ascomycetous, Basrah, DNA sequencing, Iraq, Molecular identification, Soil

INTRODUCTION

Yeasts are cosmopolitan in nature. They have been found in soil with widely different texture, chemical composition, humidity, and hydrogen ion concentration at numerous geographic locations, atmospheric condition, whether in uncultivated or cultivated soils. In most cases especially on agricultural land, soils should be considered as a reservoir for yeasts from sources compared to other habitats, i.e., air, fresh water, and plants.[1] Yeasts are a group of fungi in which unicellular type is predominant. Most of the yeasts are represented in subdivision ascomycota of the kingdom eumycota. As a group of microorganisms, yeasts have wide distribution, in particular soil has taken into account as one of the most important reservoirs of yeasts.[2] Ascomycetous yeasts encompass some of the most economically important organisms. As well as, their key roles in the production of alcoholic drinks and biofuels. Oleaginous yeasts such as *Yarrowia lipolytica* are promising agents for biofuel production.[3] A number of soil-born yeast genera have been intensely studied, such as *Cutaneotrichosporon* and *Y. lipolytica*.[4] *Y. lipolytica* is widespread in nature. It commonly isolates from oil-polluted soil and seawater, due to the ability of yeast to utilize hydrophobic substrates such as hydrocarbons, fatty acid, and lipids which are substantial role in technological methods (mycoremediation) for the mass production of biofuel.[5] Furthermore, a number of yeasts have found to be serious infectious agent to human, such as *Candida* spp. and *Trichosporon* spp. these two genera might find in soil but clinically relevant yeasts are uncommon or less frequency, and they may be introduced to soil with animal feces and dump.[6] Genera belonging to the ascomycetes such as *Pichia*, *Wickerhamomyces*, *Debaryomyces*, *Candida*, *Metschnikowia,* and *Aureobasidium* yeasts are unknown in soil but precious. A number of these genera have been frequently found in soil associated with other genera but not constant solely. Cantrell *et al*.[7] Miceli *et al*.,[8] have reported the successful use of yeasts as plant growth promoters and biological control agents. Fu *et al*.[9] have reported that potential antagonists of soil-borne plant pathogen yeasts, but only a few of tested species are true soil yeasts that are thought to have key role in

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biocontrol, i.e., *Galactomyces candidum* isolated from rhizosphere of *Drosera spatulata*, which was exhibited significant antagonistic results against *Glomerella cingulata* in culture. Gross *et al*.[10] have pointed that special six yeast species (*Aureobasidium pullulans*, *Candida subhashii*, *Cyberlindnera sargentensis*, *Hanseniaspora* sp., *Metschnikowia pulcherrima,* and *Pichia kluyveri*) have been found in soil showing antagonistic role against fungi that cause phyllosphere diseases. Therefore, these yeasts were a promising candidate for biocontrol application against phyllosphere fungi.

The aim of this research was to isolate and identify some ascomycetous yeasts from different locations in Basrah/Iraq using morphological, physiological, biochemical, and molecular methods for accurate classification.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from both cultivated and uncultivated lands, 3 test tubes (15 ml) were taken and 9 ml sterile distilled water was added with each tube. The tubes were plugged properly and labeled. Then, 1 g of soil sample was weighed and transferred into each test tube to give the first dilution 1:10, which was shacked for 5 min in the vortex to get homogenous soil suspension. One ml of the first soil suspension was transferred into new test tube containing 9 ml sterile distilled water and mixed gently to get dilution 1:100. Similarly, 1 ml of the second soil suspension was serially transferred into new test tube containing 9 ml sterile distilled water and mixed gently to get the final dilution of 1:1000. This process was repeated 4 times in which serial dilution was changed with normal saline, peptone water, and phosphate-buffered saline, respectively. Thereafter, 1 ml of soil suspension of each dilution with different solvent was aseptically poured in different culture media potato dextrose agar, yeast extract peptone glucose agar (YPGA), Dichloran Rose Bengal agar (DCRBA), malt extract agar (MEA), and potato carrot agar plates. The plates were gently rotated to spread the suspension on each medium surface. The plates were incubated at 25°C for 72 h. Slides of each growing colony were prepared to observe under a microscope. Observation of colony color and cell morphology on CHROM agar *Candida* (HiMedia, India) was used for yeast identification following the mycological literature.[11] While, biochemical identification was performed using VITEK 2 Compact Yeast Biochemical Card (Biomerieux, France) according to the manufacturer's directions.

In terms of molecular identification, a total genomic DNA of the yeast isolates was isolated and purified according to the procedure of mini kit (Geneaid, Taiwan). Internal transcribed spacer (ITS 1-5.8S-ITS2) region rDNA gene from the genomic DNA was amplified by polymerase chain reaction (PCR) technique according to the methods described by Mirhendi *et al*.[12] The common primers were used for amplification, the forward primer internal transcribed spacer (ITS1) F-5-TCC GTA GGT GAA CCT GCG G-3 and reverse primer ITS4 R-5-TCC TCC GCT TAT TGA TAT GC-3. The amplified ITS then was sequenced (Macrogen, Korea), and the yeast isolates were identified by Blast related to National Center for Biotechnology Information (NCBI). The accession numbers were deposited in DNA Data Bank of Japan (DDBJ).

Phylogenetic Analysis

The obtained ITS sequences were compared with the related species on the top of the Blast database. The phylogenetic analyses were conducted using MEGA version 7 software (Tamura *et al*., 2011). The evolutionary distances were calculated using the maximum composite likelihood method by bootstrap method with 1000 replications.

Accession Number of Some Nucleotide Sequences

The nucleotide sequences from the 4 yeasts were deposited in NCBI GenBank under accession Nos: LC474109, LC4773130, LC474381, LC473125, LC473127, LC473129, and LC474108 for those resulting from ITS1-5.8S-ITS2 region.

RESULTS AND DISCUSSION

Aureobasidium melanogenum (Hermanides-Nijhof) Zalar, Gostincar and Gunde-Cim Figure 1

Colonies on MEA at 25°C for 2 days smooth soon covered with slimy masses of conidia, cream, or pink later becoming brown or black that reached 3–10 mm diam. Sexual morph: Not observed in the present study. Asexual morph: Yeast cells ellipsoidal occur singly, 4–6 µm× 8–12 µm; hyphae are hyaline and septate frequently becoming dark-brown with age and forming chains of one to two-celled thick-walled, and dark pigment arthroconidia. The arthroconidia represent *Scytalidium* anamorph of *Aureobasidium* on Corn Meal Agar (CMA) medium at 25 for 5–7 days

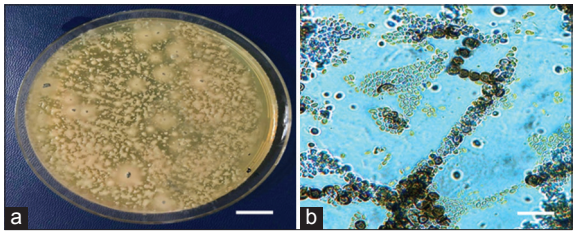

Figure 1: *Aureobasidium melanogenum* (a) colonies on malt extract agar, 2 days, 25°C bar = 10 mm (b) vegetative cells, arthroconidia bar = 10 µm

no pseudohyphae, colony on CHROM agar *Candida* green colored diazonium blue B (DBB) test −ve

- Halotolerance concentration 5% NaCl YPGA (+)
- Osmotolerance YPGA 50% glucose (−)
- Cardinal temperature: 37+, 25+, 15 (−).

Specimen examined: Living culture isolated from surface sediment soil, Al-Qurnah, Basra, Iraq.25.6.2018. (Strain AAN 20).

There are several studies described *A. melanogenum* (formerly known as *A. pullulans* var. *melanogenum*) isolated from soil and recorded in Russia, Greece, Thailand, and Japan.[13] In the current study, the anamorph of this species was recorded for the 1st time. The full description of this species is given here associated with the phylogenetic tree [Figure 2]. According to Gunde-Cimermana *et al*.,[14] *A. melanogenum* tolerates up to 10% NaCl and grows in 10°C and 35°C, whereas our isolates were not able to grow at 15°C. The explanation of that may be attributed to these strains were isolated from a region known with a high temperature (Basrah Province); therefore, it was unable to grow at low temperature. Yanwisetpakdee *et al*.[15] reported that *A. melanogenum* osmotolerance and have the ability to grow in culture medium with moderate concentration of YMA (30% glucose). Likewise, our isolate was not growing at YPGA with 50% glucose; this concentration contains a high percentage of glucose compared with 30%.

A long with Jiang *et al*.,[16] *A. melanogenum* is a black yeast such as species, particularly known in its biotechnological significance as a producer of the melanin yeast strain XJ5-1 of *A. melanogenum* isolated from the Taklamakan Desert was having an important role for the yeast survival in stressed environments, i.e., hot temperature, high salts, and ultraviolet (UV) irradiation. Numerous yeasts including *A. melanogenum* have the ability to synthesize sugar trehalose, which might be acted as a reserve carbohydrate, but its primary role is an extremely efficient protectant and chemical chaperone that reinforces the resistance of cellular elements against adverse condition.[17] A recent study like Argüelles *et al*.[18] has also demonstrated that trehalose as serves of antifungal target, a metabolic regulator, or signaling molecule. *A. melanogenum* has an unusually large spectrum of extracellular enzymatic activities which are several of them known in their biotechnological interest. These enzymes include amylases, cellulases, lipases, proteases, xylanases, β-fructofuranosidases, maltosyltransferase, mannanases, and laccases. A strain of *A. pullulans* is used for the production of acyclic peptide that has specific antifungal activity (aureobasidin). According to Molnárová *et al*.[19] and Gostinčar *et al*.,[20] *A. melanogenum* is the main fungus found in a spontaneously formed biofilm on an oil-treated wood. This dark-colored biofilm functions as a protective coating.

A case study performed by Mittal *et al*.[21] reported that advanced AIDS patients that suffering from fungemia were caused by *A. melanogenum* as pathogenic yeast.

***Galactomyces pseudocandidum* de Hoog and M.T. Sm. Figure 3**

Synonyme: *Galactomyces pseudocandidus*

Colonies on MEA at 25°C for 2 days 5–20 mm diameter. Asexual morph *G. pseudocandidum*: White, flat, dry, and powdery to finely hairy. Expanding hyphae, up to 5 µm, form rounded apices, produce much narrower hyphae at nearly right angles, and soon disarticulate into rectangular arthroconidia 6–12 µm × 3–6 µm, ellipsoidal, hyaline, chlamydospore-like cells may be present, and they may develop into asci. Asci are forming a single podium, ellipsoidal, often some what they have two sides that don't match, subhyaline walls, mostly immature, or develop a single ascospore that is subspherical, 5–6 µm, rough-walled, and with an irregular exosporium ascospores (portion of the spore that interact with the environment of host). On CMA medium at 25 for 5–7 days showed pseudohyphae; colony on CHROM agar *Candida* white-colored.

- DBB test −ve
- Halotolerance concentration 5% NaCl YPGA (+)

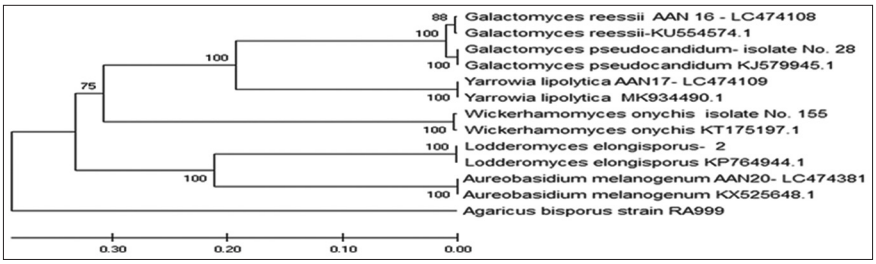

Figure 2: Evolutionary history of yeast strains with verified sequences covering the internal transcribed spacer (ITS1-ITS2) region rDNA using the UPGMA method. Ex-type strains are indicated by an asterisk. *Agaricus bisporus* strain RA999 used as out group. The bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, and the analysis was carried out with 13 nucleotide sequences

- Osmotolerance YPGA 50% glucose (−)
- Cardinal temperature: 37+, 25+,15−

Specimen examined: Living culture isolated from Sediment soil, Al-Hammar Marshes- Al-Burgha, Basrah, Iraq.1.12.2017. (Strain AAN 22).

Although, *G. pseudocandidum* has been identified by the PCR method in plenty times. A recent study Rozitis and Strade[22] reported the ability of *G. pseudocandidum* to degrade pharmaceutical wastewater in percent efficiency of 23.8% in waste degradation from water pre-treatment process.

During study phylogenetic analysis [Figure 2] clarified interspecies transfer was found in *G. pseudocandidum* and *Galactomyces reessii* in two clades.[23] Isolated *G. pseudocandidum* from wetland and confirmed its efficiency in mycoremediation, degraded dye, and treated rich dye textile of wastewater, which was showing high efficiency in treatment of wetland in Colombia. Wang *et al*.[24] pointed out that yeasts like fungi can grow in an environment with 50–70% glucose. In contrast to this finding, our study confirmed that *G. pseudocandidum* was unable to grow in media containing 50% glucose.

G. reessii (Van der Walt) Redhead and Malloch, Figure 4

Colonies on MEA at 25°C for 2 days 5–25 mm diam. Asexual morph (anamorph: *Geotrichum*) flat, white, dry, and cottony; expanding hyphae are submerged and without dichotomous branching. The main branches are 6–9 µm wide, with lateral branches 4–6 µm wide, which soon disarticulate into rectangular arthroconidia

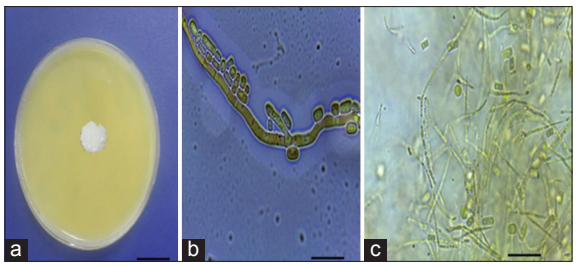

Figure 3: *Galactomyces pseudocandidum* (a): Colony on malt extract agar, 2 days, 25°C. Bar = 10 mm. (b): Arthroconidia bar =10 µm, (c) ascospore bar = 5 µm

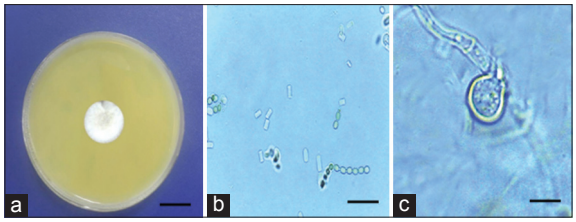

Figure 4: *Galactomyces reessii* (a): Colony on malt extract agar, 2 days, 25°C. Bar = 10 mm. (b): Arthroconidia bar =10 µm, (c) ascospore bar = 5 µm

4–15 µm, asci subspherical, 7 µm × 10 µm, one spored. Ascospores broadly ellipsoidal, 5–6 µm × 6–7µm, pale golden-brown, finely warted, with an irregular, locally inflated exosporium, colony on CHROM agar *Candida* violet-colored; budding is absent. Chlamydospores may be formed on CMA medium.

- DBB test −ve
- Halotolerance concentration 5% NaCl YPGA (+)
- Osmotolerance YPGA 50% glucose (−)
- Cardinal temperature:37+, 25+, 15−

Specimen examined: Living culture isolated from Sediment soil, Al-Faw, Basrah, Iraq.11. 2.2018. (strain AAN 16).

As stated by Suwannarach *et al*.,[25] *G. reessii* formerly name (*Dipodascus reessii)* has been recorded in both sexual and asexual states. They also pointed out that the identification of this species has been confirmed by cultural morphology associated with DNA sequencing for isolates obtained from tomato as pathogen. Garnier *et al*.[26] isolated *Galactomyces* from diary products and clarified their ability for growing in halotolerance environment. Interestingly, this finding is in accordance with the results of our study. Lee *et al*.[27] evaluated the effect of essential cosmetics that formed from *Galactomyces* ferment filtrate to improve human skin. This finding showed 9.59% of acne decrease and 2.49% increase in facial skin brightness after using this cosmetic.

Lodderomyces elongisporus (Recca and Mrak) Van der Walt, Figure 5

Colonies on MEA at 25°C for 2 days 1–2 mm diam. Tannish-white, butyrous, smooth and glistening, and single colonies are low convex with a raised center. With circular margins, asexual morph (*Candida parapsilosis*) the yeast cells spherical but they are usually ellipsoidal to elongate, 3–6 µm × 4–7 µm, and occur singly, in pairs, or in small clusters, asci one rarely two spored long-ellipsoid 2 µm × 5 µm, observed on MEA 5% after 10 days at 25°C. On CMA medium at 25 for 5–7 days showed abundant well-branched pseudohyphae with blastoconidia 3 µm × 5 µm. True hyphae are not formed, colony on CHROM agar *Candida* green colored,

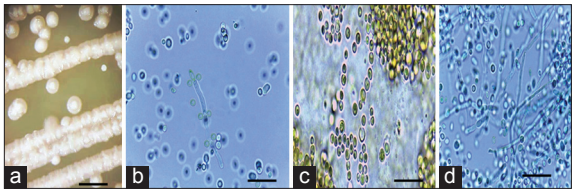

Figure 5: *Lodderomyces elongisporus* (a): Colonies on malt extract agar, 2 days, 25°C. Bar = 2 mm. (b) Yeast cells: Bar = 5 µm, (c) ascospores bar = 5 µm, (d): Blastoconidia bar = 5 µm

- DBB test −ve
- Halotolerance concentration 5% NaCl YPGA (+)
- Osmotolerance YPGA 50% glucose (−)
- Cardinal temperature:37−, 25+,15−

Specimen examined: Living culture isolated from surface sediment, Karmat Ali/Shat Al –Arab/Mhmedia, 9.1.2018 (Isolate No. 136) and agriculture soil, Karmat Ali, Basra, Iraq. 4. 1.2018. Basra, Iraq. (Strain AAN 14).

L. elongisporus, an ascomycetous fungus, has been known as teleomorph of *C. parapsilosis*; however, subsequent taxonomic studies suggested that *Candida orthopsilosis* is the anamorphic phase.[28] During study [Figure 2] shows that *L. elongisporus* appeared in a single clade associated with *G. pseudocandidum*, whereas other ascomycetous species were appeared in different clades on the phylogenetic tree. This finding is in accordance with Kurtzman and Robnett[29] who stated that the relationships among ascomycetous genera have been uncertain.

***Wickerhamomyces onychis* (Yarrow) Kurtzman, Robnett and Basehoar-Powers Figure 6**

Synonym: *Pichia onychis.*

Colonies on (MEA) at 25°C for 2 days a 1–2 mm diam., cream colored, glistening, and butyrous. The yeast cells ovoid, 2–4 µm × 3–10 µm, and occur singly or in pairs. Ascospores hat-shaped 2–5 µm, were observed on 5% MEA and V8 agars after 10 days at 25°C. On CMA medium at 25 for 5–7 days showed pseudohyphae, colony on CHROM agar *Candida* white-colored.

- DBB test −ve
- Halotolerance concentration 5% NaCl YPGA (+)
- Osmotolerance YPGA 50% glucose (−)
- Cardinal temperature:37+, 25+, 15−.

Specimen examined: Living culture isolated from Sediment soil, Al-Faw, Basra, Iraq.11. 2.2018. (Strain AAN 24).

W. onychis is one of the few yeasts with lactic acid bacteria (LAB) that exhibited important technological features and high antibacterial activity against human and food-borne pathogenic bacteria. Furthermore, LAB produced various antioxidants for fermented food products,[30] this fact may be due to their antimicrobial properties through lactic acid, hydrogen peroxide, diacetyl, and bacteriocin release. Hence, LAB application was reported as suitable and easy biotechnology to prevent fruit alteration.[31]

***Y. lipolytica* (Wick., Kurtzman and Herman) Van der Walt and Arx Figure 7**

Colonies on MEA at 25°C for 2 days 5–10 mm diam., butyrous to hyphal, tannish white in color, the yeast cells spherical, ellipsoidal or elongate, 2–4 µm × 2–8 µm, and occur singly, in pairs, or in small clusters. On CMA

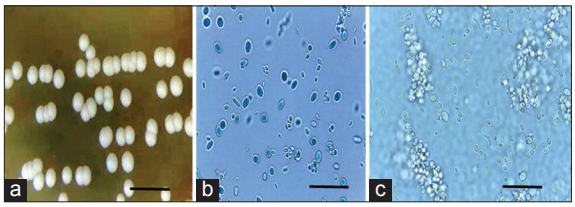

Figure 6: *Wickerhamomyces onychis* (a): Colonies on malt extract agar, 2 days, 25°C. Bar = 2 mm. (b) Yeast cells: Bar =10 µm, (c) release hat shape ascospore bar = 5 µm

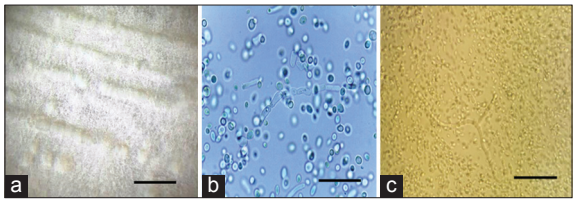

Figure 7: *Yarrowia lipolytica* (a): Colonies on malt extract agar, 2 days, 25°C. Bar = 10 mm. (b) Yeast cells: Bar =10 µm, (c) pseudohyphae bar = 10 µm

medium at 25 for 5–7 days showed pseudohyphae, and true hyphae are usually present as well; colony on DCRBA 5 mm diam. after 9 days, butyrous, white-colored reverse rose in color yeast cells spherical, ellipsoidal or elongate, 2 µm × 8 µm, occur singly, in pairs, or in small clusters colony on CHROM agar *Candida* green colored.

- DBB test −ve
- Halotolerance concentration 5% NaCl YPGA (+)
- Osmotolerance YPGA 50% glucose (−)
- Cardinal temperature: 37+, 25+, 15−

Specimen examined: Living culture isolated from surface sediment, Al-Faw, Basra, Iraq. 11. 2.2018; (isolate No.6 and. 67); agriculture soil, Karmat Ali, 4.1.2018 (isolate No.1); surface sediment, Al-Hammar Marshes – Al-Burkha 1.12.2017 (isolate No.26); agriculture soil, Abu-Alkhaseeb, 12.2. 2018 (isolate No.27); sand soil, Al-Bargesia, 15.4.2018 (isolate No. 35); surface sediment, Al-Faw, 22.4.2018 (isolate No.29); surface sediment, Chabaish marshes Abu - Subat, 21.11.2017, Thi-Qar (isolate No.28); agriculture soil, Abu-AlKhaseeb, 19.4.2018 (isolate No.44); Sand soil, Safwan, 1.3.208 (isolate No.9); Sand soil, Om-Qaser, 1.3.2018 (isolate No.38); agriculture soil, Al-Hartha, 9.1.2018. (isolate No.43); surface sediment, Al-Sabbaghia Al-Chebiash, Thi-Qar 24.8.2018 (isolate No.47); surface sediment, Shatt Al-Arab – Siba, 19.6.2018 (isolate No.51); surface sediment, Al-Faw, 2.2.2018 (isolate No.52); agriculture soil, Abu-Alkhaseeb, 1.3.2018 (isolate No.53); agriculture soil, Karmat Ali, 15.3.2018 (isolate No.54); surface sediment, Al-Faw, 11.2.2018; (strain AAN 17).

Knutsen *et al*.[32] performed a deep study on *Yarrowia* and closely related *Candida* species using a wide range of techniques, such as sequence analysis of ITS region, PCR, and fingerprinting analysis. Groenewald *et al*.[33]

indicated the two phases teleomorph (*Y. lipolytica*) and anamorph (*Candida lipolytica*). Yeast oils have arisen as an appropriate exceptional raw material to generate biodiesel because of their parallel composition shared raw materials, i.e., vegetable oils.

Molecular Study

The results of sequences for nine strains compared with available sequences in GenBank, ITS homology percentages were 99–100% for different isolates in Basrah-Iraq Table 1.

Identification of Yeast Strains

ITS1-ITS2 5.8S rDNA gene

ITS1-ITS2 5.8S rDNA gene of eight yeast isolates was shown on agarose gel electrophoresis under UV transilluminator at the position 300–700 bp by comparing with standard DNA ladder [Figure 8].

Alleles in *Y. lipolytica*

The ITS regions occur in multiple copies in the fungal genome.[34] Changing in copy numbers

Table 1: Molecular characterization Strains No., Accession No., and homology percentages

ITS-5.8S rDNA–ITS2			
No.	**Strains**	**Accession no.**	**Homology (%)**
AAN16	*Galactomyces reessii*	LC474108	100
AAN17	*Yarrowia lipolytica*	LC474109	100
AAN14	*Lodderomyces elongisporus*	LC473130	100
AAN20	*Aureobasidium melanogenum*	LC474381	100
AAN24	*Wickerhamomyces onychis*	In process	100
AAN22	*Galactomyces pseudocandidum*	in process	100
AAN9	*Debaryomyces hansenii*	LC473125	99
AAN11	*Pichia fermentans*	LC473127	99
AAN12	*Pichia fermentans*	LC473129	99

ITS: Internal transcribed spacer

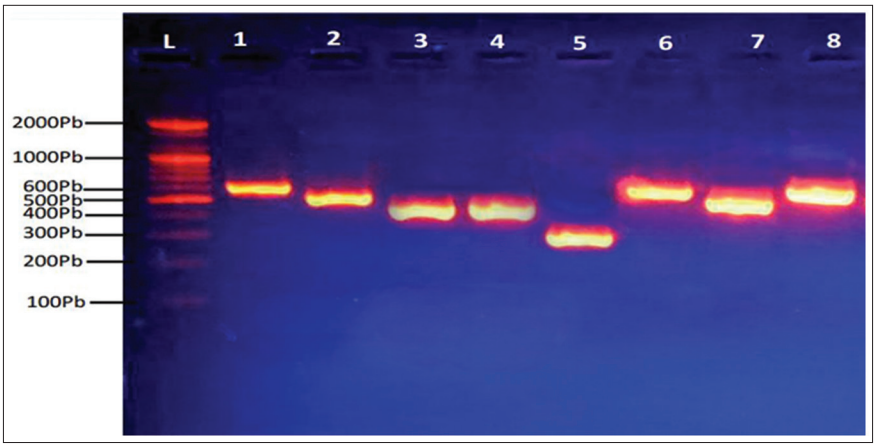

Figure 8: Agarose gel electrophoresis 2% of polymerase chain reaction products for internal transcribed spacer (ITS1 – ITS2) regions (including 5.8S rDNA gene): Lane L: (100 bp) DNA ladder, lane 1: *Wickerhamomyces onychis* (527 bp), lane 2: *Galactomyces pseudocandidum* (523 bp), lane 3: *Pichia fermentans* (392 bp), lane 4: *P. fermentans* (408 bp), lane 5: *Galactomyces reessii* (330 bp), lane 6: *Aureobasidium melanogenum* (550 bp), lane 7: *Lodderomyces elongisporus* (517 bp) and lane 8: *Debaryomyces hansenii* (586 bp) for ascomycetes yeasts isolates

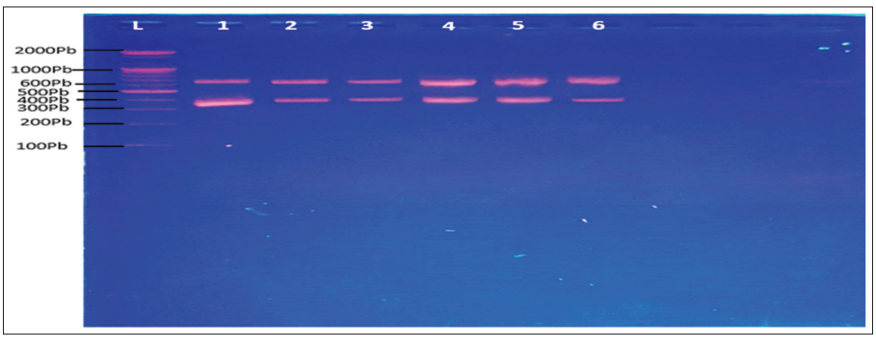

Figure 9: Agarose gel electrophoresis 2% of polymerase chain reaction products for ITS internal transcribed spacer (ITS1–ITS2) regions (including 5.8S rDNA gene). Lane L: 100 bp DNA ladder, Lane 1–6 *Yarrowia lipolytica* isolates at the position 400 and 700 bp

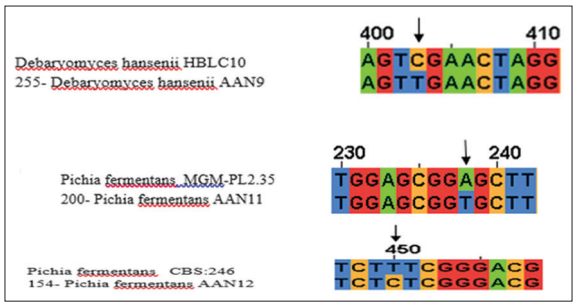

Figure 10: Different mutation in new strains *Debaryomyces hansenii* AAN9, *Pichia fermentans* AAN11, and *P. fermentans* AAN1

in gene sequences were identified, as allele on different chromosomes is known polymorphism, if more than one allele occupied gene's locus within a population.[35] Therefore, two bands were appeared in the gel electrophoresis because this yeast has more than one picture for gene in different chromosomes [Figure 9].

Invention of New Strains

Three isolates were recorded in DDBJ and NCBI as a new strains differed from their type strains in some nucleotide position [Figure 10] *D. hansenii* AAN9 new strain 255, *D. hansenii* Hblc 10 which available in NCBI showed similarity with 99% and difference in one nucleotide at the position 403 bp transition mutation (T instead C) *P. fermentans* AAN 11 new strain 200 *P. fermentans* MGM-Pl2 which available in NCBI showed similarity with 99% and difference in one nucleotide at the position 238 bp transversion mutation (T instead A), *P. fermentans* AAN12 new strain 154, *P. fermentans* CBS-246 which available in NCBI showed similarity with 99% and difference in one nucleotide at the position 450 bp transition mutation (C instead T).

CONCLUSIONS

The current study considered to be the first in isolating ascomycetous yeasts from Iraqi soil and revealed biodiversity in soil samples studied from provinces Basrah and Dhi-Qar and the use of PCR in the identification of yeasts considered to be fast accurate and develop the method.

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