A new record of interesting basidiomycetous yeasts from soil in Basrah province/Iraq.**

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Abstract

Seventy-five soil samples were collected from different sites in Basrah province, southern Iraq to investigate the presence of basidiomycetous yeasts using the dilution-plate method. The growing isolates on culture media were identified based on morphological and biochemical features, and was confirmed by molecular data. Twelve yeast species belong to six different genera were identified. The most frequent isolated species were belonging to genus *Naganishia* with five species. All the described species are recorded for first time from Iraq. New strains were also obtained in the present study and registered in the Japanese Gene Bank.

Key words: Basidiomycetes Yeasts, Soil, Basrah .

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1. Introduction

A number of studies and reviews have been investigated the frequency of occurrence, diversity and importance of yeasts associated with soil worldwide. Yurkov [1] has reported that basidiomycetous yeasts generally encountered more abundantly in soil samples than ascomycetous yeasts. Soil rich with organic matter usually contain numerous yeast colonies compered to amendment (fertilized) agricultural soils , such as pigmented yeasts *Cystobasidium ,Rhodotorulla , Rhodosoridiobolus, Sporolomyces* and *Vishniacozyma* species [2]. The relevance of yeasts to soil function is not yet fully understood although it is known that they influence soil aggregation , contribute to nutrient cycle, and interact with the vegetation [3].

Many previous studies have been isolated and identified basidiomycetous yeasts by biochemical and molecular methods from different soils in various location around the world[4, 5] . The aim of this study was to record a number of newly soil basidiomycetous yeasts in Iraq

2- Materials and methods

Soil samples (75) were collected from different location in several regions in Basrah, during the period from 24-10-2017 to 24 – 8 – 2018 The sample were placed in sterile plastic bags and brought to the laboratory and stored at 4-7^{o} C until processed. The soil dilution method was employed by using four types of diluents, which were distilled water, normal saline (0.85%), peptone water (0.1%), and phosphate buffer (0.1M, pH.7). For each sample , several dilutions were made (10^{-1} to 10^{-3}) , then 1 ml of each dilution was cultured on yeast extract peptone glucose agar (YPGA) ,potato dextrose agar (PDA), dichloran rose bengal agar (DCRBA),malt extract agar (MEA).The petri plates were incubated at 15,25,37 C ^{o}for 7 days , morphological features were selected from primary cultures , after purify grown on Potato Dextrose Agar then stored at 4 ^{o}C. The isolated strains were identified morphophysiologically using the taxonomic key proposed by Pitt and Hocking [6] and Kurtzman *et al*. [7]. Living cultures for all the reported species and strains were deposited in Mycology laboratory , Department of Biology , College of Science , University of Basrah.

Molecular identification

Genomic DNA was extracted according to the instructions provided by the manufacturer of Presto Mini gDNA yeast kit (Geneaid, Taiwan) after cultivation of single colony on PDA for 48 h. at 25 °C. The PCR designed by [8] was used to amplify the internal transcribed spacer (ITS 1-5.8S-ITS2) region of the isolated yeasts using universal primers ITS1 F-5-TCC GTA GGT GAA CCT GCG G-3 and ITS4 R-5-TCC TCC GCT TAT TGA TAT GC-3 . The amplification of target region was carried out in a total volume of 50 µl contained Master Mix (Bioneer, Korea), 2µl of each primer,10 µl of genomic DNA as a template and 36 µl Nuclease-free water. The reaction was performed using (Applied Bio System, USA) thermal cycler and the PCR conditions were 94 °C for 5 min followed by 25 cycles at 94 °C for 30 sec , 56 °C for 45 sec and 72 °C for 1 min then followed by 7 min. final extension was done at 72 °C . The PCR product was detected by visualized on 2 % Agarose gel stained with ethidium bromide electrophoresis

sequencing

A twenty µl of PCR product ITS1-ITS2 region of each sample were send to Macrogen Company for purification and sequencing. All yeast isolates sequences were identified compared with the standard sequences that deposited in the NCBI using BLAST tool

The data analysis by Chromas software

3- Results and discussion

Twelve yeast species were identified by morphological and biochemical methods and was confirmed by molecular data. Below is a brief description of new registered species :-

***Cutneotrichosporon dermatis* Sugita, Takash., Nakase & Shinoda Fig.1**

Colonies on malt extract agar (MEA) growing rapidly reaching 10 mm diameter in 2 days at 25 C° , yellowish white, semi-shiny, butyrous , wrinkled margin with frings. The yeast cells on MEA at 2 days' ovoid, ellipsoid to elongate2-15 µm, singly, in pairs or in clusters. Basidia were observed on MEA 5 % after 10 days at 25 C°. On corn meal agar (CMA) at 25 for 5-7 days arthroconidia and mycelia present, CHROMagar Candida color: dark brown, Diazonium blue B test (DBB) = +ve

Specimen examined: Living culture isolated from agriculture soil of Abu-Alkhaseeb ,Basrah,Iraq 19.4.2018(Strain AAN 25).

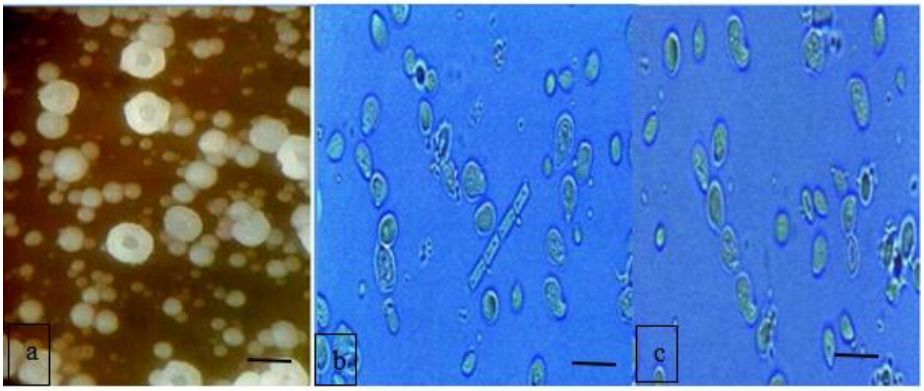


Figure (1) ***Cutneotrichosporon dermatis*** (a) colonies on MEA, 2 days, 25C, scale bar = 2mm (b) vegetative cells, (c) arthroconidia, scale bar = 5 µm

Cutneotrichosporon species have been isolated from different habitats, including predominantly in tropical and temperate areas. This organism can be found in substrates such as soil, decomposing wood, air, rivers, lakes, seawater, cheese, scarab beetles, bird droppings, bats, pigeons, and cattle. In humans, these fungal species occasionally are part of the gastrointestinal and oral cavity microflora and can transiently colonize the respiratory tract and skin.[9].

Cystobasidium benthicum **(Nagah., Hamam., Nakase & Horikoshi) Yurkov, Kachalkin, H.M. Daniel, M. Groenew., Libkind, V. de Garcia, Zalar, Gouliam., Boekhout & Begerow, Fig.2**

Synonym: ***Rhodotorula benthica*** **Nagah,Haman,Nakase and Horikoshi**

Colonies on MEA 1-2 mm diam, light pink, glistening, smooth, mucoid. The yeast cells on MEA at 2 days subglobose to ovoid, occur singly or in pairs, 2-7µm.Basidiospores were formed on MEA 5 % after 10 days at 25 C °. Colony on CHROMagar Candida white colored, DBB= +ve. On CMA at 25 C° for 5-7 days pseudohyphae and true hyphae. growth on YPDA containing NaCl 5%, growth at 5 C°.

Specimen examined: Living culture isolated from agriculture soil, Al-Zubair, Basrah,Iraq 11.2.2018, (Strain AAN5)

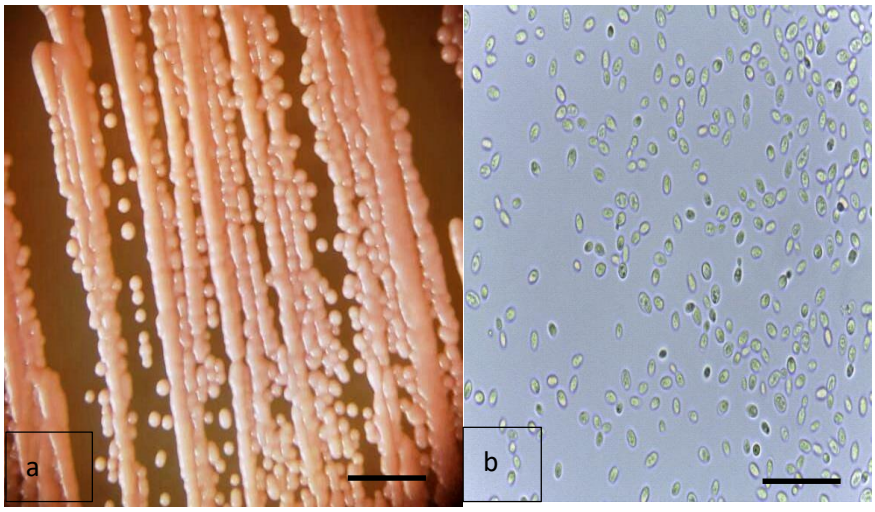


Figure (2) ***Cystobasidium benthicum*** (a) colonies on MEA , 2 days, 25C, scale bar = 2mm (b) vegetative cells, scale bar= 5 µm

Nagahama *et al* . [10] reported the isolation of seven strains of this species from tubeworms belonging to the genus *Lamellibrachia* sp. that collected from deep-sea floor. Since then all available information suggest that this species has not been found in other substrates, it seems that *R. benthica* is a marine species associated with *Lamellibrachia* sp. Several species of *Cyctobasidium* were isolated from Arab Gulf and East Ongul Island, East Antarctica .According to Tsuji *et al* . [11] *Cyctobasidium* species are halotolerant yeasts. This finding is in accordance with our results, this species was exposed to different tests and it found to be able to grow at 5 °C, which could be also psychrotolerant.

Cystobasidium minutum **(Saito) Yurkov, Kachalkin, H.M. Daniel, M. Groenew., Libkind, V. de Garcia, Zalar, Gouliam., Boekhout & Begerow. Fig.3**

Colonies on MEA after 2 days at 25 C 1-2 mm diameter, light pink, glistening, smooth, mucoid, with entire margin. The yeast cells on MEA at 2 days subglobose to ovoid, 3-6 µm, occur singly or in pairs Basidia were formed on MEA 5 % after 10 days at 25 C °. On CMA at 25 C °for 5-7 days no pseudohyphae ,Colony on CHROM agar Candida light rose colored ,DBB= +ve

Specimen examined: Living culture isolated from sediment soil, Al-Hammar Marshes, Basrah, Iraq 21.11.2017, (Strain AAN 10).

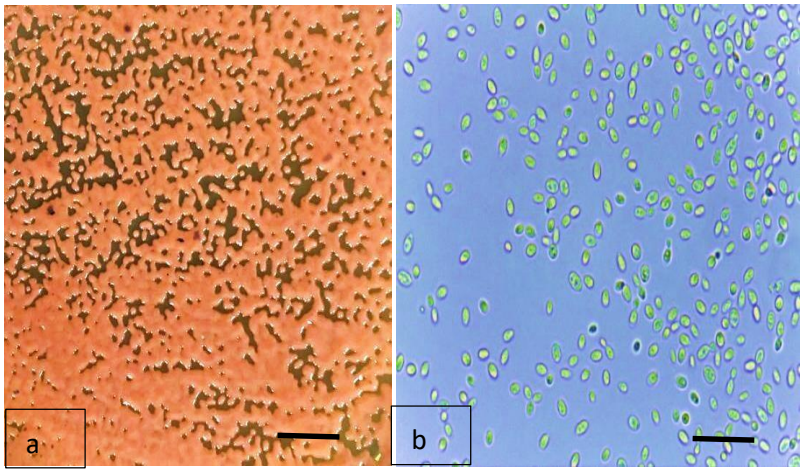


Figure (3) ***Cystobasidium minutum*** (a) colonies on MEA, 2 days, 25C, scale bar = 2mm (b) vegetative cells, scale bar= 10 µm.

This yeast species that is commonly isolated from air, freshwater, seawater all over the world. This species has also been implicated in rare human infections. It also isolated from food and fruits. This pigmented yeast may have important role in biological control of fruit disease because capability of this yeast for production rhodotorulic acid [12]. This is the first record of the species from the soil in Iraq.

Filobasidium oeirense **(Á. Fonseca, Scorzetti & Fell) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout. Fig.4**

Colonies on MEA after 2 days at 25 C 2-3 mm diameter, hyaline to pinkish-cream, soft to mucoid, glossy, with smooth surface. The yeast cells on MEA at 2days sub globose or broadly ellipsoidal, 4-11 µm, occur singly or in short chains. Cells have small to moderately size capsules. On MEA 5% basidia present, 10-30 µm. On CMA medium at 25C^{o} for 5-7 days pseudohyphae absent, Colony on CHROM agar *candida* dark rose colored, DBB= +ve.

Specimen examined: Living culture isolated from sediment soil, Abu-Alkhaseeb ,Basrah, Iraq 12.2.2018.,(Strain AAN 26)

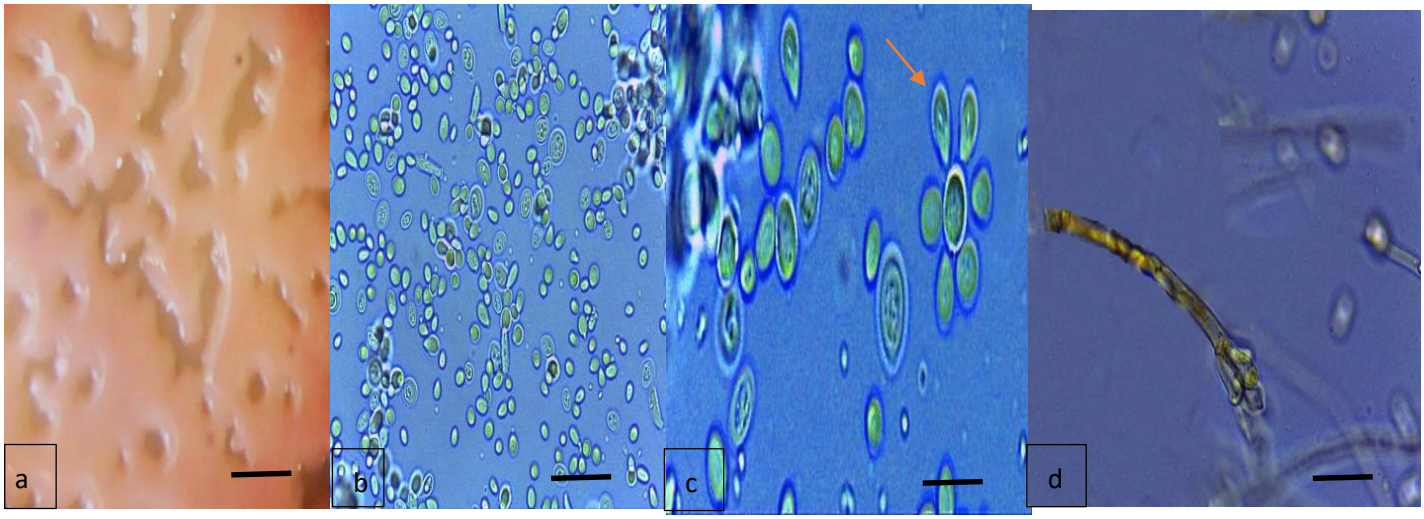


Figure (4) ***Filobasidium oeirense*** (a) colonies on MEA, 2 days, 25C, scale bar = 2mm (b) vegetative cells, scale bar= 10 µm (c): basidium (circular arrangement) scale bar= 10 µm, (d) Mature basidium with basidiospores, scale bar= 10 µm

This species has been reported for the first time in Australia, on the surface of undamaged grapes wine by[13]. In terms of our results, to the best of our knowledge, this is the first report of the species from soil sediments in Iraq and probably represent the second report in the world.

Naganishia adeliensis **(Scorzetti, I. Petrescu, Yarrow & Fell) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout.**
Fig.5

Colonies on MEA after 2 days at 25 C 1-2 mm diameter. white to cream, soft to mucoid, with a smooth and glossy surface, entire to lobate margin. The yeast cells on MEA at 2 days spherical, subglobose or ovoid, 2-4 µm. Cells with small capsules. Pseudohyphae absent on CMA, Colony on CHROM agar Candida rose colored, DBB= +ve,

Specimen examined: The strain (AAN 7) isolated from agricultural soil of Al-Midaina ,Basrah, Iraq in March 30th 2018.

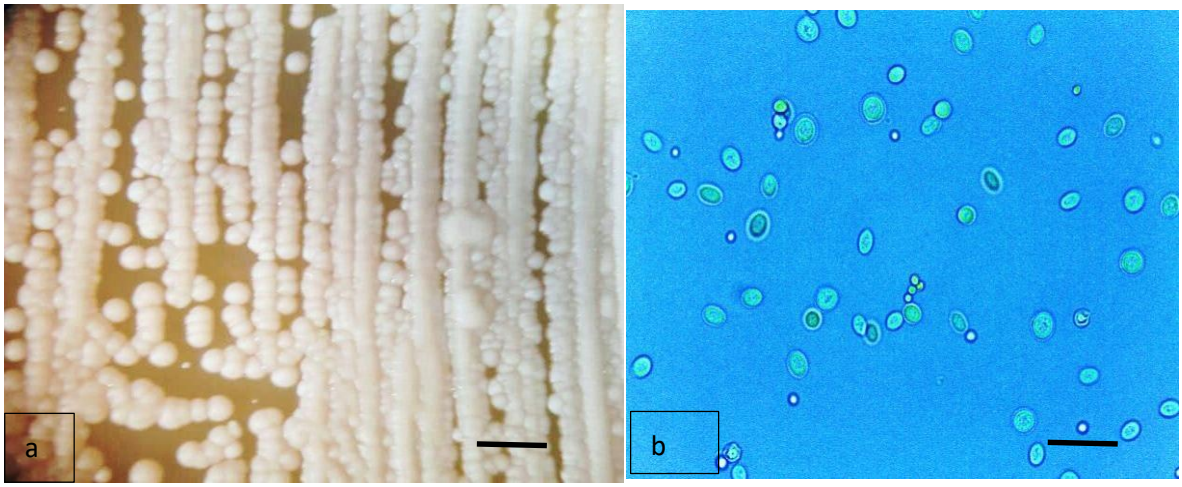


Figure (5) ***Naganishia adeliensis*** (a) colonies on MEA, 2 days, 25C, scale bar = 2mm (b) vegetative cells, scale bar = 5 µm.

The present species was isolated from decayed algae in the icepack at the Antarctic station ,cerebrospinal fluid of patient with acute myeloid leukemia and suffering from meningitis ,sheep feces in Spain , and glacial meltwater river in Patagonia, Argentina [7].Kamari

[14] have isolated *N. adeliensis* from Eucalyptus tree at percentage of 25% in Iran . Generally, this yeast is known to be psychrotolerant as it was isolated at 5 ,15,25,37 C. Psychrotolerant organisms are similar to mesophiles (growing at 20-40C), but are able to tolerate lower temperatures albeit with slower growth rates [15]. Some of the yeasts that present in soil can be pathogenic or harmful to humans and other animals, and may produce secondary metabolites including mycotoxins [16]. In the recent study, the ability of the strain (AAN 7) for producing killer toxin (by culturing it on methylene blue medium) was tested and the result was positive (+). Seemed to be the first record in Iraq and probably the first time in the world as soil yeast.

Naganishia albidosimilis **Vishniac and Kurtzman** **Fig.6**

Colonies on MEA after 2 days at 25 C 1-2 mm diam., cream to rose colored, mucoid, with smooth and glossy surface. The yeast cells on MEA at 2 days ovoid or broadly ellipsoidal, 3-5 µm. Cells with medium-sized capsules. On CMA medium at 25 for 5-7

days pseudohyphae present, true hyphae absent , Colony on CHROM agar Candida violet colored ,DBB= +ve

Specimen examined: Living culture isolated from agriculture soil, Al-Midaina ,Basrah, Iraq 3.3.2018.,(Strain AAN 4) .

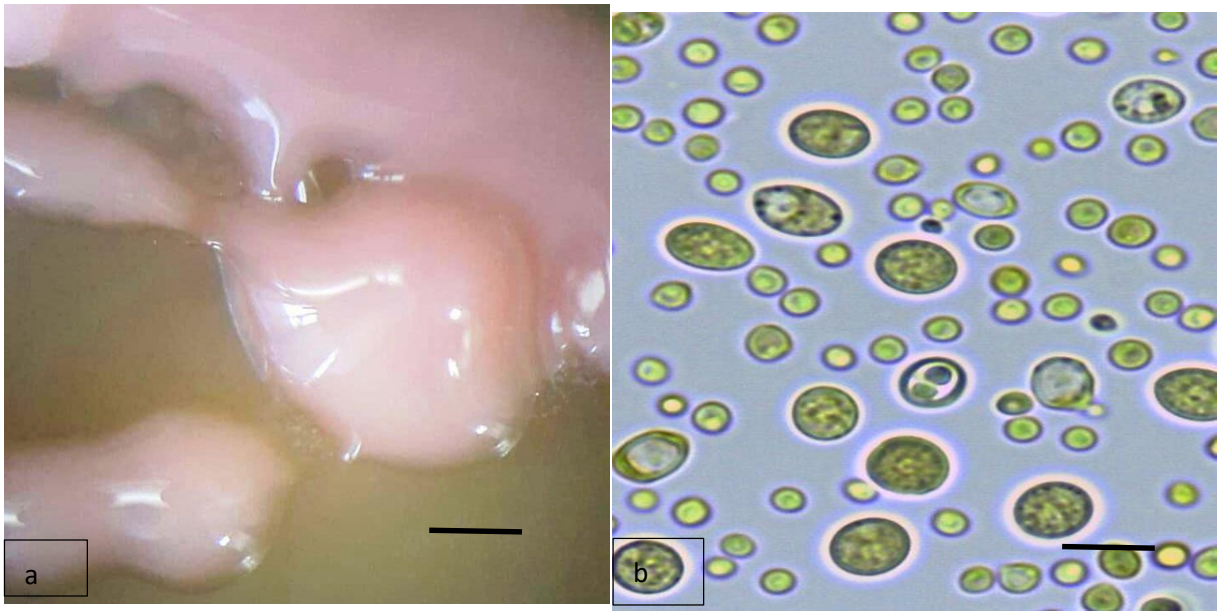


Figure (6) ***Naganishia albidosimilis*** (a) colonies on MEA, 2 days, 25C, scale bar = 2mm (b) vegetative cells, scale bar= 10 µm.

The type strain of *Cryptococcus albidosimilis* synonyme of *N. albidosimilis* was isolated from soil in Antarctica and the species was also found in ice[17], this suggesting that it is well adapted to low-temperature environments. Several strains isolated from soil and wood in the Ross Sea region of Antarctica by Arenz *et al.* [18] were labeled as *C. albidosimilis* Petrescu *et al* . [19] reported the production of cold adapted xylanase from *C.adeliae* similar to that of *C. albidosimilis* .The molecular structure of this xylanase is characterized by high plasticity which make it thermosensitive.

In our study *Naganishia albidosimilis* AAN 4 strain was isolated from Basrah- Al-Midaina which is well known of its high temperature region and it was recorded for the first time in world from cold environment[16] therefor isolation conditions for this isolate requires incubation at 5 C^{o} for 2 months.

***Naganishia liquefaciens* Saito and Ota** **Fig.7**

Colonies on MEA after 2 days at 25 C 1-2 mm diam., cream to pinkish-cream, mucoid, a glossy, smooth surface. The yeast cells on MEA at 2 days subglobose or broadly ellipsoidal, 3-8 µm, occur singly, cells often with moderately sized capsules. On CMA medium at 25 C °for 5-7 days no pseudohyphae, sexual stage absent ,Colony on CHROMagar candida violet colored , DBB= +ve,

Specimen examined: Living culture isolated from sediment soil, Karmat Ali,Basrah, Iraq 9.1.2018.,(Strain AAN23) .

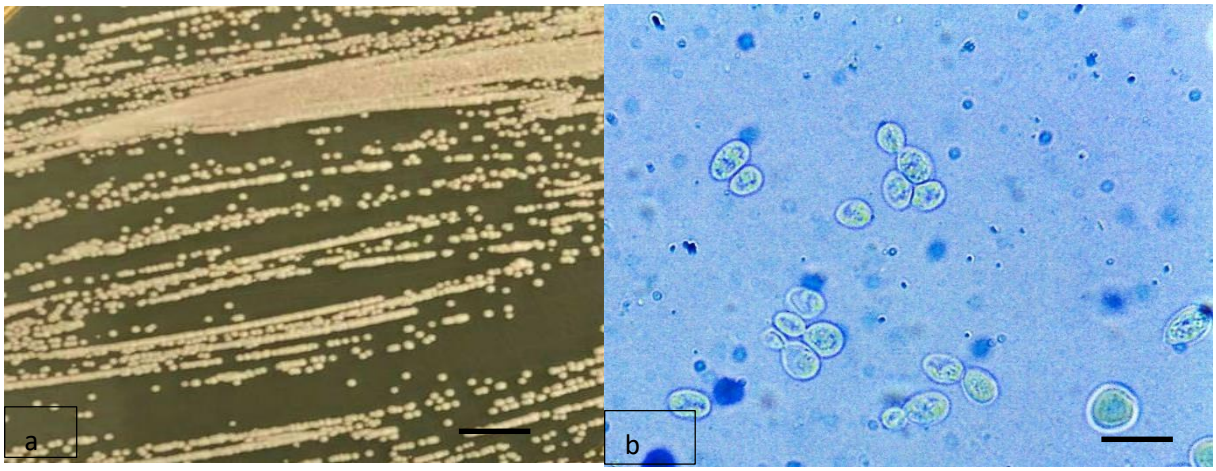


Figure (7) ***Naganishia liquefaciens*** (a) colonies on MEA, 2 days, 25C, scale bar = 2mm(b) vegetative cells, scale bar= 10 µm.

Type strain of *Torulopsis liquefaciens* (recent name *Naganishia liquefaciens*) was isolated by K. Saito from sake-moto in Japan [7] also it was isolated from human skin in Portugal and seawater in California [20] *Naganishia liquefaciens* was tested on different culture media. Interestingly it was successfully grow at 37 °C suggesting that it may be has clinical relevance as a pathogenic yeast. Our result was inconsistent evidence with Sugita et al. [21], who reported that *N. liquefaciens* was isolated from cold environments and had no a significant clinical importance due to its unable to grow at 35 °C. Biotechnologically, this species has a special concern, due to its ability to accumulate lipid as a novel oleaginous by pre-digested municipal waste activating sludge (PWAS) [22].

Naganishia uzbekistanensis (Á. Fonseca, Scorzetti & Fell) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout. **Fig.8**

Colonies on MEA after 2 days at 25 C 1-2 mm diam., white to pinkish and butyrous, slightly glossy, smooth surface. The yeast cells subglobose or broadly ellipsoidal, 3-4 µm, occur singly, Without capsule. On CMA medium at 25 C° for 5-7 days no pseudohyphae, Colony on CHROMagar Candida greenish violet Colored, DBB= +ve,

Specimen examined: Living culture isolated from agriculture soil, Abu-Alkhaseeb,Basrah, Iraq 16.4.2018.,(Strain AAN3).

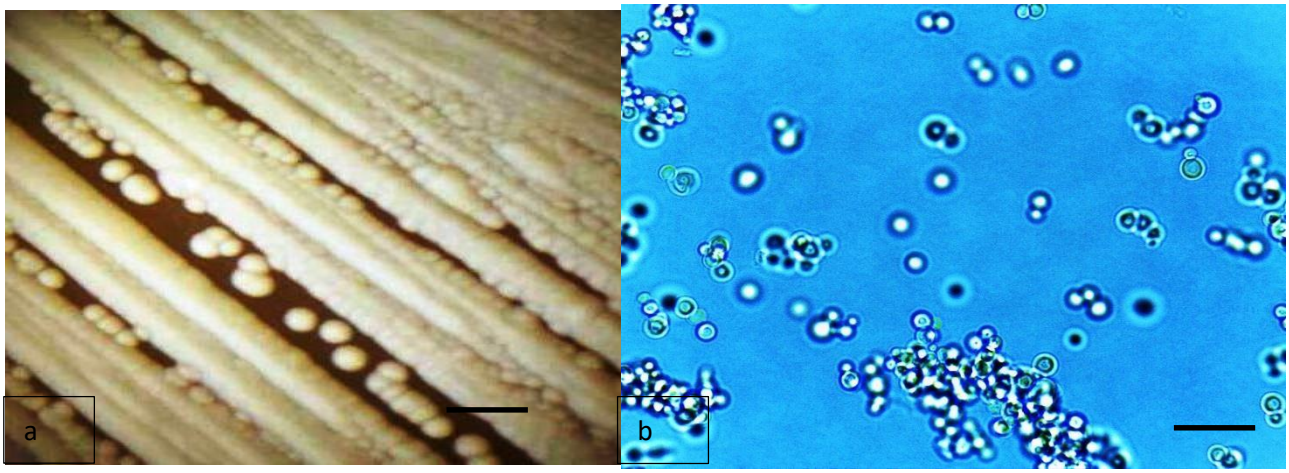


Figure (8) ***Naganishia uzbekistanensis*** (a) colonies on MEA, 2 days, 25C bar = 2mm(b) vegetative cells,)bar= 10 µm.

Cryptococcus usually grow on conventional isolation media and their cells with small or no capsules. Cutaneous and systemic infections due to *Cryptococcus* were in human and animals [23].The first isolation of *Cryptococcus uzbekistanesis* synonym of N. *uzbekistanensis* was from immunocompromised patient with lymphoma. No previous report of its isolation from environment sources in North America. Consequently, this finding might be the second report of isolation *Naganishia uzbekistanensis* from soil in the Middle East. our isolate can be able to grow at 37°C and can cause disease.

Naganishia vishniacii **Vishniac and Hempfling** **Fig.9**

Colonies on MEA after 2 days at 25 C° 2 mm diam., cream colored, butyrous, with a dull, smooth surface The yeast cells on MEA at 2 days broadly ellipsoidal, 4-12µm. On CMA

medium Pseudohyphae absent, Colony on CHROM agar *candida* bluish – violet colored, DBB= +ve,

Specimen examined: Living culture isolated from sediment soil, Al-Faw,Basrah, Iraq 1.3.2018.,(Strain AAN19).

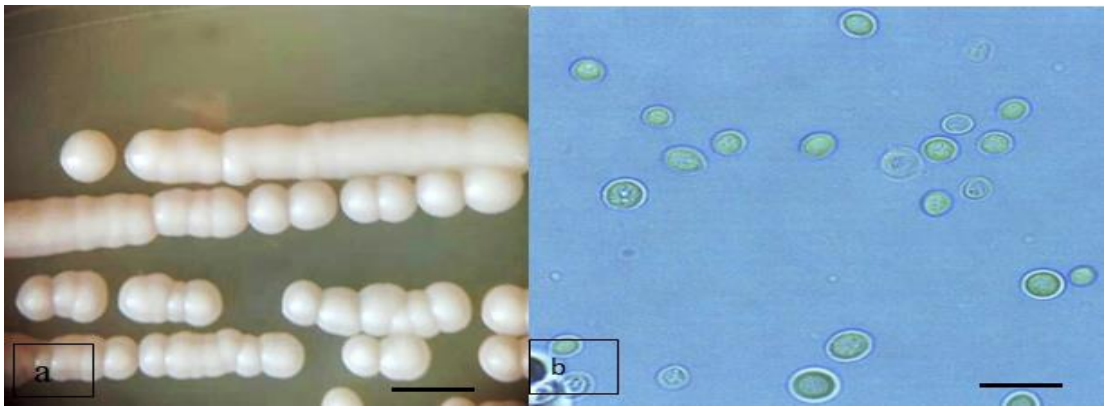


Figure (9) ***Naganishia vishniacii*** (a) colonies on MEA, 2 days, 25C bar = 2mm(b) vegetative cells, bar= 10 µm

Our isolate was different in the dimension of vegetative cell (5.5-7.5 µm) compared to the original descrption of the species. *Naganishia vishniacii* was isolated from soil sediment in Faw district, North of Basra province as halotolerance and acidophilic isolate due to its growing on YPDA with 5% NaCl and media with acetic acid respectively.

Naganishia vishniacii was isolated from extreme terrestrial environment. Their ability to withstand high levels of UV radiation and low pH values combined with their unique ability to grow during extreme freeze-thaw cycles indicates that they are adapted to live where these three stressors are stay. Also, given that they are halotolerant (rather than halophilic), psychrotolerant (rather than psychrophilic), and metabolically versatile indicates that they are flexible in their response to water potential, temperature, and nutrient availability. Therefore, given the evidence available so far, our working model for why *Naganishia* species are so prevalent on these high elevation volcanoes is that they are flexible "opportunitrophs" [24, 25] that can grow during rare periods of water

nd nutrient availability. The type species was originally described from soil in Antarctica [26]

Rhodotorula diobovata **Newell and Hunter** **Fig.10**

Colonies on MEA after 2 days at 25 C 1-3 mm diam. ovoidal, convex elevating with entire margin, mucoid with smooth/glossy appearance. orange-red to dark-red. The yeast cells on MEA at 2 days subglobose, ovoid, ellipsoid or elongate. 3-6 µm, occurred singly or in pairs. On CMA medium at 25 ^{0}C for 5-7 days pseudohyphae absent, Colony on CHROMagar Candida pink colored, DBB= +ve .

Specimen examined: Living culture isolated from sediment soil of Al-Hammar Marshes, Basrah, Iraq 19.11.2017., (Strain AAN13).

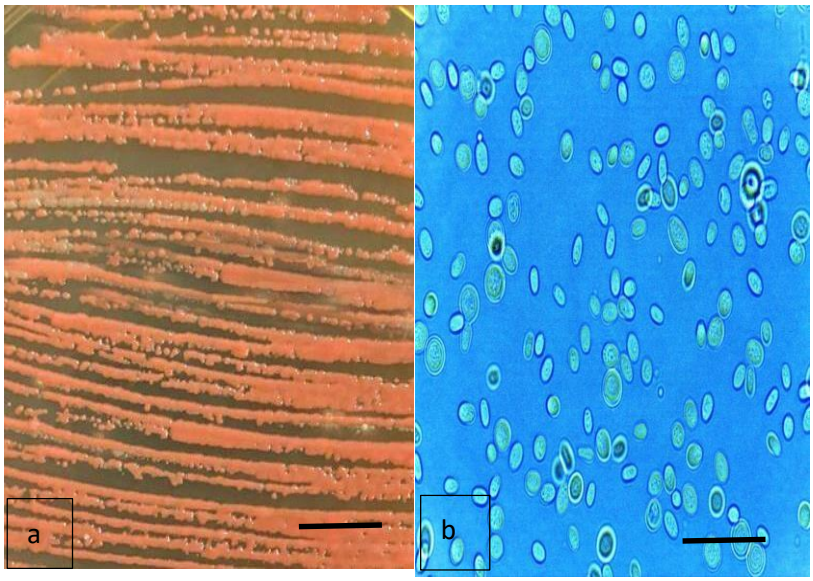


Figure (10) ***Rhodotorula diobovata*** (a) colonies on MEA, 2 days, 25C bar = 2m (b) vegetative cells, bar= 5 µm.

Rhodotorula sp. produce extracellular lipase and protease [27]. Ability to grow rapidly at refrigeration and adapted for cold temperature means *Rhodotorula diobovata* commonly caused spoilage for yoghurt , cream , butter and cheeses [28]. Whereas, the first record was reported in Saudi Arabia, as the isolation and characterization of carotenoid

producing yeasts was in the Qassim region from samples of ice cream, soft cheese and yoghurt [29] The present isolates considered second record in Middle East, Asia.

***Symmetrospora foliicola* Shivas and Rodr.** **Fig.11**

Colonies on MEA after 2 days at 25 C 1-2 mm diam. butyrous, smooth or somewhat shiny, red (incarnate), with entire margin. The yeast cells are ellipsoidal to subglobose, 5-10 µm, singly Budding polar or multilateral, and shows per current or sympodial proliferation On CMA medium at 25 C° for 5-7 days pseudohyphae present and ballistoconidia are ellipsoidal 10 µm ,

Specimen examined: Living culture isolated from sediment soil of Al-Faw , ,Basrah, Iraq 1.5.2018.,(Strain AAN21) Colony on CHROMagar Candida bluish – rose colored,DBB= +ve

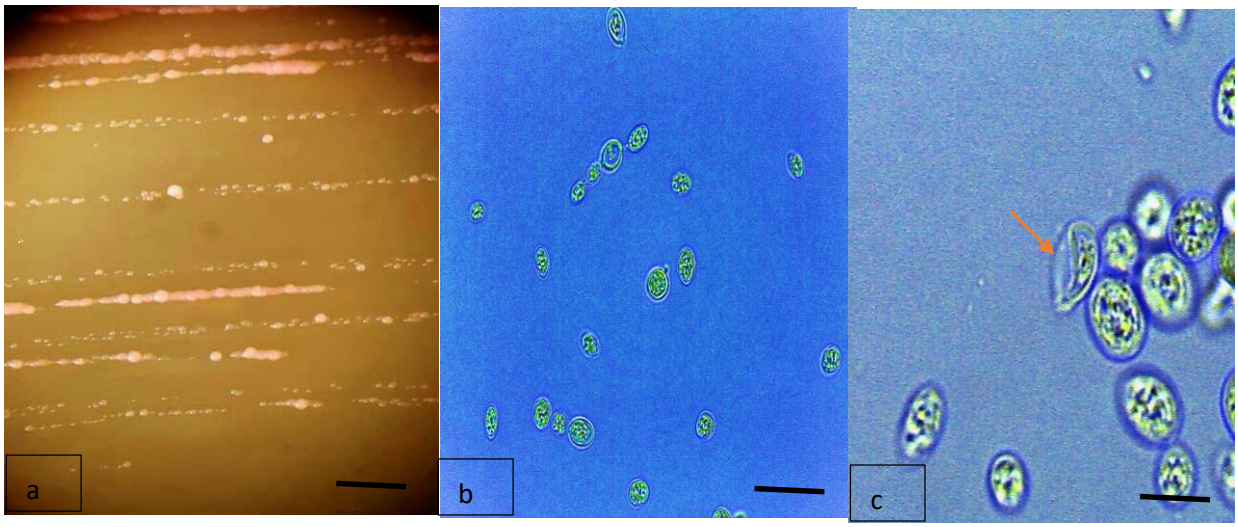


Figure (11) ***Symmetrospora foliicola*** (a) colonies on MEA, 2 days, 25C bar = 2mm (b) vegetative cells,)bar= 10 µm, (c) → Ballistoconidia are ellipsoidal, bar= 10 µm.

This species was originally isolated Sample from leaves of Banksia collina (Proteales, Proteaceae), New South Wales, Australia [30]. Our finding of this strain may represents the first record from soil and second report in the world. It was isolated from a sediment with high rate of salinity and high acidity as well as was easily growing at 37 °C. All these features make this strain as halophilic and acidophilic yeast. It's possible causing

disease for human. In addition to, these features may be important for producing secondary metabolites, which may have many applications in biotechnology.

Vishniacozyma carnescens **Verona and Luchetti** **Fig.12**

Colonies on MEA after 2 days at 25 C° 2-3 mm diam. convex, shiny, pale yellowish-brown to grayishcream, smooth, butyrous to mucoid, and with an entire straight to crenulate margin. The yeast cells broadly ellipsoidal, ovoid, pyriform or subglobose,2-6µm, On CMA medium at 25 C° for 5-7 days pseudohyphae absent. Colony on CHROMagar Candida rose colored, DBB= +ve

Specimen examined: Living culture isolated from sediment soil of Al-Qurna,Basrah, Iraq 25.6.2018.,(Strain AAN27) .

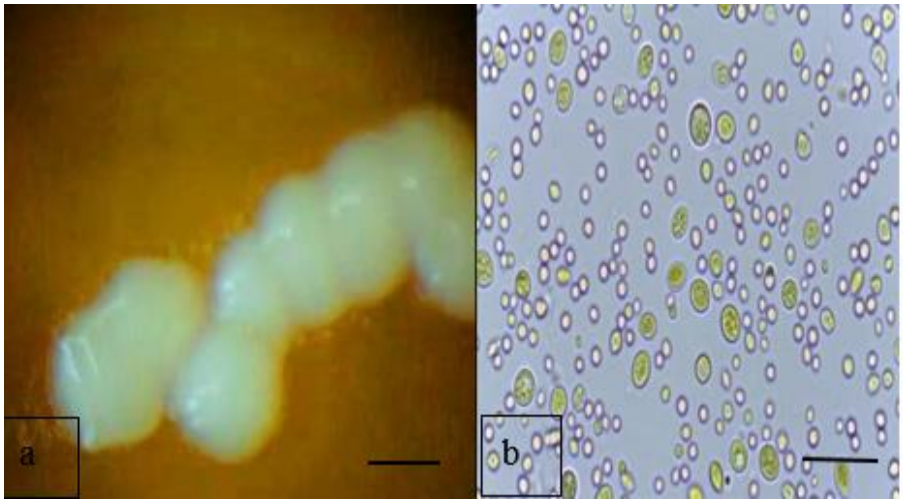


Figure (12) ***Vishniacozyma carnescens*** (a) colonies on MEA, 2 days, 25C bar = 2mm (b) vegetative cells, bar= 5 µm.

This specimen was isolated from muscatel grapes , seawater in Portugal[31] soil in an alluvial forest in Austria[32] and glacial ice in Spitsbergen [17]. *Cryptococcus carnescens* synonyme of (*V. carnescens*) is physiologically similar to *C. laurentii* but also to other *Cryptococcus* species in the victoriae clade, from which it cannot be distinguished between them in morphology appearance. Differ from *C. Laurentii* by inability to assimilate ethanol , creatinine, and to grow at 30 C by *C. carnescens* .[7] Two genera of yeasts were encountered in high frequency on dichloran rose bengal Agar in the air of both citrus and grapevine plantations and these were *Cryptoccocus* (4

species) *and Rhodotorula* (3 species). *C. carnescence, C. flavescens* and *C. laurentii* were isolated from grapevine air only [33].

Cryptococcus carnescens was isolated from flowers of six different host plants during two years and from nectar [34].

The present species was isolated from a region with high salinity to be cultured in different media, such as yeast extract peptone glucose agar with 5% NaCl and 50% glucose, as a result it might be halotolerance and osmotolerance respectively

Molecular identification

ITS1-ITS2 5.8S rDNA gene is gold standard to identify yeast isolates .Its quick reliableness technique in a very comparison with biochemistry ways , additional more providing a formation regarding the evolutionary relationships [35] figures13,14 .

Total genomic DNA of yeasts

Figure (13) shows DNA bands of 9 isolates observed under UV transilluminator identification of yeast isolates.

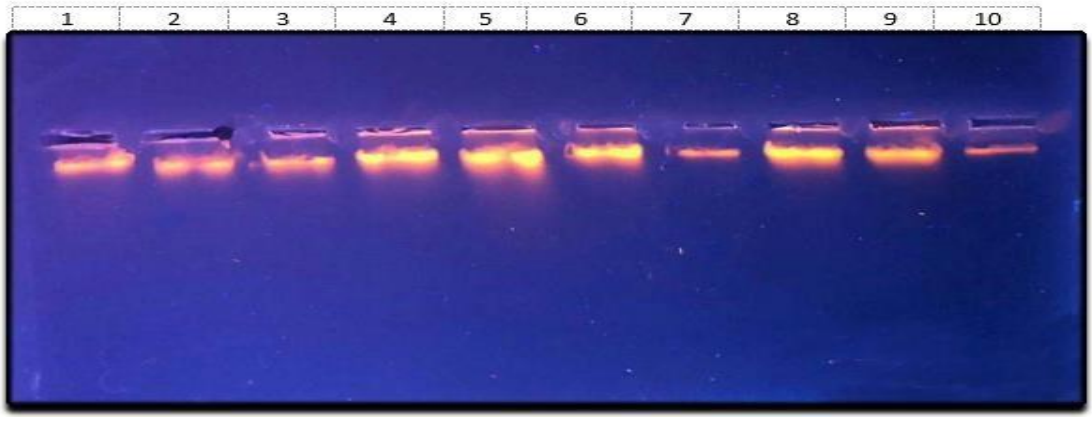


Figure13: 0.8% of agarose gel electrophoresis shows genomic DNA bands of yeast isolates under UV transilluminator.

ITS1-ITS2 5.8S rDNA gene

ITS1-ITS2 5.8S rDNA gene of 131 yeast isolates were shown on agarose gel electrophoresis under UV transilluminator at the position 300-700 bp by comparing with standard DNA ladder (Figure 14)

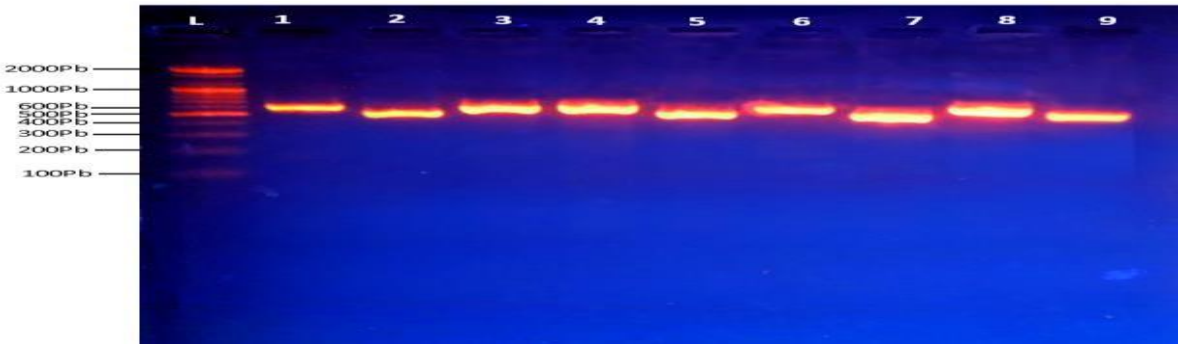


Figure (14): Agarose gel electrophoresis 2 % of PCR products for internal transcribed spacer ITS1 – ITS2 regions (including 5.8S rDNA gene) : Lane L: (100 bp)DNA ladder, Lane 1: *Naganishia vishniacil* (600 bp) , Lane 2: *Cystobasidium benthicum* (500 bp) , Lane 3: *Symmetrospora folicola* (600 bp), Lane 4: *Naganishia liquefaciens*(600 bp), Lane 5: *Nagishiana uzbekistanensis* (500 bp), Lane 6: *Filobasidium oeirense* (600 bp) , Lane 7: *Nagishiana carnescens* (500 bp),Lane 8: *Naganishia adeliensis*(600 bp) and Lane 9 : *Cystobasidium minutum* (500 bp) for basidiomycetes yeasts isolates

Discovery of new global strains

Table 1 shows the nine isolates that have been recorded officially into the Japanese Genbank with different accession numbers. In addition to, additional 4 isolates have been deposited in the Genbank but we still waiting for their accession numbers.

Table (1) : Molecular characterization type strains No., Accession No. and homology percentages

ITS-5.8S rDNA –ITS2				
No.	Type strains No.	Type strains	Accession No.	Homology (%)
1	*AAN5*	*Cystobasidium benthicum* strain	LC473093.1	99%
2	*AAN8*	*Cystobasidium benthicum* strain	LC473096.1	99%
3	*AAN10*	*Cystobasidium minutum* strain	LC473126.1	98%
4	AAN 4	*Naganishia albidosimilis* strain	LC473092.1	99%

5	AAN19	*Naganishia albidosimilis* formerly name*: N. vishniacii*\ strain	LC474380.1	99%
6	*AAN3*	*Naganishia uzbekistanensis*	LC473091.1	99%
7	*AAN7*	*Naganishia adeliensis* strain	LC473095.1	99%
8	*AAN25*	*Cutneotrichosporon dermatis*	In process	100%
9	*AAN26*	*Filobasidium oeirense*	In process	100%
10	*AAN23*	*Naganishia liquefaciens*	In process	100%
11	*AAN13*	*Rhodotorula diobovata*	LC473129.1	100%
12	*AAN21*	*Symmetrospora foliicola*	LC474382.1	100%
13	*AAN27*	*Vishniacozyma carnescens*	In process	100%

Many strains discovered during current study Figure (15,16,17,18,19)

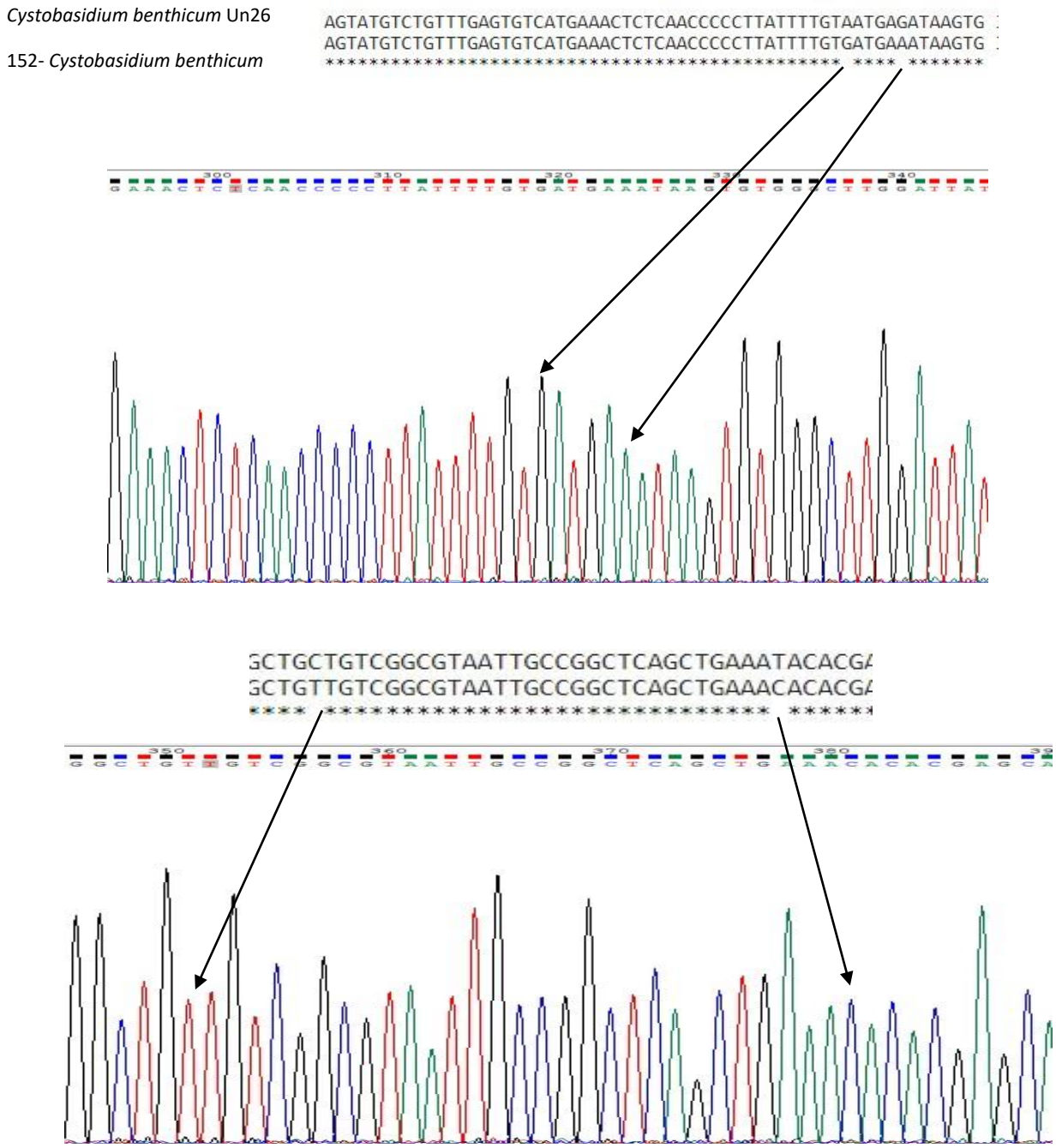


Figure (15) : Comparison of 5.8S rDNA gene internal transcribed spacer ITS1-ITS2 nucleotide sequence for isolate No. 152 *Cystobasidium benthicum* AAN5 LC473093.1 from present study with their type strain Un26, Transition mutation (G instead A) at the position 319 bp , Transition mutation (A instead G) at the position 324bp, Transition mutation (T instead C) at the position 351 bp and Transition mutation(C instead T) at the position 381 bp

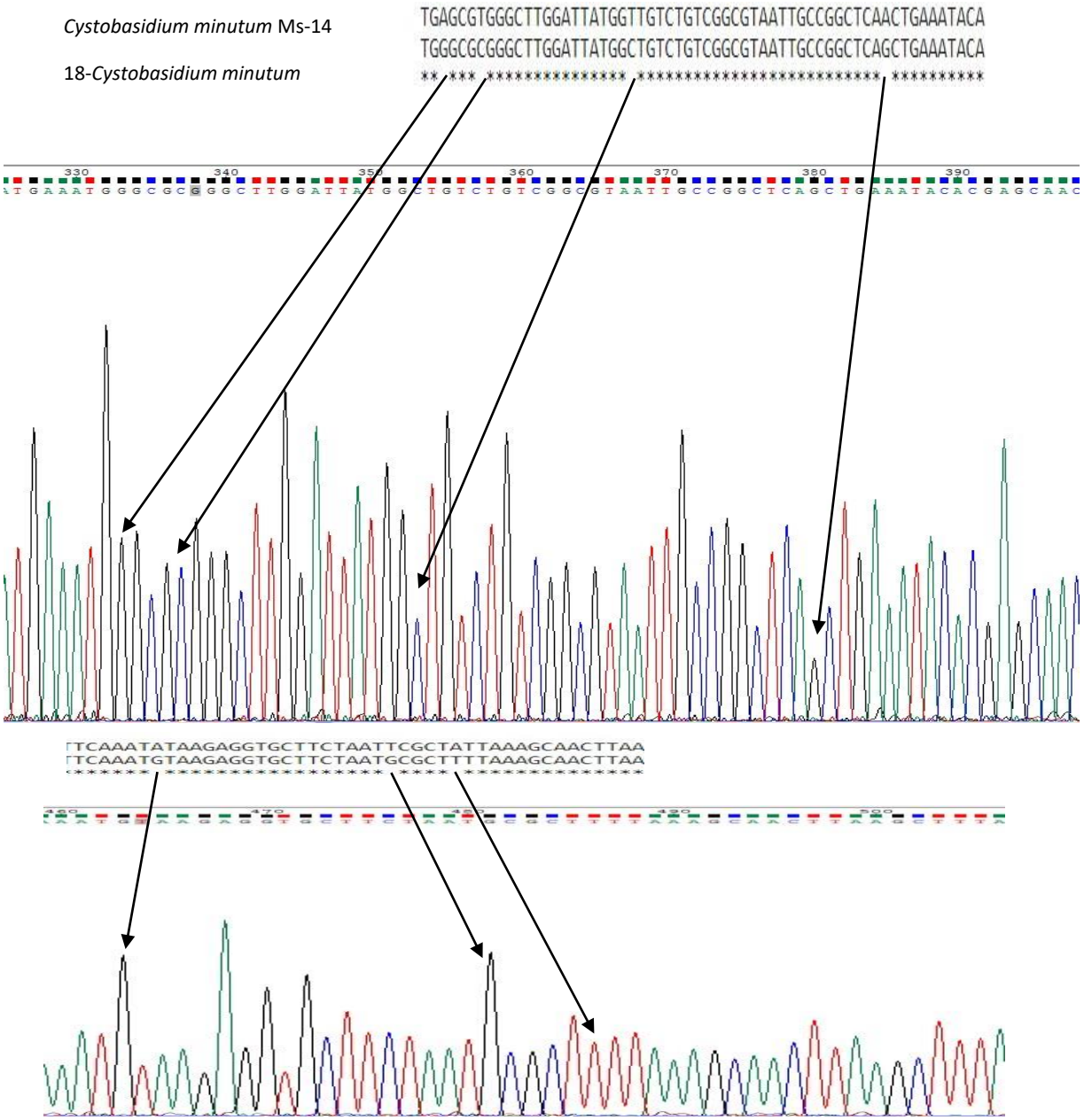


Figure (16): Comparison of 5.8S rDNA gene internal transcribed spacer ITS1-ITS2 nucleotide sequence for isolate No. 18 *Cystobasidium minutum* AAN10 LC473126.1 from present study with their type strain Ms-14, Transition mutation(G instead A) at the position 333 bp, Transition mutation(C instead T) at the position 336 bp, Transition mutation(C insteadT) at the position 353 bp Transition mutation(G instead A) at the position 380 bp, Transition mutation(G instead A) at the position 463 bp, Transversion mutation(G instead T) at the position 481 bp and Transversion mutation(T instead A) at the position 486 bp

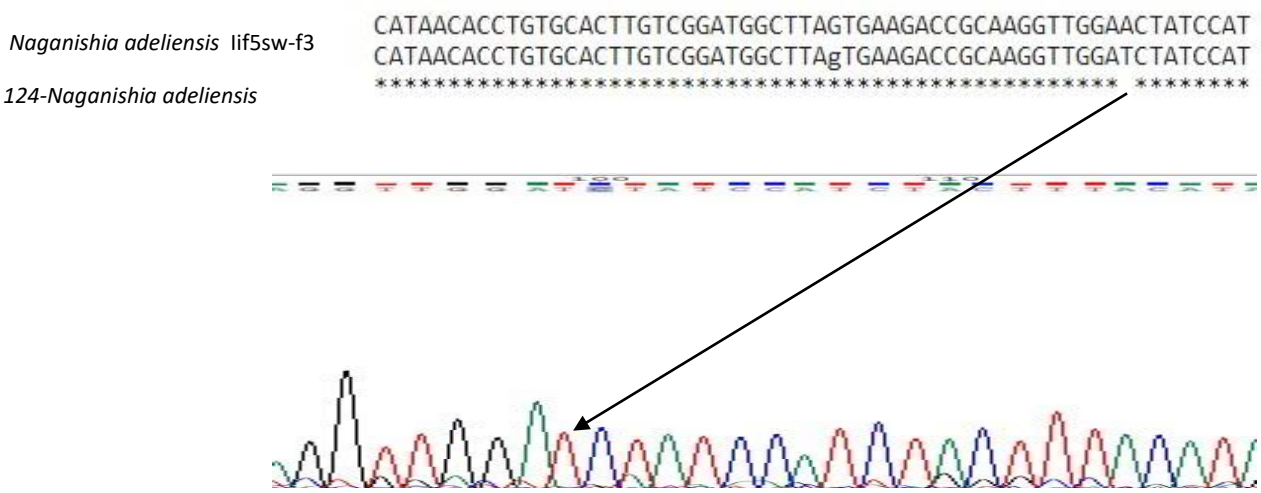


Figure (17): Comparison of 5.8S rDNA gene internal transcribed spacer ITS1-ITS2 nucleotide sequence for isolate No. 124 *Naganishia adeliensis* AAN7 LC473095.1 from present study with their type strain Iif5sw-f3, transversion mutation (T instead A) at the position 99 bp

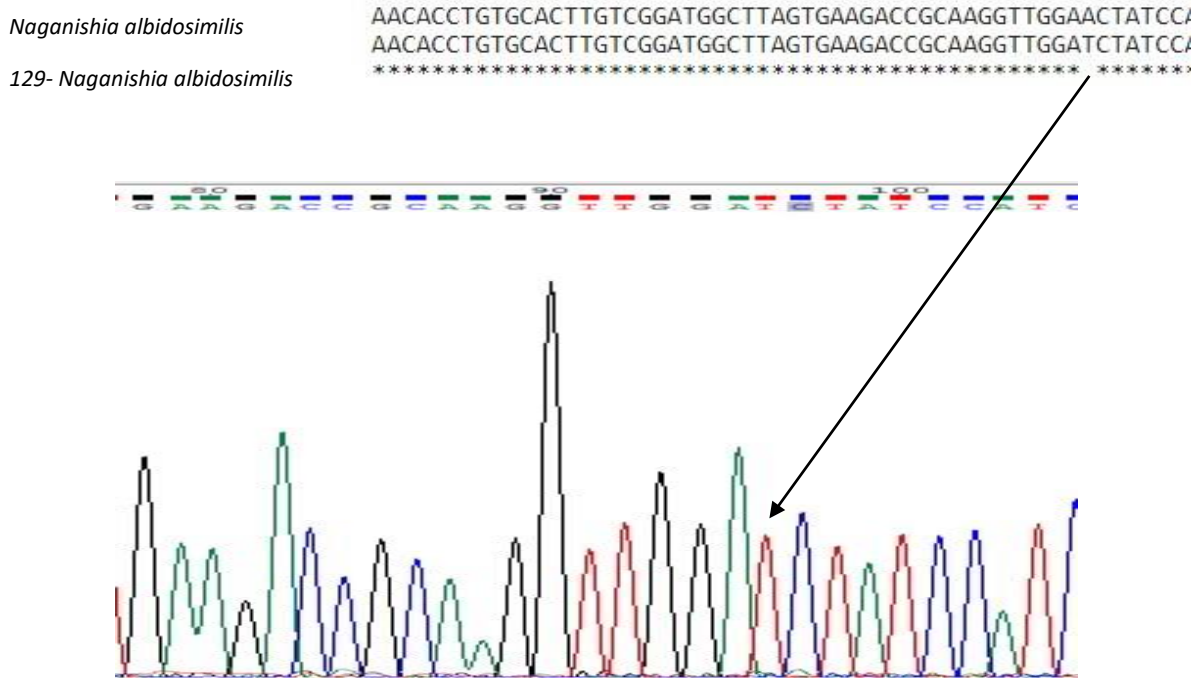
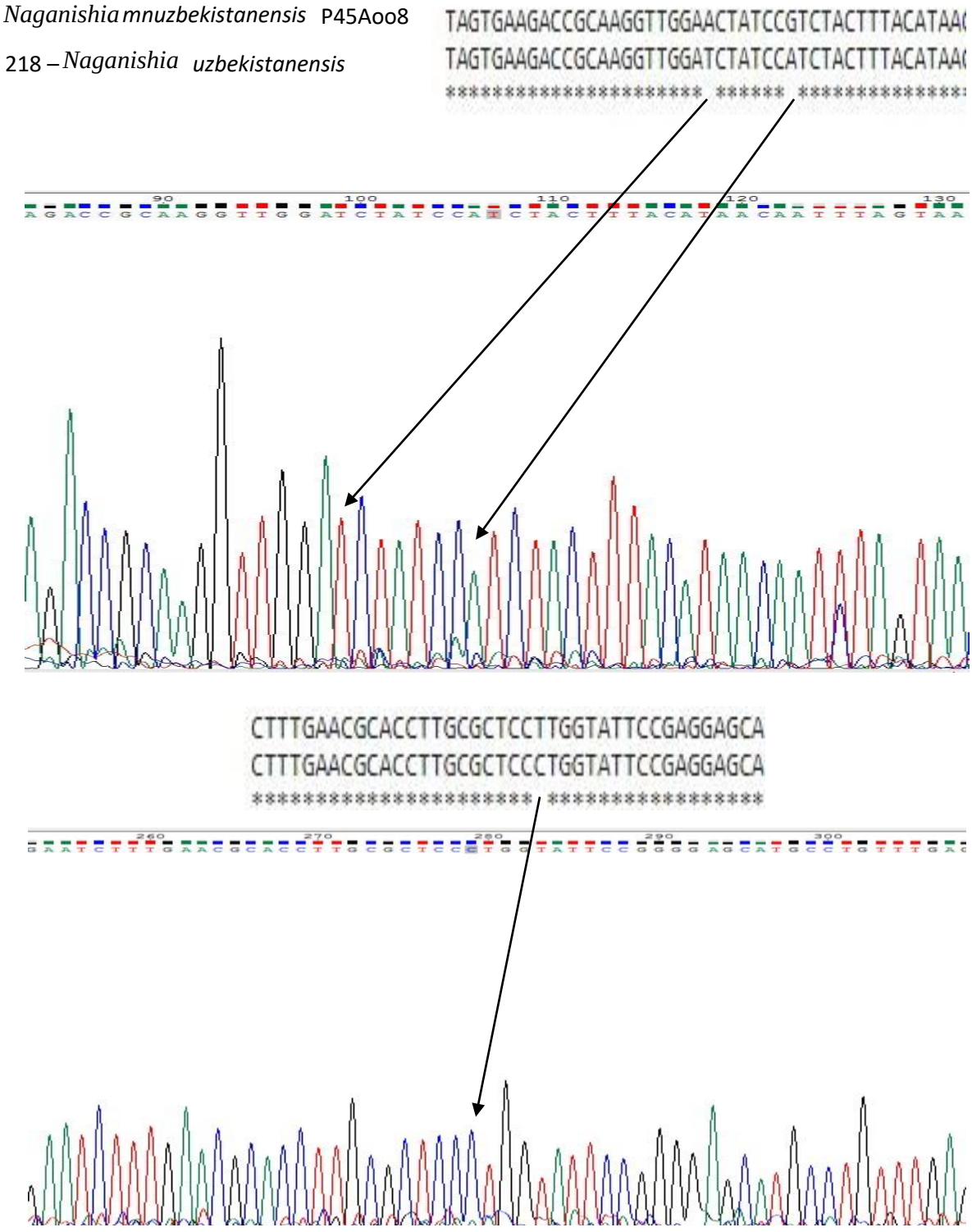


Figure (18): Comparison of 5.8S rDNA gene internal transcribed spacer ITS1-ITS2 nucleotide sequence for isolate No. 218 *Naganishia albidosimilis* AAN19 LC474380.1 from present study with their type strain, transversion mutation (T instead A) at the position 96 bp


Naganishia mnuzbekistanensis P45Aoo8
218 – Naganishia uzbekistanensis
TAGTGAAGACCGCAAGGTTGGAACTATCCGTCTACTTTACATAA
TAGTGAAGACCGCAAGGTTGGATCTATCCATCTACTTTACATAA
CTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCA
CTTTGAACGCACCTTGCGCTCCCTGGTATTCCGAGGAGCA

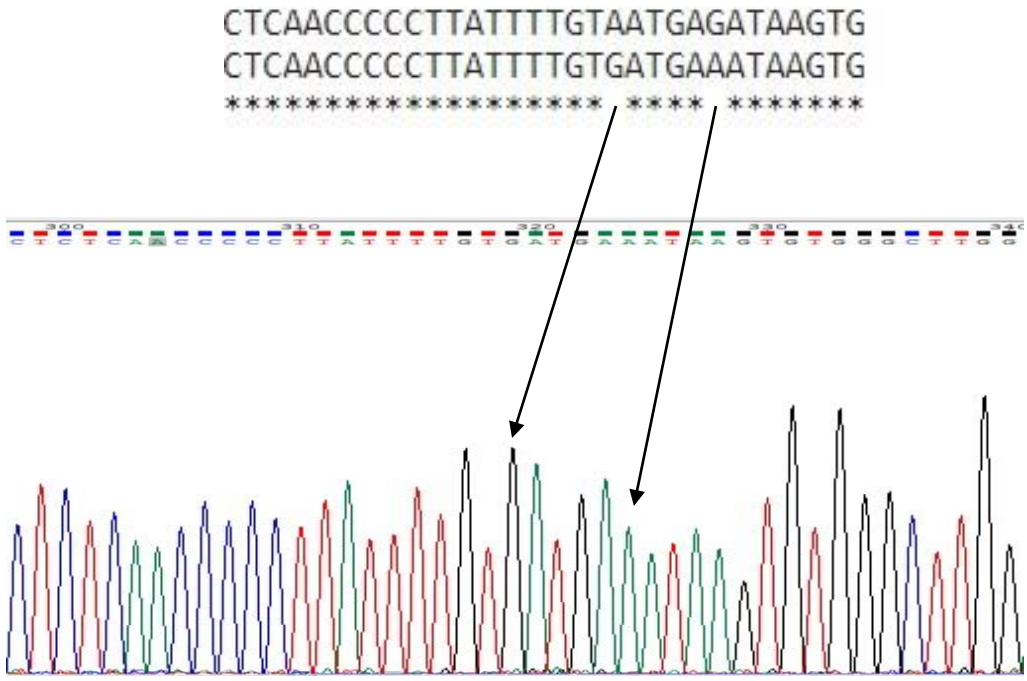


Figure (19 : Comparison of 5.8S rDNA gene internal transcribed spacer ITS1-ITS2 nucleotide sequence for isolate No. 218 *Nagishiana uzbekistanensis* AAN3 LC473091.1from present study with their type strain P45A008, transversion mutation (T instead A) at the position 99 bp , Transition mutation (A instead G) at position 106bp, Transition mutation (C instead T) at the position 279bp, Transition mutation(G instead A)at the position 319bp and Transition mutation (A instead G) at the position 324 bp.

Phylogenetic analysis

The results shown phylogenetic tree of basidiomycetes strains which it appeared 12 branches for 12 species the results shown closely related among *Naganisha* species. during this tree using *Lodderimyces elongispora* as out group belong to ascomycetous yeast Fig.20

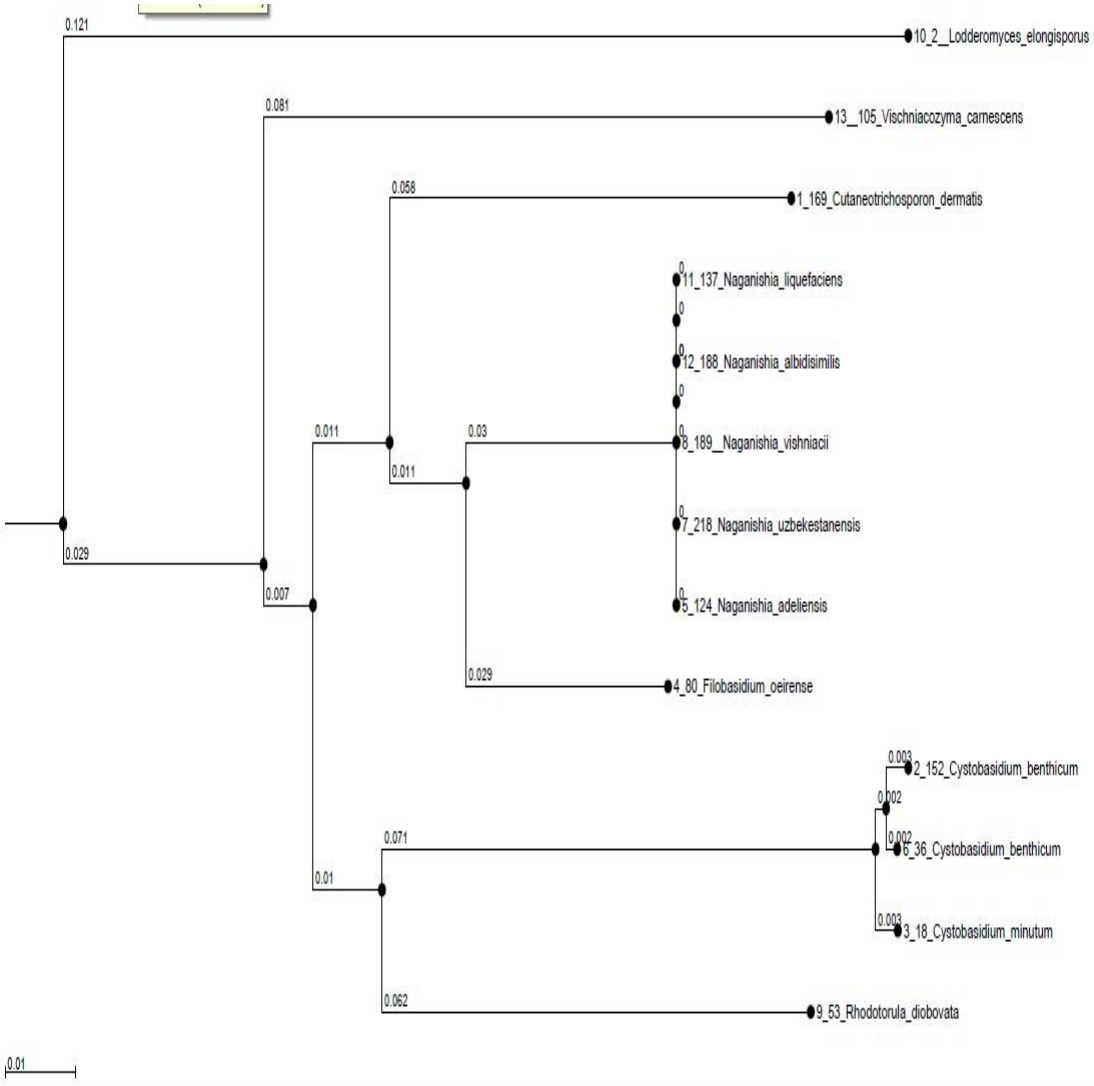


Figure (20) Rooted Neighbor Joining Phylogenetic tree constructed from alignment of ITS1-ITS2 rDNA gene sequences produced from a MAFFT alignment. This N-J tree showing the distribution and Phylogenetic relationships of 12 species isolated from soil. All horizontal branch lengths were drawn to scale. Bootstrap values after 1000 repetitions are indicated.

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تسجيل جديد لخمائر بازيدية مهمة من تربة محافظة البصرة / العراق

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الخلاصة

تم خلال الدراسة الحالية جمع ٧٥ عينة تربة من مناطق مختلفة من محافظة البصرة جنوبي العراق للتحري عن وجود الخمائر البازيدية باستعمال طريقة التخفيف. شخصت العزلات النامية على الاوساط الزرعية بالاعتماد على الصفات المظهرية والبايوكيمياوية، وتم تأكيد التشخيص بالبيانات الجزيئية. سجلت في هذه الدراسة ١٢ نوعا تعود لستة اجناس من الخمائر وكان اكثرالاجناس ترددا بالأنواع هو الجنس Naganishia بخمسة أنواع.

جميع الانواع المشخصة تسجل لأول مرة في العراق. كذلك سجلت سلالات جديدة في بنك الجينات الياباني.

الكلمات المفتاحية: الخمائر البازيدية، التربة البصرة.