# Phenotypic and genotypic identification of yeasts isolated from some dairy products

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## ABSTRACT

Yeast classification is traditionally based on their physiological and biochemical profiles. Recently, molecular methods have been successfully applied to yeast strain typing and identification. The aim of this paper was to characterize four yeast strains isolated from dairy products by classical and molecular methods. The preliminary examination involved macroscopic appearances of colonies on solid media and microscopic feature of the cells. Physiological characterization was mainly performed by assessing the ability to use certain sugars semi-anaerobically, the ability to use organic compounds as sole carbon source for aerobic growth, urea hydrolysis, growth at high concentrations of glucose and the ability to growth at non-optimal temperatures (37°C and 42°C). From results obtained it could be stated that, although there were some variations in biochemical patterns all tested yeasts were classified either as *S. cerevisiae* or and *G. candidum* with 95 – 99 % of probability. For molecular identification only three specific primer pairs and one universal primer were used. The identification was carried out based on ITS 1 partial sequence, 5.8 S rRNA gene and ITS 2 complete sequence and large subunit rRNA gene sequence analysis. The results obtained showed that the product of *S. cerevisiae* scored 600 bp in lan (1and 2) using 5.8 S rRNA gene with primer ITS and NL2, while *G. candidum* scored 600 bp in lan (3and 4) using 18S rDNA gene with primer (18S ITS1 – 28S ITS 2). However, the sequence analysis of the four identified yeast strains was submitted to Genebank in the NCBI database. They have been accepted to be deposited and released in Genebank under four new accession numbers, actually KY441458, MF380234 for *S. cerevisiae* and MF383376, MF383368 for *G. candidum* strains. In conclusion, Strains were locally isolated from Egyptian resources to increase the additive value of the Egyptian microbial wealth.

**Keywords:** Dairy products; *G. candidum; S. cerevisiae.*

## INTRODUCTION

Nowadays the impact of yeasts in foods is beyond original and popular notions of bread, beer and wine fermentations by *Saccharomyces cerevisiae*. There is an increasing interest in using yeasts as new sources for improvement of food properties such as: flavor, vitamins content and as agents for the control of food spoilage by their anti-fungal activity (Querol and Fleet, 2006).

In addition, the use of yeasts as potential probiotics, have also been reviewed (Psomas *et al*., 2001 and Kumura *et al*.2004). In this concern it is believed that dairy products are ideal for delivering the probiotics, therefore probiotic yeasts have been increasingly incorporated into dairy products as dietary adjuncts.

Yeasts are traditionally characterized and identified by morphological and physiological criteria (Kurtzman and Fell, 1998). However, these conventional criteria are often unable to discriminate at a subspecies level and provide doubtful identification (Psoma *et al*., 2001 and Van der AaKuhle *et al*., 2001).

Recently, molecular methods have become available, it extends from determining DNA composition to sequencing of parts and even the whole genome of yeast (Kurtzman, 2006). However, molecular techniques have been increasingly and successfully applied to yeast strains typing and identification (Iosepa *et al*., 2000; Pataro *et al*., 2000 and Ouwehand *et al*., 2002). Therefore, the target of the present work was to characterize the tested yeast strains by classical (morphology, biochemical features) and molecular methods.

## MATERIALS AND METHODS

### Materials

Yeast strains: Yeast strains named Y30, Y42, Y67 and Y72 were previously isolated from dairy products such as: cream, raw milk and milk ripe.

As reference strains we used Saccharomyces cervisiae ATCC MYA-795 and Geotrichum candidum ATCC ADE-115, were obtained from

Botany Dept., Fac. of Science, Al-Azhar Univ. Assiut.

**Media**

Glucose peptone yeast extract Agar (GPY Agar): This medium is composed of: glucose 40g; peptone 5g, yeast extract 5g, and 20g of agar: pH 5 – 6.

Carbohydrates: Glucose, sucrose, lactose, maltose, galactose, D-Xylose were provided from SIGMA, USA, while, glycerol, manitol, methanol, citrate, and starch were delivered from Difco Laboratories, Detroit, Michigan, USA.

**Nitrogen compounds**

L-lysine, ethylamine, tryptophan, nitrite and nitrate were obtained from SIGMA, USA.

**Methods**

**Morphological characteristics of tested yeast cultures**

Fresh yeast cultures were cultivated on YPGA medium in petri dishes, and the surface of the colonies were observed. The yeasts were also inoculated in liquid YPG medium for determination of their characteristics The microscopic appearance of the cells was examined after growth in the YPG medium for 2-3 days at 25°C (Guilliermond, 2003).

**Fermentati Tested strains on of carbohydrates:**

The ability of yeast to ferment different sugars were tested using 2% (w/v) sugar solution were determined by using Durham tubes in fermentation basal medium as described by Suh *et al*. (2007), bromothymol blue was added, inoculated with 0.1 mL of cell suspension and incubated at 25-28 ºC for 28 days.

**Assimilation of carbon and nitrogen sources:**

The ability of yeast cultures to grow aerobically on carbon or nitrogen as the sole source of energy where cared out by replica plate method as described by (Kurtzman *et al* 2011). Yeast nitrogen base (YNB) and Yeast carbon base (YCB) as described by Lodder and Kreger (1952) were used for testing the assimilation of either carbon or nitrogen source by yeasts. The plates which containing different carbon or nitrogen source in carbon or nitrogen basal agar medium was inoculated by the test yeast cultures.

Inspection of the colonies growth, and compared with control plates (without carbon or nitrogen sources) after incubation period of 2 – 6 days at 28°C was adopted.

**Complementary tests**

Growth at non-optimal temperatures (37°C and 42°C): Yeast cultures were checked for their growth ability at 37°C and 42°C on GPY agar medium after 4 days, of incubation.

Growth on high osmotic pressure media :Slops were prepared of 1 % yeast extract and 2 % agar some tubes containing 50 % or 60% (w/v) glucose or 10 % NaCl plus 5 % glucose. The slops were inoculated lightly, incubated at 25°C and examined for up to 4 weeks.

Tolerance of 1% acetic acid: A lapful of the cell suspension streaked onto agar plate contain 1% acetic acid, the plates were incubated at 25°C, and examined after 3 and 6 days for the development of colonies.

Hydrolysis of urea: Yeast culture were inoculated onto a slant of Christensen's urea agar (Christensen, 1946), compared with control tube of the basal medium without urea. The cultures inspected daily for up to 4 days and the results recorded positive when a deep pink color develops in the tube of test medium but not.

**Buffers and solutions used DNA extraction:**

Tris-Borate-EDTA 5X buffer (TBE), pH 8: This buffer contains 0.29 g of Na-EDTA, 5.4 g of Tris-HCl, 2.75 g of boric acid and 100 mL distilled water (pH 8).

**Ethidium Bromide Stock Solution**

This solution is composed of 1g Ethidium bromide dissolved in 100 mL.

**The Loading Dye (5x)**

The loading dye consists of Na-EDTA, pH8.0 (500mM), 2mL glycerol (100%), 5mL bromophenol blue (2%), 0.75 mL xylene cyanol (2%) 0.75 mL distilled water.

**Gel Preparation (1% agarose gel)**

Agarose gel (1%) is prepared by adding 1g of agarose, to 100mL Tris-Borate-EDTA (TBE). The solution was boiled to dissolve the agarose in a microwave oven for 1 – 3 min, and cooled down to 45°C. then 3 µL of ethidium bromide (1%) was added and let for solidification at room temperature.

**Genomic DNA Extraction**

Total genomic DNA was extracted by using Zymo Research Fungal/Bacterial DNA MiniPrep™ Kit (Catalog No. D6005) and Thermo Fisher Scientific

Gene JET Genomic DNA Purification Kit (K0721) were purchased from Sigma Company, Egypt.

**Primers used for yeast identification**

Universal primer used: Primer 18SF (5'-GCATATCAATAAGCGGAGGAAAAG) and28 SR (5'-GGTCCGTGTTTC AAGACGG).

**Specific primer used**

A-The ITS region was amplified using primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and NL2 (5'-CTC TCT TTT CAA AGT GCT TTT CAT CT-3') according to Baleiras Couto *et al*. (1996).

B- 5.8SrRNAregion was amplified using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') according to McCullough *et al*. (1998).

C- Primers NL1 (5'-GCATATCAATAAGCGGAGGAAA AG) and NL4 (5'-GGTCCGTGTTCAAGACGG) according to Boekhout *et al*. (1994).

**Molecular identification**

**DNA Extraction from Yeast Cells**

A- DNA was extracted according to manufacture instructions using ZR fungal/Bacterial DNA MiniPrep™ Kit (Catalog No. D6005-ZR crop, India).

B-Yeast genomic DNA purification protocol was carried out according to manufacture instructions using (Thermo Genomic Purification Kit "K0721, USA").

**PCR amplification of 26S rRNA gene and 5.8S ITS region**

Amplification reactions were prepared in total volumes of 25 µL containing 12.5 µL Go Taq Master Mix, a pair of specific primers at the concentration of 0.25µmolof each primer,100ng of Template DNA and nuclease free water up to 25µL. PCR apparatus (Medison, USA). The PCR temperature profile was applied as follows: denaturation cycle of 94°C for 1 min., followed by 35 cycles of 94°C for 30s. Annealing temperature was tested at 50°C for 2 min and extension at 74°C for 1.5 min. and a final elongation cycle to 72°C for 4 min.

**Amplified fragments visualization**

Gel electrophoresis of 1.5 %( w/v) agarose was used for migrating the amplified DNA fragments. Gels were stained with 0.5 µg/mL ethidium bromide. For elimination, 3 Kb DNA ladder markers from Thermo Scientific Gene was also loaded on the gel for fragment size comparison. DNA bands were visualized under UV light and quantified using spectrophotometer (Genway 630).

**Purification of PCR products**

The electrophoresed PCR products were purified using Gene JETTMPCR Purification Kit (Thermo, K0701, Germany) gel extraction kit following the manufacture instructions.

**Sequence analysis**

PCR product sequencing was achieved by GATC Biotech using ABI 3730xl DNA Sequencer (Konstanz, Germany). Applying forward and reverse primers as will be described by combining the traditional Sanger technology. Sequence similarity search was performed using the NCBI BLAST online tool (http://ncbi.nlm.nih.gov/BLAST/) against the nucleotide collection (nr/nt) database.

**Sequences submissions and accessions number**

Sequences of this study have been submitted to NCBI using BankIt tool (http://www.ncbi.nlm.nih.gov/BankIt/)with the published data in the NCBI database accession number.

**Phylogenetic tree construction:**

Phylogenetic tree was constructed based on the 18SrRNAintergenicspacers(ITS)sequence comparisons length polymorphism of the PCR-amplified and sequences from database using BLAST tree constructed in www.clcbio.com using CIC workbench 7.5 system based on Neighbor Joining method.

## RESULTS AND DISCUSSION

Four yeast strain named Y30, Y42, Y67 and Y72 besides two references strains were traditionally characterized and identified by morphological and physiological criteria according to the keys of identification of Barnett *et al*. (1990), then they were confirmed and renamed according to Kurtzman and Fell (1998) and Suh *et al*. (2007).

Their cell morphology and culture characteristics are presented in Table 1. All tested strains possessed oval cells, also two strains (Y67 and Y72) showed cylindrical shape. However, from the data obtained it was shown that strains Y30 and Y42 were vegetative reproduction by multilateral budding beside that Y67 and Y72 strains develop pseudohypha formed by budding and elongation.

Also, from information gathered in Table (1), it is clearly indicated that all tested strains showed white colonies. In addition, strains Y30 and Y42 showed colony with smooth surface, while the rest cultures formed powdery colonies.

However, the morphological and microscopical examination of the tested strains revealed that some similarities with yeast species already characterized in the literature and with the reference strains as *S. cerevisiae* and *G. candidum* were observed.

Further physiological analyses were performed for a preliminary identification of the tested strains.

The fermentation tests (Table 2) showed that Y30 and Y42 strains could catabolize glucose, galactose, sucrose and maltose by fermentation while Y67 and Y 72 strains could use no sugar. Moreover, all tested cultures failed to ferment D-xaylose. However, the obvious disparity between the tested cultures could be explained by the fact that various yeast strains showed great variability in their ability to grow on different carbon sources (Barnett *et al*., 1983).

Regarding the assimilation ability, obvious differences between tested strains were detected (Table 3). It could be gathered from results obtained that all tested cultures assimilated glucose and galactose, our finagling are in agreement with those reported by Hayford and Jespersen (1999). In contrast the tested strains were unable to assimilate lactose, starch, methanol, manitol and citrate. While, only Y30 and Y42 strains could consume either sucrose or maltose.

Moreover, in order to obtain a complete physiological characterization for the tested cultures, assimilation of nitrogen compounds was carried out, where 5 nitrogen compounds were used.

As shown from Table 4, it could be noticed that all tested cultures failed to assimilate either nitrate or nitrite as a sole source of nitrogen. On the other hand, Y67 and Y72 strains were able to assimilate ethylamine and showed different response to L-lysine and tryptophan. In contrast, Y30 and Y42 strains were unable to assimilate ethylamine. This finding is consistent with previous results of Rajkowska and Kunicka-Styezynska (2010).

Continuously, serial of complementary tests were carried out in order to complete the physiological features of the tested cultures. Results obtained are tabulated in Table 4. Viewing of these results, it might be observed that growth at non-optimal temperatures (37°C and 42°C) declared that only Y30 strain was able to grow at 37°C, while the rest tested cultures were failed to grow at 42°C. This finding means that the tested culture was not thermotolerant, this statement is in contrary with that reported by Ghindea *et al*. (2009).

In addition, tested strains were examined for resistance to high concentration of glucose. Our results showed that strains Y30 and Y42 grew on medium containing 50 %. In contrary, Y67 and Y72 strains were failed to grow at ether 50 % or 60 % glucose. In this respect, Ghindea *et al*. (2000) reported that all tested strains grow well on YPGA medium containing 50 % and 60 % glucose. Furthermore, it was evident from the results obtained that all tested strains failed to grow in medium containing either 10 % NaCl+5 % glucose or 1 % acetic acid and failed to hydrolysis urea.

From the foregoing results it could be tested that classical taxonomy analysis showed a great similarity between Y30 and Y42 strains and *Saccharomyces cerevisiae* according to Barnett *et al*. (2000), also the present data suggest a possible affiliation between Y67 and Y72 strains and those given for *Geotrichum candidum*. Four an accurate identification of the studied further molecular analysis is necessary to be done. The first step in this approach was the isolation of plasmid DNA.

In this study three specific primer pairs and one universal primer were used. In silico results showed that two primer pairs (ITS1 – NL2) and (ITS1 – ITS4) exhibited sensitivity and specificity primers for *Saccharomyces cerevisiae*, while the third specific primer (NL1 – NL4) and the universal primer (18S ITS1-28S ITS1) showed sensitivity and specificity for *Geotricum candidum* strains.

Results of amplified PCR fragments using 5.8S and 18S to four tested yeast strains are shown in Figure (1). The identification of strains was carried out based on ITS1 partial sequence, 5.8S rRNA gene and ITS1 complete sequence and large subunit rRNA gene sequence analysis.

It is very clear from the results obtained that the product of S. *cerevisiae* scored 600 bp in lan (1and 2) by using 18S and primers (ITS1 – NL2) and (ITS1 – ITS4). These results agreed with those reported by McCullough *et al*. (1998). Also, *G. candidum* scored 600 bp in lan (3 and 4) of PCR using primer (18SITS1-28SITS2) rRNA. Alignments of sequences using BLASTN-NCBI: Alignments sequences of *S. cerevisiae* AA2strain Y30: BLASTN analysis of *S. cerevisia*e Y30 sequences is shown in Figure (2) using (ITS1-NL2) primer. This obtained sequence produced significant alignment with other accessions of NCBI-databases using BLASTN (http://ncbi.nlm.nih. gov/BLAST/) against

nucleotide database indicating high similarity with approximately 100 strains of *S. cerevisiae* as shown in Figure (2); whereas it was scored the highest similarity with accession EU268656.1 (99% identical and 96 % Query cover) and with accessions MG017570.1, MG017580.1 and MG017546.1 (99% similarity and 99% Query cover). Alignments sequence of *S. cerevisiae* AAA3 strain Y42: BLASTN analysis of *S. cerevisiae* Y42 sequence was shown in Figure (3). This obtained sequence characterized with significant alignment with other accessions of NCBI-databases using BLASTN (http://ncbi.nlm.nih.gov/BLAST/) against nucleotide database, indicating high similarity to strain of *S. cerevisiae* as shown in Figure (3), whereas it was scored the highest similarity with accession JQ771726.1, HQ443686.1 and KX237671.1 (100% identical).

Alignments sequence G. candidumGG1 strain Y67. BLAST analysis of G. candidumGG1 Y67 sequence was shown in Figure (4). Against nucleotide database indicating similarity to strain *G. candidum*, highest similarity was scored with accession MF383368.1 (99% identical and 98 % Query cover) as shown in Figure (4), and accession numbers JQ713185.1 and JN974267.1 (99% similarity 100% Query cover). Alignments sequences of *G. candidum* AAA strainY72:

BLAST analysis of *G. candidum*Y72 sequence was shown in Figure (5). The obtained sequence revulted in significant alignment with other accessions of NCBI-databases using BLASTN (http://ncbi.nlm.nih.gov/BLAST/)against nucleotide database indicating similarity to strain of *G. candidum*. Highest similarity with accession MF383376.1 and KF112070.1 (99% identical and query coverage 91%) as shown in Figure (5).

**Database submissions and accession numbers.**

Four yeast isolates were molecularly identified as: *S. cerevisiae* AA2, *S. cerevisiae* AAA3, *G. candidum* GG1 and *G. candidum* AAA, their sequence analysis results were submitted to Genebank in the NCBI database. They have been accepted to be deposited and released in Genebank under four new accession numbers as shown in Table (5), and Figures (6-9).

**Phylogenetic relationship of the Genus Saccharomyces and Geotrichum**

Phylogenetic tree was constructed based on the 18S ribosomal RNA sequence comparisons length polymorphism of the PCR-amplified and sequences from database using BLAST tree construct in https://www.ncbi.nlm.nih.gov/blast/ treeview, based on Fast Minimum Evolution.

## CONCLUSION

The PCR method can allow the highly sensitive detection of specific yeast. Such method possesses a significant impact on the analysis of gut community structure, emphasizing the species-specific primers for *Saccharomyces* spp**.** Sequencing the 18S-ITS region provides a rapid identification and intraspecific phylogenetic studies of strains *Saccharomyces* spp. Strains were locally isolated from Egyptian resources to increase the additive value of the Egyptian microbial wealth.

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**Table 1.** Morphological and microscopically characteristics of tested yeast strains.

| Item | Characteristic of vegetative cells | | | | | | | | | Characteristic of vegetative reproduction | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Growth in solid medium | | | | Growth in liquid medium | | | | | budding | | |
| Tested strains | Description of colonies | | | | Cell shape | | | Hypha | | | | |
| | Color | Surface | Margin | Elevation | Oval | Spherical | Cylinder | True | Pseudo | Monopolar | Biopolar | Maltilaeral |
| *S. cerevisiae** | wh | smoth | entire | Convex | + | - | + | - | - | - | - | + |
| Y30 | wh | smoth | entire | Convex | + | + | - | - | - | + | - | + |
| Y42 | wh | smoth | entire | Convex | + | + | - | - | - | - | - | + |
| *G. candidum** | wh | powdery | filament | flat | + | - | + | - | + | - | - | - |
| Y67 | wh | powdery | filament | flat | + | - | + | - | + | - | - | - |
| Y72 | wh | powdery | filament | flat | + | - | + | - | + | - | - | - |

*: Reference strain; wh: white

**Table 2.** Fermentation profiles of tested yeasts.

| Tested strains | Glucose | Galactose | Lactose | Sucrose | Maltose | D-xylose |
|---|---|---|---|---|---|---|
| *S. cerevisiae** | + | + | - | + | + | - |
| Y30 | s | + | - | + | + | - |
| Y42 | s | + | ± | + | + | - |
| *G. candidum** | - | - | - | - | - | - |
| Y67 | - | - | - | - | - | - |
| Y72 | - | - | - | - | - | - |

*: Reference strain; s: strong positive

**Table 3.** Physiological characterization of tested yeast strains.

| Item | Assimilation of carbon compounds | | | | | | | | | | | | Assimilation of nitrogen | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tested strains | Glucose | Sucrose | Galactose | Lactose | Maltose | Starch | D-Xylose | Methanol | Ethanol | Glycerol | Manitol | Citrate | L-lysine | Ethyl amine | Tryptophan | Nitrate | Nitrite |
| *S. cerevisiae** | + | + | + | - | + | - | W | - | + | - | - | - | - | - | - | - | - |
| Y30 | + | + | + | - | + | - | w/- | - | + | w | - | - | - | - | - | - | - |
| Y42 | + | + | + | - | + | - | w/- | - | w | - | - | - | - | - | - | - | - |
| *G. candidum** | + | +/w | + | - | - | - | + | - | + | + | - | - | w | + | w | - | - |
| Y67 | + | w | + | - | - | - | + | - | + | + | - | - | w/- | + | w | - | - |
| Y72 | + | w | + | - | - | - | + | - | + | + | - | - | w/- | + | w | - | - |

*: Reference strain; w: Weak positivew/-: Weakor negative

**Table 4.** Physiological characterization (complementary tests) of tested yeast strains.

| Tested strains | Growth at 37°C | Growth at 42°C | Starch formation | Glucose 50% | Glucose 60% | NaCl 10% + 5% glucose | Acetic acid 1% | Hydrolysis of urea |
|---|---|---|---|---|---|---|---|---|
| *S. cerevisiae** | + | - | - | + | w | - | - | - |
| Y30 | + | - | - | + | w | - | - | - |
| Y42 | w | - | - | + | w | - | - | - |
| *G. candidum** | +/w | - | - | w | - | - | - | - |
| Y67 | w | - | - | w/- | - | - | - | - |
| Y72 | w | - | - | w/- | - | - | - | - |

*: Reference strain; w: Weak positive w/-: Weakor negative

**Table 5.** Sequence features and accession numbers.

| No. | Source | Released Data | Locus | Length | Strain | Accession No. |
|---|---|---|---|---|---|---|
| 1 | Raw milk | 11. 1. 2017 | 26S rRNA | 589bp | AA2 | KY441458 |
| 2 | Fruit yoghurt | 26. 1. 2017 | 26S rRNA | 608bp | AAA3 | KF380234 |
| 3 | Local cream | 26. 6. 2017 | 26S rRNA | 563bp | GG1 | MF383376 |
| 4 | Milk ripe | 26. 6. 2017 | 26S rRNA | 686bp | AAA | MF383368 |

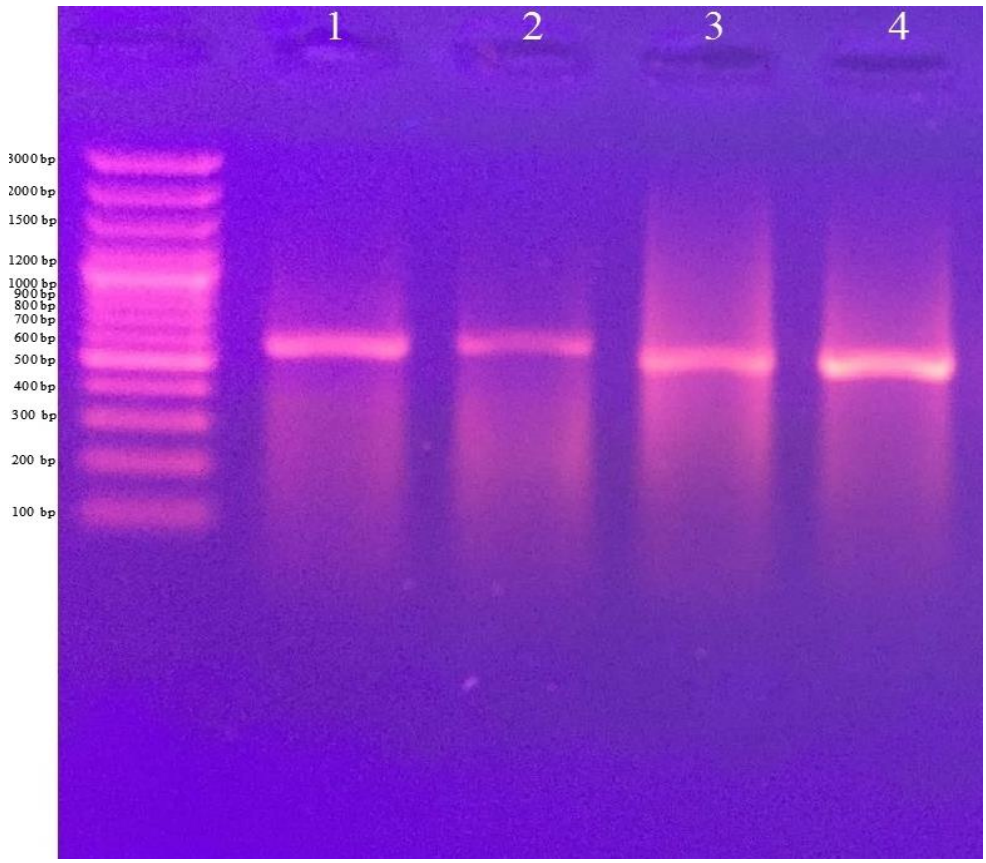


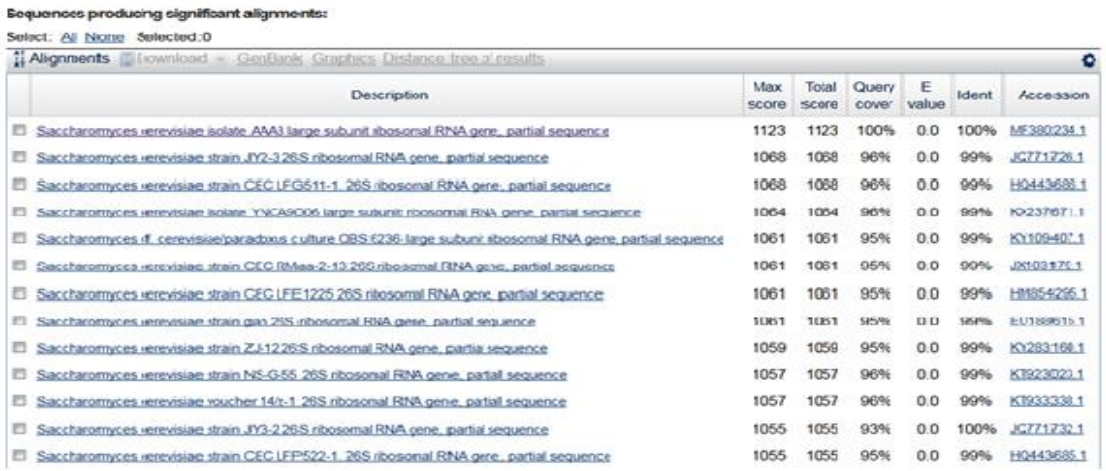

**Fig. 1.** PCR amplified fragments. (1) using 18S for *S. cerevisiae* AA2, (2) *S. cerevisiae* AAA3,(3) using 18S *G. candidum* GG1 and (4) *G. candidum*AAA.

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

| | Description | Max score | Total score | Query cover | E value | Ident | Accession |
|---|---|---|---|---|---|---|---|
| | Saccharomyces cerevisiae isolate AA2 large subunit ribosomal RNA gene, partial sequence | 1088 | 1088 | 100% | 0.0 | 100% | KY441458.1 |
| | Saccharomyces cerevisiae isolate SFMG9 26S ribosomal RNA gene, partial sequence | 1061 | 1061 | 99% | 0.0 | 99% | MG017570.1 |
| | Saccharomyces cerevisiae isolate SFM39 26S ribosomal RNA gene, partial sequence | 1057 | 1057 | 99% | 0.0 | 99% | MG017580.1 |
| | Saccharomyces cerevisiae strain 4 26S ribosomal RNA gene, partial sequence | 1057 | 1057 | 99% | 0.0 | 99% | HM107797.1 |
| | Saccharomyces cerevisiae isolate SFM5 26S ribosomal RNA gene, partial sequence | 1055 | 1055 | 99% | 0.0 | 99% | MG017546.1 |
| | Saccharomyces cerevisiae isolate SFM47 26S ribosomal RNA gene, partial sequence | 1053 | 1053 | 99% | 0.0 | 99% | MG017588.1 |
| | Trichosporon faecale 26S ribosomal RNA gene, partial sequence | 1053 | 1053 | 96% | 0.0 | 100% | KX380576.1 |
| | Saccharomyces cerevisiae strain NL32 isolate 113 large subunit ribosomal RNA gene, partial sequence | 1053 | 1053 | 96% | 0.0 | 100% | KY511895.1 |
| | Saccharomyces sf. cerevisiae/paradoxus culture CBS 2270 large subunit ribosomal RNA gene, partial sequence | 1053 | 1053 | 99% | 0.0 | 99% | KX109419.1 |
| | Saccharomyces cerevisiae strain Yli-33 26S ribosomal RNA gene, partial sequence | 1053 | 1053 | 97% | 0.0 | 99% | KU862640.1 |
| | Saccharomyces cerevisiae strain NL32 26S ribosomal RNA gene, partial sequence | 1053 | 1053 | 97% | 0.0 | 99% | HM191663.1 |
| | Saccharomyces cerevisiae strain r2 26S ribosomal RNA gene, partial sequence | 1053 | 1053 | 96% | 0.0 | 100% | HM107791.1 |
| | Saccharomyces cerevisiae strain r1 26S ribosomal RNA gene, partial sequence | 1053 | 1053 | 98% | 0.0 | 99% | HM107789.1 |
| | Saccharomyces cerevisiae isolate SFM43 26S ribosomal RNA gene, partial sequence | 1051 | 1051 | 99% | 0.0 | 99% | MG017584.1 |
| | Saccharomyces cerevisiae isolate SFM36 26S ribosomal RNA gene, partial sequence | 1051 | 1051 | 99% | 0.0 | 99% | MG017576.1 |

**Fig. 2.** BLASTN similarity regions and percentage with *S. cerevisiae* AA2 sequence**.**



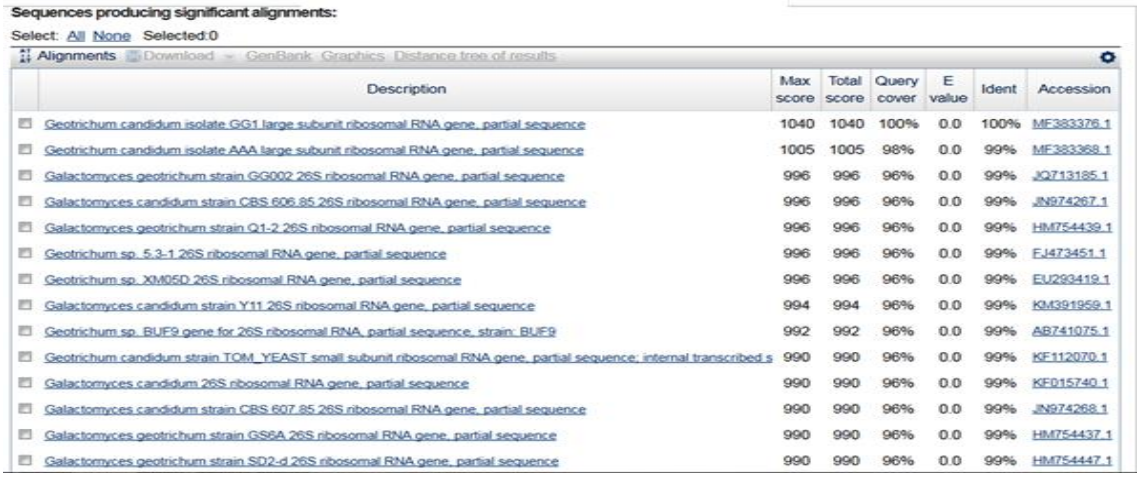
**Sequences producing significant alignments:**

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
|---|---|---|---|---|---|---|
| Saccharomyces cerevisiae isolate AAA3 large subunit ribosomal RNA gene, partial sequence | 1123 | 1123 | 100% | 0.0 | 100% | MF380234.1 |
| Saccharomyces cerevisiae strain JY2-3 26S ribosomal RNA gene, partial sequence | 1068 | 1068 | 96% | 0.0 | 99% | JC771726.1 |
| Saccharomyces cerevisiae strain CEC LFG511-1, 26S ribosomal RNA gene, partial sequence | 1068 | 1068 | 96% | 0.0 | 99% | HQ443688.1 |
| Saccharomyces cerevisiae isolate YKCA6006 large subunit ribosomal RNA gene, partial sequence | 1064 | 1064 | 96% | 0.0 | 99% | KX237671.1 |
| Saccharomyces cerevisiae/paradoxus culture CBS 6236 large subunit ribosomal RNA gene, partial sequence | 1061 | 1061 | 95% | 0.0 | 99% | KY109407.1 |
| Saccharomyces cerevisiae strain CEC RMaa-2-13 26S ribosomal RNA gene, partial sequence | 1061 | 1061 | 95% | 0.0 | 99% | JX103175.1 |
| Saccharomyces cerevisiae strain CEC LFE1225 26S ribosomal RNA gene, partial sequence | 1061 | 1061 | 95% | 0.0 | 99% | HM854256.1 |
| Saccharomyces cerevisiae strain gan 26S ribosomal RNA gene, partial sequence | 1061 | 1061 | 95% | 0.0 | 99% | EU188915.1 |
| Saccharomyces cerevisiae strain ZJ-12 26S ribosomal RNA gene, partial sequence | 1059 | 1059 | 95% | 0.0 | 99% | KY283168.1 |
| Saccharomyces cerevisiae strain NS-G-55 26S ribosomal RNA gene, partial sequence | 1057 | 1057 | 96% | 0.0 | 99% | KT923020.1 |
| Saccharomyces cerevisiae voucher 14/r-1 26S ribosomal RNA gene, partial sequence | 1057 | 1057 | 96% | 0.0 | 99% | KT933338.1 |
| Saccharomyces cerevisiae strain JY3-2 26S ribosomal RNA gene, partial sequence | 1055 | 1055 | 93% | 0.0 | 100% | JC771732.1 |
| Saccharomyces cerevisiae strain CEC LFP522-1, 26S ribosomal RNA gene, partial sequence | 1055 | 1055 | 95% | 0.0 | 99% | HQ443685.1 |

**Fig. 3.** BLASTN similarity regions and percentage with *S. cerevisiae*AAA3 sequence.



**Sequences producing significant alignments:**

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
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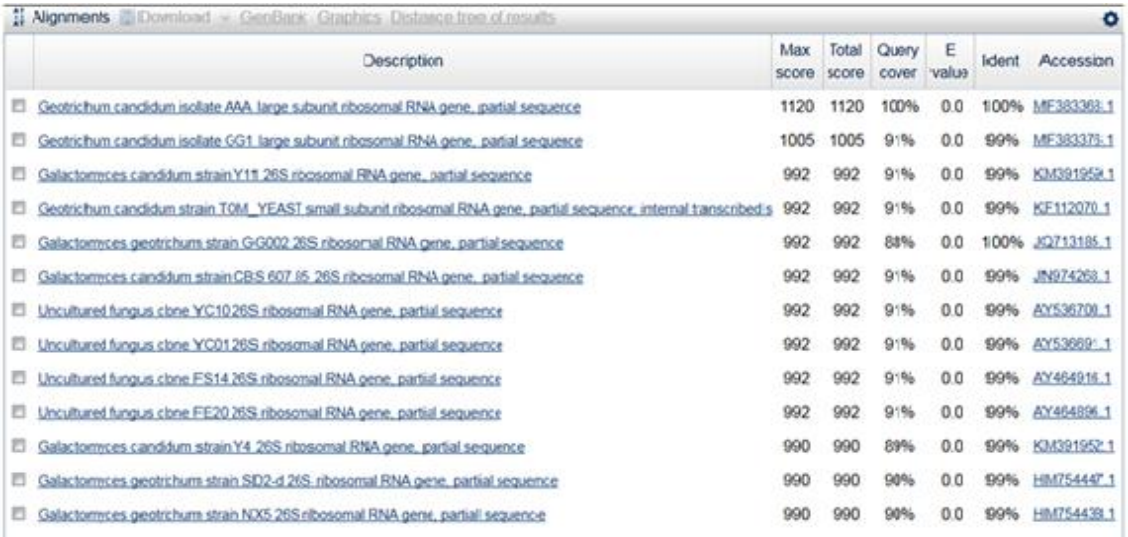
| Geotrichum candidum isolate GG1 large subunit ribosomal RNA gene, partial sequence | 1040 | 1040 | 100% | 0.0 | 100% | MF383376.1 |
| Geotrichum candidum isolate AAA large subunit ribosomal RNA gene, partial sequence | 1005 | 1005 | 98% | 0.0 | 99% | MF383368.1 |
| Galactomyces geotrichum strain GG002 26S ribosomal RNA gene, partial sequence | 996 | 996 | 96% | 0.0 | 99% | JQ713185.1 |
| Galactomyces candidum strain CBS 606.85 26S ribosomal RNA gene, partial sequence | 996 | 996 | 96% | 0.0 | 99% | JN974267.1 |
| Galactomyces geotrichum strain Q1-2 26S ribosomal RNA gene, partial sequence | 996 | 996 | 96% | 0.0 | 99% | HM754439.1 |
| Geotrichum sp. 5.3-1 26S ribosomal RNA gene, partial sequence | 996 | 996 | 96% | 0.0 | 99% | FJ473451.1 |
| Geotrichum sp. XM05D 26S ribosomal RNA gene, partial sequence | 996 | 996 | 96% | 0.0 | 99% | EU293419.1 |
| Galactomyces candidum strain Y11 26S ribosomal RNA gene, partial sequence | 994 | 994 | 96% | 0.0 | 99% | KM391959.1 |
| Geotrichum sp. BUF9 gene for 26S ribosomal RNA, partial sequence, strain: BUF9 | 992 | 992 | 96% | 0.0 | 99% | AB741075.1 |
| Geotrichum candidum strain T0M_YEAST small subunit ribosomal RNA gene, partial sequence; internal transcribed s | 990 | 990 | 96% | 0.0 | 99% | KF112070.1 |
| Galactomyces candidum 26S ribosomal RNA gene, partial sequence | 990 | 990 | 96% | 0.0 | 99% | KF015740.1 |
| Galactomyces candidum strain CBS 607.85 26S ribosomal RNA gene, partial sequence | 990 | 990 | 96% | 0.0 | 99% | JN974268.1 |
| Galactomyces geotrichum strain GS6A 26S ribosomal RNA gene, partial sequence | 990 | 990 | 96% | 0.0 | 99% | HM754437.1 |
| Galactomyces geotrichum strain SD2-d 26S ribosomal RNA gene, partial sequence | 990 | 990 | 96% | 0.0 | 99% | HM754447.1 |

**Fig. 4.** BLASTN similarity regions and percentage with *Geotrichum candidum* GG1 sequence.



Alignments Download GenBank Graphics Distance tree of results

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
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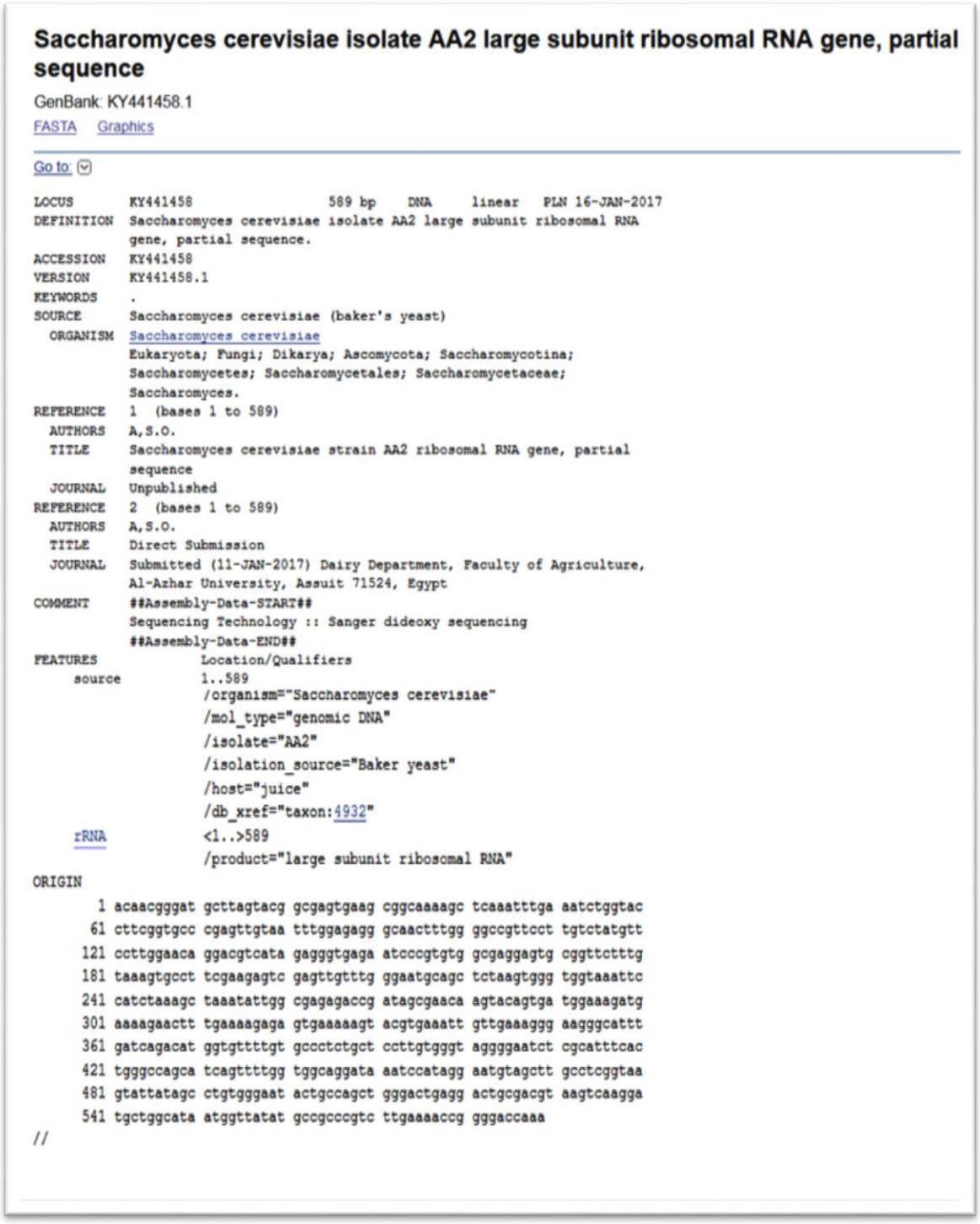
| Geotrichum candidum isolate AAA large subunit ribosomal RNA gene, partial sequence | 1120 | 1120 | 100% | 0.0 | 100% | MF383368.1 |
| Geotrichum candidum isolate GG1 large subunit ribosomal RNA gene, partial sequence | 1005 | 1005 | 91% | 0.0 | 99% | MF383376.1 |
| Galactomyces candidum strain Y11 26S ribosomal RNA gene, partial sequence | 992 | 992 | 91% | 0.0 | 99% | KM391959.1 |
| Geotrichum candidum strain T0M_YEAST small subunit ribosomal RNA gene, partial sequence; internal transcribed s | 992 | 992 | 91% | 0.0 | 99% | KF112070.1 |
| Galactomyces geotrichum strain GG002 26S ribosomal RNA gene, partial sequence | 992 | 992 | 88% | 0.0 | 100% | JQ713185.1 |
| Galactomyces candidum strain CBS 607.85 26S ribosomal RNA gene, partial sequence | 992 | 992 | 91% | 0.0 | 99% | JN974268.1 |
| Uncultured fungus clone YC10 26S ribosomal RNA gene, partial sequence | 992 | 992 | 91% | 0.0 | 99% | AY536709.1 |
| Uncultured fungus clone YC01 26S ribosomal RNA gene, partial sequence | 992 | 992 | 91% | 0.0 | 99% | AY53669[illegible].1 |
| Uncultured fungus clone FS14 26S ribosomal RNA gene, partial sequence | 992 | 992 | 91% | 0.0 | 99% | AY464916.1 |
| Uncultured fungus clone FE20 26S ribosomal RNA gene, partial sequence | 992 | 992 | 91% | 0.0 | 99% | AY464896.1 |
| Galactomyces candidum strain Y4 26S ribosomal RNA gene, partial sequence | 990 | 990 | 89% | 0.0 | 99% | KM391952.1 |
| Galactomyces geotrichum strain SD2-d 26S ribosomal RNA gene, partial sequence | 990 | 990 | 90% | 0.0 | 99% | HM754447.1 |
| Galactomyces geotrichum strain NX5 26S ribosomal RNA gene, partial sequence | 990 | 990 | 90% | 0.0 | 99% | HM754438.1 |

**Fig. 5.** BLASTN similarity regions and percentage with *Geotrichum candidum* AAA sequence.



**Saccharomyces cerevisiae isolate AA2 large subunit ribosomal RNA gene, partial sequence**

GenBank: KY441458.1

FASTA Graphics

Go to:

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VERSION     KY441458.1
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  ORGANISM  Saccharomyces cerevisiae
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            Saccharomycetes; Saccharomycetales; Saccharomycetaceae;
            Saccharomyces.
REFERENCE   1  (bases 1 to 589)
  AUTHORS   A,S.O.
  TITLE     Saccharomyces cerevisiae strain AA2 ribosomal RNA gene, partial
            sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 589)
  AUTHORS   A,S.O.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-JAN-2017) Dairy Department, Faculty of Agriculture,
            Al-Azhar University, Assuit 71524, Egypt
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            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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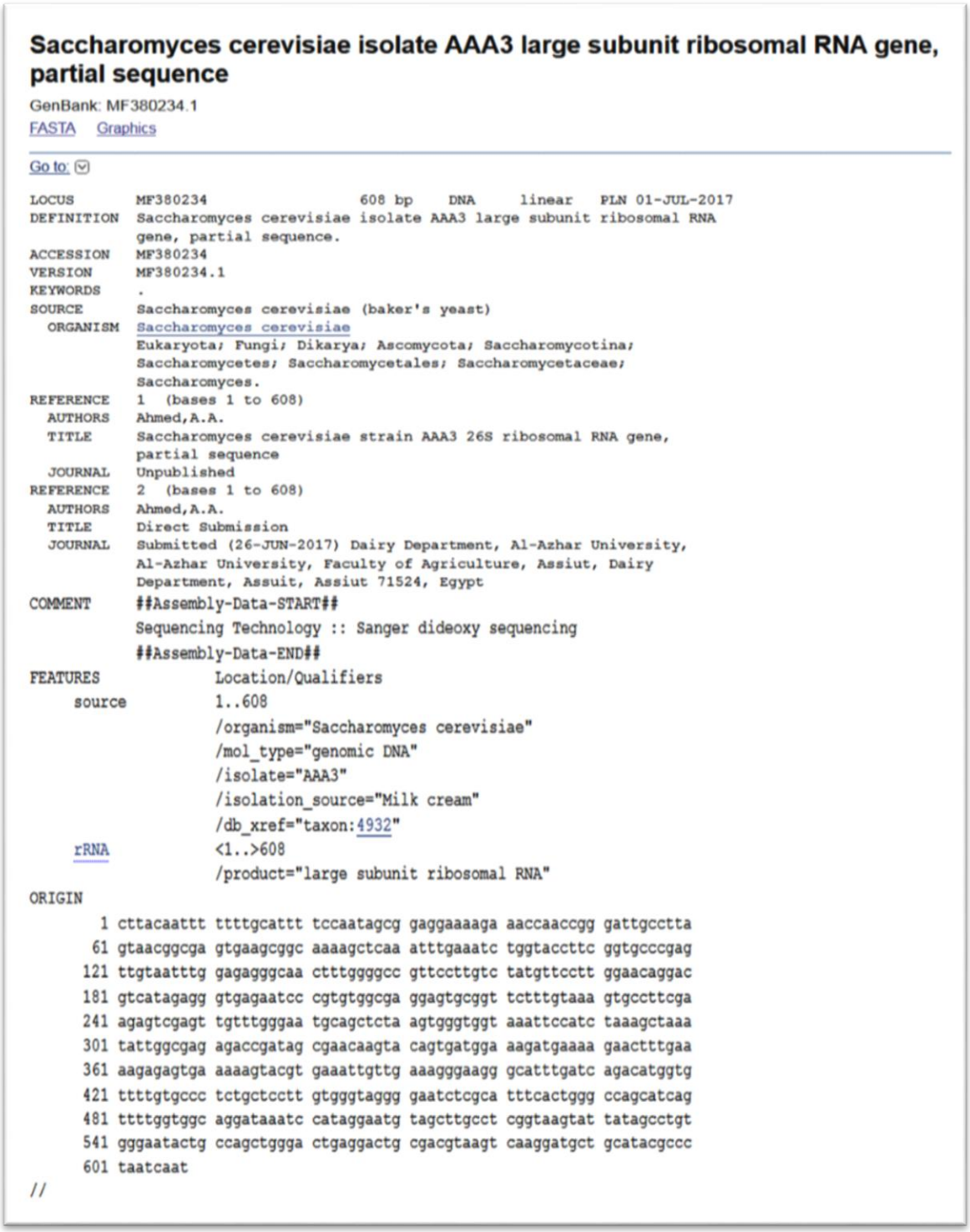
**Fig. 6.** NCBI flat file for Egyptian *Saccharomyces cerevisiae*Y3018S ribosomal RNA gene, partial sequence.



**Saccharomyces cerevisiae isolate AAA3 large subunit ribosomal RNA gene, partial sequence**

GenBank: MF380234.1

FASTA Graphics

Go to:

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DEFINITION  Saccharomyces cerevisiae isolate AAA3 large subunit ribosomal RNA
            gene, partial sequence.
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VERSION     MF380234.1
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SOURCE      Saccharomyces cerevisiae (baker's yeast)
  ORGANISM  Saccharomyces cerevisiae
            Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina;
            Saccharomycetes; Saccharomycetales; Saccharomycetaceae;
            Saccharomyces.
REFERENCE   1  (bases 1 to 608)
  AUTHORS   Ahmed,A.A.
  TITLE     Saccharomyces cerevisiae strain AAA3 26S ribosomal RNA gene,
            partial sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 608)
  AUTHORS   Ahmed,A.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-JUN-2017) Dairy Department, Al-Azhar University,
            Al-Azhar University, Faculty of Agriculture, Assiut, Dairy
            Department, Assuit, Assiut 71524, Egypt
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            ##Assembly-Data-END##
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**Fig. 7.** NCBI flat file for Egyptian *Saccharomyces cerevisiae*Y42 18S ribosomal RNA gene, partial sequence.

**Geotrichum candidum isolate GG1 large subunit ribosomal RNA gene, partial sequence**

GenBank: MF383376.1

FASTA Graphics

Go to:

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DEFINITION  Geotrichum candidum isolate GG1 large subunit ribosomal RNA gene,
            partial sequence.
ACCESSION   MF383376
VERSION     MF383376.1
KEYWORDS    .
SOURCE      Geotrichum candidum (Galactomyces candidum)
  ORGANISM  Geotrichum candidum
            Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina;
            Saccharomycetes; Saccharomycetales; Dipodascaceae; Geotrichum.
REFERENCE   1  (bases 1 to 563)
  AUTHORS   Ahmed,A.A.
  TITLE     Galactomyces geotrichum strain GG1 26S ribosomal RNA gene
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 563)
  AUTHORS   Ahmed,A.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-JUN-2017) Dairy Department, Al-Azhar University,
            Al-Azhar University, Faculty of Agriculture, Assiut, Dairy
            Department, Assuit, Assiut 71524, Egypt
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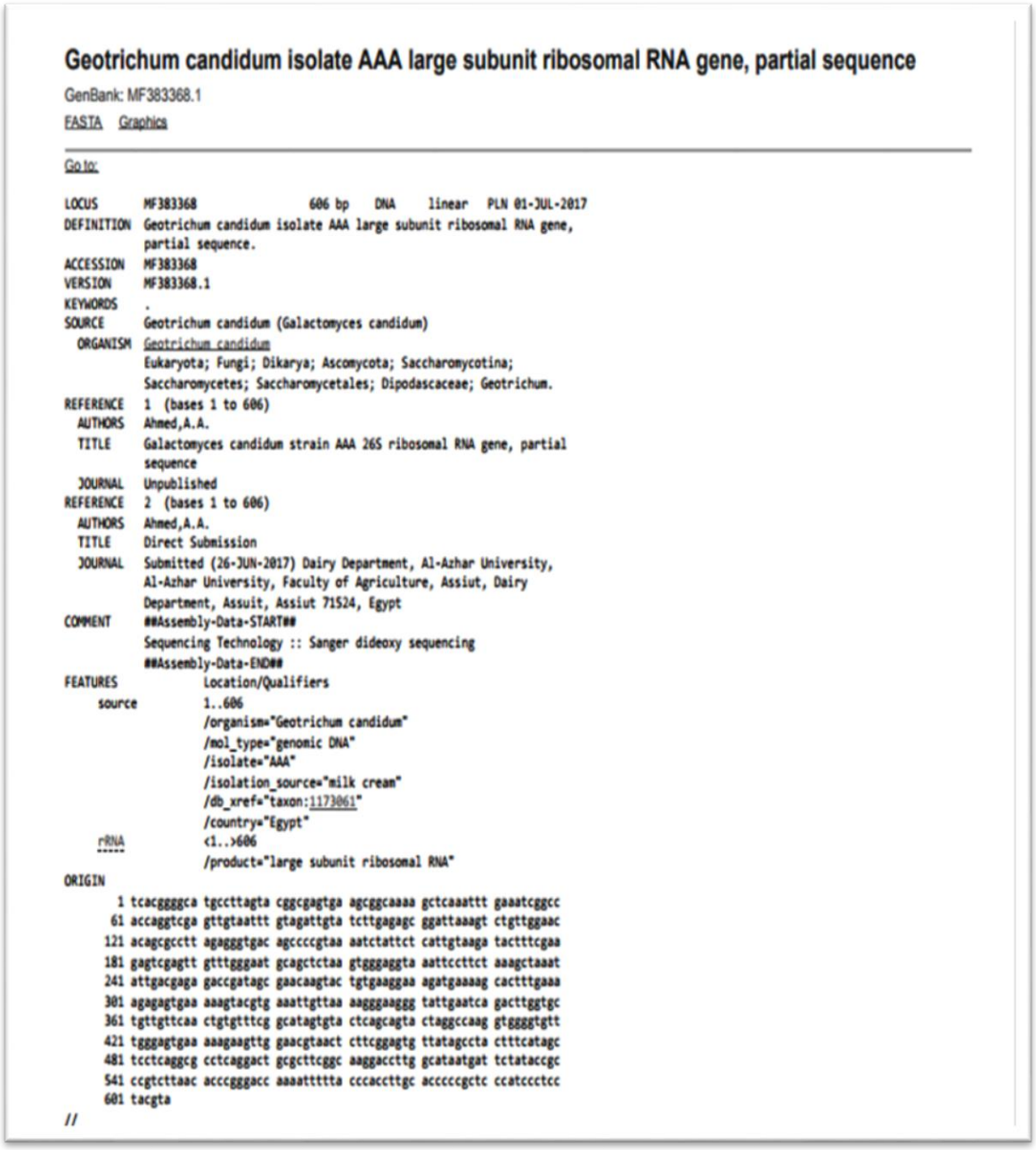
**Fig. 8.** NCBI flat file for Egyptian *Geotrichum candidum*Y67 18S ribosomal RNA gene, partial sequence.



**Geotrichum candidum isolate AAA large subunit ribosomal RNA gene, partial sequence**

GenBank: MF383368.1

FASTA Graphics

Go to:

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            partial sequence.
ACCESSION   MF383368
VERSION     MF383368.1
KEYWORDS    .
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  ORGANISM  Geotrichum candidum
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REFERENCE   1  (bases 1 to 606)
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  TITLE     Galactomyces candidum strain AAA 26S ribosomal RNA gene, partial
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  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 606)
  AUTHORS   Ahmed,A.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-JUN-2017) Dairy Department, Al-Azhar University,
            Al-Azhar University, Faculty of Agriculture, Assiut, Dairy
            Department, Assuit, Assiut 71524, Egypt
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**Fig. 9.** NCBI flat file for Egyptian *Geotrichum candidum*Y72 18S ribosomal RNA gene, partial sequence.

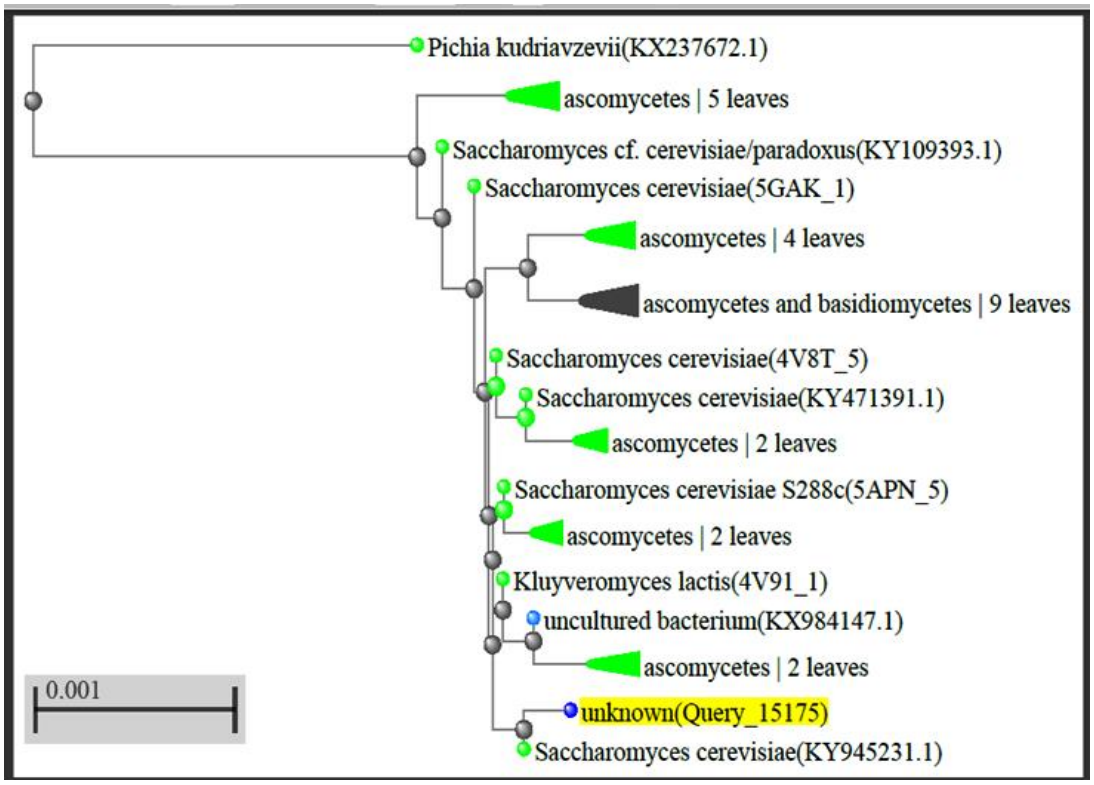


**Fig. 10.** Phylogenetic tree based on sequence distance analysis of 589bp positions of the 18S rDNA region in *Saccharomyces cerevisiae* strain Y30.

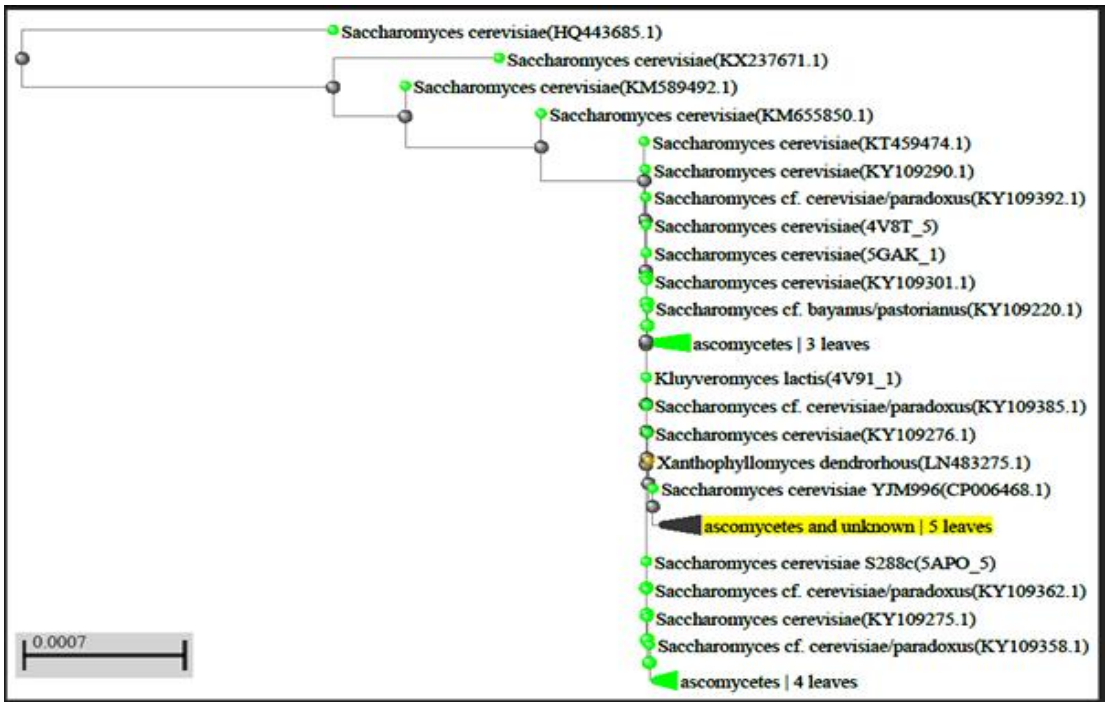


**Fig. 11.** Phylogenetic tree based on sequence distance analysis of 608bp positions of the 18S rDNA-ITS2 region in *Saccharomyces* strain Y42.

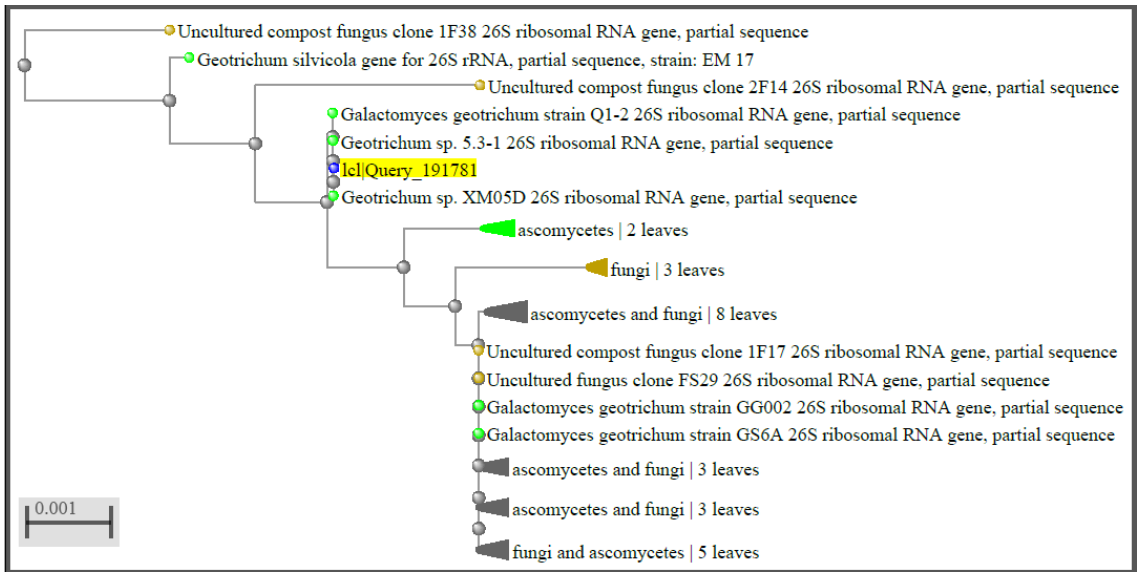


**Fig. 12.** Phylogenetic tree based on sequence distance analysis of 563bp positions of the ITS1-18S rDNA-ITS2 region in *Geotrichum candidum* strain Y67.

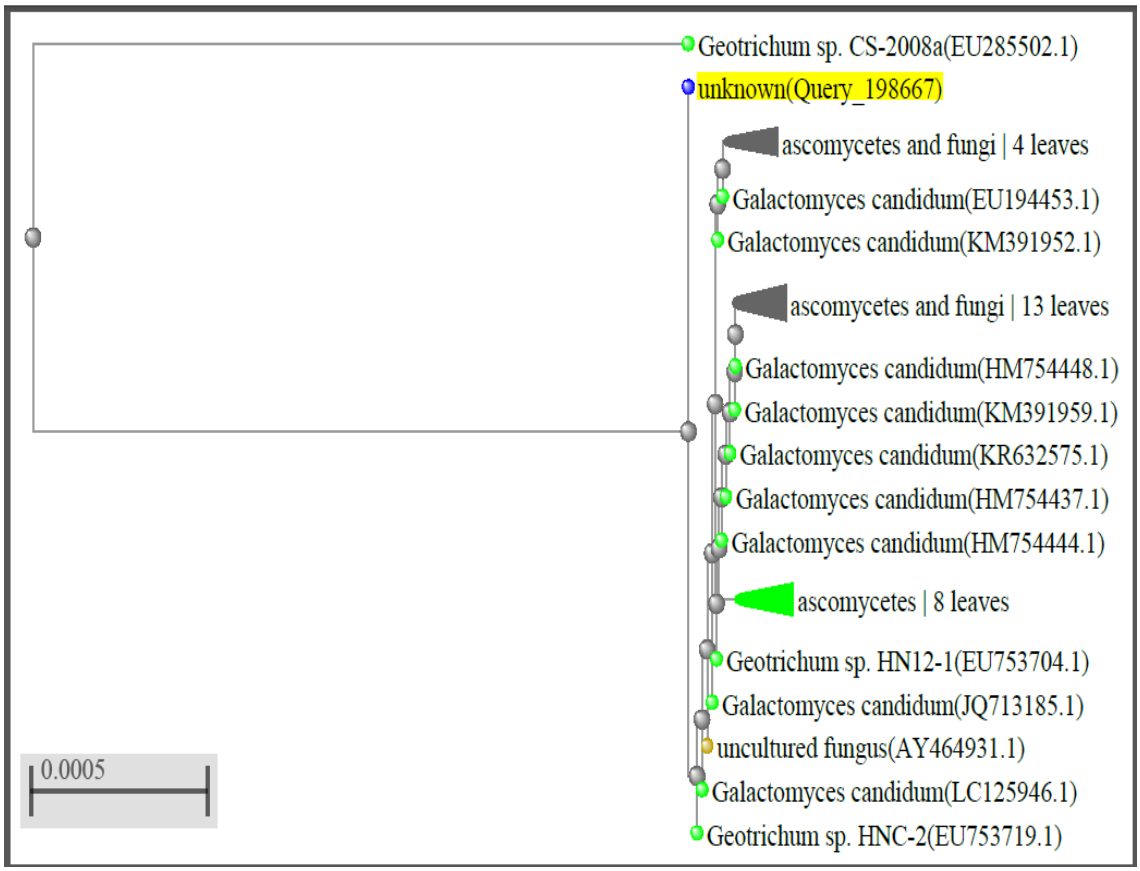


**Fig. 13.** Phylogenetic tree based on sequence distance analysis of 606bp positions of the ITS1-18S rDNA-ITS2 region in *Geotrichum candidum* strain Y72.

# التعريف بالصفات المظهرية والجينية للخمائر المعزولة من بعض منتجات الالبان

**خاطر عبد الفتاح احمد خاطر[١]، ياسر عبد التواب احمد [٢]، احمد عبد الدايم احمد [٢]**

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[٢] قسم الالبان، كلية الزراعة، جامعة الازهر، اسيوط، مصر

**الملخص العربي**

يتم تصنيف سلالات الخمائر تقليديا باستخدام الاختبارات الظاهرية والكيموحيوية وحاليا باستخدام الطرق الحديثة باستخدام البصمة الوراثية بنظام الـ PCR. والدراسة الحالية تهدف الى اجراء تصنيف لاربعة سلالات من الخمائر المعزولة من بعض منتجات الالبان المحلية بمدينة اسيوط. تم التعريف الاولي للعزلات بالفحص المجهري للخلايا وكذا طبيعة وشكل المستعمرات النامية على البيئات الصلبة حيث اوضحت النتائج ان مستعمرات العزلات الاربع ظهرت بشكل بيضاوي وباللون الابيض، كما تبين ان سلالتين تتكاثران بالتبرعم متعدد الاقطاب. تم تعريف العزلات بالاختبارات الكيموحيوية عن طريق تخمير السكريات وتمثيل المصادر الكربونية والنيتروجينية بالاضافة الى الاختبارات التكميلية مثل القدرة على النمو في تركيزات مرتفعة من الجلوكوز (٥٠ ٪، ٦٠ ٪)، القدرة على النمو على درجتي ٣٧°م ،٤٢°م وكذا النمو في ١ ٪ حمض الخليك وتحليل اليوريا. اظهرت نتائج تجارب الطريقة التقليدية المستخدمة في التصنيف تشابها بين السلالتين Y30, Y42 وسلالة *S. cerevisiae* المرجعية وكذا انتماء السلالتين Y67, Y72 الى نوع *G. candidum*. تم اجراء تصنيف تأكيدي للسلالات الاربعة المختبرة عن طريق البصمة الوراثية بنظام PCR على النحو التالي: تم عزل الـ DNAباستخدام Kit للعزل وهي: ZR Fungal/Bacterial DNA Min Prep ™ Kit (Catalog No. D6005) و Thero Gene JET genomic DNA Purification Kit (K0721). . وتم استخدام ٤ بادئات (primers) منها بادئان عامان (Universal primers) وهما 18sF & 28 sF وبادئان متخصصان (Specific primers) وهما ITS1 & NL2 وقد اظهرت هذه البادئات نتائج ايجابية في تعريف سلالات الخميرة وقد اظهرت التجارب الاولية ان درجة حرارة ٥٠°م هي الافضل لعمل البادئات. نجح في التعرف على التتابعات النيكلوتيدية Nucleotide sequenceالخاصة بالبادئات المستخدمة (Primers) وتم ارسال ناتج الـ PCR للسلالات الاربعة المختبرة لعمل تتابع للنيكلوتيدات (Sequencing) لقطع الـ DNA التي قام البادئ بالالتحام عليها وتكرارها. تم مقارنة نتائج الـ Sequencing المتحصل عليها مع تلك المسجلة في بنك الجينات NCBI ومن خلال هذه المقارنة امكن تسجيل هذه السلالات ببنك الجينات. تم ايداع هذه التتابعات النيكلوتيدية في بنك الجينات Genbank وتم الحصول على اربعة ارقام ايداع Accession numbers على النحو التالي: رقم ايداع KY441458 تحت اسم- *Saccharomyces cerevisiae*AA2Y30 ورقم ايداع MF380234 تحت اسم *Saccharomyces cerevisiae*AAA3Y42 - ورقم ايداع MF383376 تحت اسم - *Geotrichum candidum*GG1Y67 ورقم ايداع MF383368 تحت اسم *Geotrichum candidum*AAAY72 -.