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# *Fonsecazyma quercina* sp. nov., a novel yeast species isolated from Persian oak (*Quercus brantii*) branch in Iran

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**Abstract**

A taxonomic study of yeast isolates collected from the Persian oak (*Quercus brantii*) branch in the Zagros oak forests (Kermanshah Province, West of Iran) revealed the presence of a novel yeast species. Morphological and physiological characteristics, as well as sequence analysis of the D1/D2 region of the large subunit rRNA gene and the internal transcribed spacer region, showed that, the novel species belonged to the genus *Fonsecazyma* (*Tremellales*, *Bulleraceae*). The name *Fonsecazyma quercina* sp. nov. is proposed for this new species. This novel species has the highest sequence similarity with *Fonsecazyma* sp. KT301 (97.58%). This isolate comprises spherical single cells and can grow in NaCl concentrations up to 1 M. It can thrive at pH levels of 4, 8, and 10 but not at a pH of 2. The isolate is psychrotolerant, exhibiting optimal growth between 6 and 25 °C but unable to grow outside of this temperature range. Regarding carbon sources, the isolate efficiently utilizes and assimilates glucose and trehalose. It also can utilize sucrose, mannose, maltose, and fructose to some extent but shows weak utilization of cellobiose, sorbitol, and rhamnose. However, this isolate cannot utilize galactose, sorbose, lactose, melibiose, arabinose, ribose, or glycine as sole carbon sources. The holotype of *Fonsecazyma quercina* sp. nov. was deposited at the Fungus Reference Collection of Herbarium Ministerii Iranici Agriculturae (IRAN) as an inactive form.

**Keywords:** Basidiomycetous yeast, D1/D2 region, psychrotolerant, *Quercus*, Zagros oak forests

# معرفی یک گونه جدید مخمر *Fonsecazyma quercina* sp. nov.، جداسازی شده از شاخه بلوط در غرب ایران*

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**خلاصه**

در این بررسی یک جدایه مخمر از نمونه شاخه بلوط ایرانی (*Quercus brantii* Lind.) در جنگل‌های بلوط زاگرس (استان کرمانشاه)، جداسازی شد. ویژگی‌های ریخت‌شناختی، فیزیولوژیکی و تجزیه و تحلیل توالی ناحیه D1/D2 ژن زیر واحد بزرگ rRNA و فاصله ترانویسی شده داخلی (ITS) نشان داد که این جدایه به جنس *Fonsecazyma* (*Tremellales*, *Bulleraceae*) تعلق دارد. همچنین بر این اساس، توالی‌های D1/D2 و ITS نشان داد که سویه IRAN 18507F متعلق به جنس مذکور بیش‌ترین شباهت (۹۷/۵۸٪) را با گونه *Fonsecazyma* sp. KT301 دارد، لذا نام *Fonsecazyma quercina* sp. nov. برای این گونه پیشنهاد شد. سلول‌های این مخمر کروی بوده و توانست تا غلظت ۱ مولار NaCl رشد کند. گونه مذکور در pH ۴، ۸ و ۱۰ رشد کرد، اما قادر به رشد در درجه اسیدی ۲ نبود. این جدایه که مقاوم به سرما است توانست به خوبی بین دماهای ۶ تا ۲۵ درجه سلسیوس رشد کند، اما در دمای خارج از این محدوده توانایی رشد نداشت. همچنین، جدایه مذکور به خوبی قادر به استفاده از گلوکز، ترهالوز، ساکارز، مانوز، مالتوز و فروکتوز به عنوان منابع کربن بود، اما از سلوبیوز، سوربیتول و رامنوز به صورت خفیف استفاده کرد و قادر به استفاده از گالاکتوز، سوربوز، لاکتوز، ملیبیوز، آرابینوز، ریبوز و گلیسین به عنوان تنها منابع کربن نبود. سویه مرجع *Fonsecazyma quercina* sp. nov. در مجموعه قارچ‌های وزارت جهاد کشاورزی (IRAN 18507F) به صورت غیرفعال نگهداری می‌شود.

**واژه‌های کلیدی:** جنگل‌های بلوط زاگرس، متحمل به سرما، مخمر بازیدیومیستی، ناحیه D1/D2، *Quercus*

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**Introduction**

The genus *Fonsecazyma* was first proposed in 2015 by Liu *et al*. (2015a), using a seven-gene dataset including the internal transcribed spacer region (ITS-rDNA), the small subunit (SSU or 18S) rRNA gene, the D1/D2 domains of the large subunit (LSU or 26S) rRNA gene, the translation elongation factor 1-α (*TEF1*), two subunits of RNA polymerase II (*RPB1* and *RPB2*), and the cytochrome b (*CYTB*) genes (Liu *et al*. 2015a). *Fonsecazyma* formed a well-supported clade (97%) that included *Cryptococcus mujuensis* CBS 10308, *C. tronadorensis* (GU560003), and *Kwoniella betulae* (KM408130). Since *C. mujuensis* and *C. tronadorensis* diverged significantly from the genus *Cryptococcus*, and *K. betulae* had no close relatives within *Kwoniella*, a new genus *Fonsecazyma* was proposed to include these three species (Liu *et al*. 2015b). Later, in 2020, *F. betulae* and *F. tronadorensis* were transferred to a new genus *Teunia* and renamed *Teunia betulae*, and *T. tronadorensis*, respectively (Li *et al*. 2020).

The type species of *Fonsecazyma* i.e., *F. mujuensis*, formed its own single-species lineage in the seven-gene phylogeny (Shin *et al*. 2006, Liu *et al*. 2015a, Li *et al*. 2020). Naming this clade *Fonsecazyma* was crucial to avoid confusion with other genera, such as *Kwoniella* and *Cryptococcus* (Li *et al*. 2020). Based on a seven-gene dataset, *Fonsecazyma* was placed in *Bulleraceae* (*Tremellales*, *Tremellomycetes*, *Agaricomycotina*, and *Basidiomycota*) and clustered with high bootstrap support (98–100%) with the genera *Pseudotremella* and *Sirobasidium*, although it remained distinct from them. Currently, the genus *Fonsecazyma* contains only one known species, *F. mujuensis* (Li *et al*. 2020).

For the identification of yeast species, conventional methods mainly rely on morphological and physiological data, which are time-consuming and may result in inaccurate species identification. Molecular biology has led to the development of new techniques for quickly and accurately identifying different types of yeasts (Middelhoven & Kurtzman 2003, Kurtzman *et al*. 2011, Kurtzman *et al*. 2015). The identification of yeast species is now routinely determined by DNA sequence analyses. The most commonly used comparisons include nucleotide sequence divergence in domains 1 and 2 (D1/D2) of the LSU and ITS (Kurtzman & Robnett 1998, Fell *et al.* 2000, Scorzetti *et al*. 2002).

The number of newly identified yeast species has increased in recent years, mainly due to the acceptance of ribosomal DNA (rDNA) gene sequence analysis, such as the D1/D2 domain and the ITS region, and the availability of databases (Scorzetti *et al*. 2002, Kurtzman *et al*. 2011). Various estimates suggest that, numerous yeast species are yet to be described (Lachance 2006, Kurtzman *et al*. 2015). Based on available evidence (Morais *et al*. 2006, Nakase *et al*. 2006), it is worth noting that, a significant proportion of these undescribed yeast species are thought to inhabit forest environments.

In the present study, a novel basidiomycetous yeast obtained from a Persian oak branch in the Zagros oak forest (Kermanshah Province, W of Iran) is described. Based on physiological, biochemical, and molecular analyses of ITS and the D1/D2 domain of the LSU rRNA gene, the isolate, namely, *Fonsecazyma quercina* sp. nov. is, therefore, classified here.

**Materials and Methods**

- Sampling and isolation

A novel yeast strain (IRAN 18507F), was isolated from bark samples of a Persian oak tree in Chahar Zebar-e-Oliya (Kermanshah Province, W of Iran), 34°13'58.8" N, 46°40'58.3" E, in Nov. 2022. Bark samples were placed in a flask with sterile distilled water and shaken for 30 min. The resulting solution was plated on potato dextrose agar containing chloramphenicol (50 ppm) to isolate the yeast. In this study, the phenotypic and genotypic characteristics of the strain were analyzed to determine its taxonomic and phylogenetic position.

- Morphological characteristics

A single colony from a streak plate culture on PDA medium was examined for its macroscopic characteristics, including color, texture, surface, elevation, and margin

according to established protocols (Kurtzman *et al*. 2011). Cell shape was further assessed using light microscopy.

- Assimilation of carbon sources

The ability of the yeast to utilize various carbon sources was assessed using Yeast extract-peptone (YEP) fermentation basal medium. Bromothymol blue indicator (4 mL of a 50 mg/75 mL solution) was added to 100 mL of YEP medium, dispensed into sterile test tubes, and autoclaved. After sterilization, a 1% solution of each carbon source including glucose, galactose, sucrose, mannose, maltose, sorbose, cellobiose, trehalose, lactose, melibiose, sorbitol, fructose, arabinose, ribose, rhamnose, and glycine was added to separate tubes, followed by inoculation with fresh yeast culture and incubation at 25 °C for up to seven days. Utilization of a carbon source was indicated by a color change from green to yellow (Schaad *et al*. 2001, Kurtzman *et al*. 2011).

- Extracellular enzymatic activity

The ability of the yeast isolate to produce extracellular enzymes was assessed by cultivating the isolate in triplicate on basal media containing specific enzyme substrates**.**

- Catalase activity

Catalase activity was assessed by mixing a yeast colony with a drop of 3% hydrogen peroxide ($H_2O_2$) and observing for immediate oxygen bubble formation (Schaad *et al*. 2001, Borkar 2018).

- Oxidase activity

To assess oxidase activity, a 1% solution of tetramethyl-p-phenylenediamine-dihydrochloride (TMPD) reagent was prepared. A filter paper soaked in this solution was placed in a petri dish, and a small amount of the yeast culture was streaked onto the soaked area. A change in colony color to blue within 10 s was considered a positive reaction, 10–60 s was considered weakly positive, and no change within 60 s was considered negative (Schaad *et al*. 2001).

- Amylase activity

Starch hydrolysis was assessed by culturing the isolate on PDA plates supplemented with 1% starch for seven days at 25° C. Grown colonies were then washed, and the agar surface was flooded with Lugol's iodine solution. A clear zone around the colony on the blue background indicates starch hydrolysis by the isolate, suggesting amylase enzyme production (Mishra & Behera 2008, Borkar 2018).

- Lipase/esterase activity

To assess lipolytic activity and production of lipase and esterase enzymes, the isolate was cultured on media containing Tween 80 (a broad-spectrum lipase substrate) and Tween 20 (an esterase substrate) in triplicate (Kumar *et al*. 2012). The medium was prepared with 10 g peptone, 5 g NaCl, 0.1 g $CaCl_2$ $2H_2O$, 1.5 g agar, and 10 mL of either Tween in one liter of distilled water. The plates were incubated at 25 °C for seven days. The presence of crystalline precipitates around the colony growth after incubation indicated positive results (Ramnath *et al*. 2017).

- Lecithinase activity

The lecithinase activity of the isolate was assessed by culturing a streak plate on PDA medium supplemented with egg yolk (lecithin substrate) in triplicate. The plates were incubated at 25 °C for seven days. The formation of a clear halo around the isolate after incubation indicated positive results, signifying lecithin hydrolysis (Maharana & Ray 2013).

- Protease activity

Protease enzyme production was assessed by culturing the isolate on PDA medium containing 2% skim milk in triplicate and incubating at 25 °C for seven days. A clear halo around the isolate after incubation indicated a positive result, signifying the presence of protease activity (Strauss *et al*. 2001, Carrasco *et al*. 2012).

- Xylanase activity

Xylanase activity was assessed by culturing the isolate on PDA medium supplemented with 0.5% xylan, followed by incubation at 25 °C for seven days. The formation of a clear halo around the colony indicated xylanase enzyme production (Carrasco *et al*. 2012).

- Gelatinase activity

Gelatinase activity was assessed by culturing the isolate on PDA medium supplemented with 1.5% gelatin.

A clear halo around the colony after seven days of incubation at 25 °C indicated a positive reaction, signifying gelatinase production (Hanane *et al*. 2022).

- Urease activity

To assess urease activity, a urea broth medium was prepared by adding 20 g of sterilized urea solution (prepared with 180 mL of distilled water) to autoclaved basal medium (containing 1 g each of peptone and dextrose, 5 g NaCl, 2 g $KH_2PO_4$, 0.012 g phenol red, and 15 g agar in 800 mL of distilled water) using a sterile PTFE membrane filter (pore size 0.22 µm). The medium was then dispensed into sterile tubes and slanted. After inoculation with the yeast isolate, the tubes were incubated at 25 °C for 48–72 h. A color change from orange to red due to ammonia production and alkalinization of the medium indicated a positive urease test (Christensen 1946, Brink 2010).

- Physiological characteristics

The isolate's growth temperature range was investigated by culturing it in triplicate on PDA medium at different temperatures (0, 4, 10, 15, 20, 25, 28, 30, and 35 °C). One replicate at 25 °C was used as a positive control. The presence or absence of growth was recorded (Borkar 2018). To assess salt tolerance, the yeast isolate was cultured on PDA medium containing 0.5, 1, 2, and 3 molar NaCl (58.44 g/mol). Petri dishes were incubated at 25 °C for seven days, and growth or no growth was observed at each salt concentration (Corte *et al*. 2006, Bulgari *et al*. 2019). The ability of the yeast isolate to grow at different pH levels (2, 4, 6, 8, and 10) was investigated by adjusting the pH of PDA medium using NaOH and HCl. After autoclaving, the medium was inoculated with the isolate and incubated at 25 °C for seven days, and the presence or absence of growth was recorded at each pH level. Three replicates were considered for each pH (Borkar 2018, Hanane *et al*. 2022).

- Molecular identification

For molecular identification of the yeast isolate, genomic DNA was extracted from a pure culture using the Genome Extraction Kit (Denazist Asia Company, Mashhad, Iran). Subsequently, the internal transcribed spacer (ITS) and D1/D2 domain of the 26S rRNA gene were amplified by polymerase chain reaction (PCR) using the primers ITS1 (5´-TCCGTAGGTGAACCTGCGG -3´) / ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) (White *et al*. 1990) and NL1 (5´-GCATATCAATAAGCGGAGGAAAAG-3´) / NL4 (5´-GGTCCGTGTTTCAAGACGG-3´) (Kurtzman & Robnett 1998), respectively. The PCR reaction mixture (final volume 25 µL) contained 12.5 µL of 2x master mix (*Taq* polymerase, dNTPs, and reaction buffer (Amplicon Company), 1.5 µL each of the primers (10 µM), 2 µL of sample DNA, and 7.5 µL of nuclease-free deionized water. The PCR program consisted of an initial denaturation at 95 °C for five min, followed by 30 cycles of denaturation at 95 °C for 40 s, annealing at 55 °C for 40 s, and extension at 72 °C for 60 s. A final extension step was performed at 72 °C for 10 min. The PCR was performed using a Biometra T-Personal thermocycler (Analytik Jena, Germany). PCR products (3 µL) were then electrophoresed on a 1% agarose gel with 1 × TAE buffer and DNA Safe Stain Dye (Cinaclon Company) for 60 min at 85 V. The PCR products were subsequently sent to Microsynth (Switzerland) for sequencing.

- Phylogenetic analysis

After sequencing, the nucleotide sequences were edited using Chromas software and subjected to a nucleotide similarity search using the NCBI BLAST service to identify closely related sequences. MUSCLE, implemented in the MEGA X software, was used to perform a multiple alignment of the ITS and D1/D2 sequences obtained in this study with sequences retrieved from GenBank (Table 1). The alignments were then manually refined using BioEdit Ver. 7.2.5 software. Gblocks Ver. 0.91b (Castresana 2000) was used to exclude poorly aligned regions from the final alignment of each dataset with the following parameters: minimum conserved sequences per position = 14, minimum block length = 5, maximum contiguous non-conserved positions = 4, minimum flanking sequences = 22, and allowed gaps = 50% under these parameters as follows: minimum

number of sequences for a conserved position = 14, minimum length of a block = 5, maximum number of contiguous nonconserved positions = 4, minimum number of sequences for a flanking position = 22, and allowed gap positions = half.

Both distance (Neighbor-joining) and cladistic (maximum parsimony and maximum likelihood) methods were used to infer the evolutionary history of the isolate, using MEGA X software for the analyses. The maximum parsimony tree was generated by the Subtree-Pruning-Regrafting (SPR) algorithm (search level 1, initial trees obtained by random addition of sequences, 10 replicates) (Nei & Kumar 2000). Gaps and missing data were excluded from the analysis (complete deletion). In maximum likelihood analysis, the percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete gamma distribution [five rate categories (+*G*, parameter = 0.142)] was used to model evolutionary rate differences among sites. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 27 nucleotide sequences. The reliability of tree topologies was evaluated by calculating 1000 bootstrap re-samplings (Felsenstein 1985).

**Table 1.** List of sequences used in the construction of phylogenetic trees

| Taxon | GenBank Accession No. | | Isolate / Strain | Country | Isolation source | Reference |
|---|---|---|---|---|---|---|
| | ITS | D1/D2 (LSU) | | | | |
| *Fonsecazyma* sp. | MK942578 | - | 12A8 | China | Flower | (Wang *et al*. 2020) |
| *Fonsecazyma* sp. | MK942577 | - | X54 | China | Flower | (Wang *et al*. 2020) |
| *Fonsecazyma* sp. | MK942579 | - | 21S4 | China | Flower | (Wang *et al*. 2020) |
| *Fonsecazyma* sp. | LC440108 | - | DMKU-XD44 | Thailand | Peat | Unpublished |
| *Fonsecazyma* sp. | KY558358 | KY558358 | KT301 | Czech Republic | Oak litter | (Mašínová *et al*. 2018) |
| *Fonsecazyma mujuensis* | NR137814 | NG058290 | CBS 10308 | China | Culture collection | (Liu *et al*. 2015) |
| *Cryptococcus* sp. (*Fonsecazyma*) | - | EU002790 | CBS 10166 | Portugal | Plant | (Liu *et al*. 2015) |
| *Cryptococcus* sp. (*Fonsecazyma*) | - | EU002791 | CBS 10167 | Portugal | Plant | (Liu *et al*. 2015) |
| *Cryptococcus* sp. (*Fonsecazyma*) | - | EU002792 | CBS 10168 | Portugal | Plant | (Liu *et al*. 2015) |
| *Cryptococcus* sp. (*Fonsecazyma*) | - | EU002793 | CBS 10169 | Portugal | Plant | (Liu *et al*. 2015) |
| *Teunia helanensis* | NR174732 | - | CGMCC 2.4450 | China | Soil | (Li *et al.* 2020) |
| *Teunia* sp. | OQ818880 | - | KBP:Y-7082 | Russia | Culture collection | Unpublished |
| *T. korlaensis* | NR174731 | MK050286 | CGMCC 2.3835 | China | Soil | (Li *et al.* 2020) |
| *Teunia* sp. | - | OP941487 | KBP:Y-6585 | Russia | Termites | Unpublished |
| *Teunia* sp. | - | OQ851887 | NYNU 23232 | China | - | Unpublished |
| *Teunia* sp. | - | OP470195 | XZY194-1 | China | *Quercus* | Unpublished |
| *Kwoniella mangrovensis* | NR073332 | AF444742 | CBS 8507 | USA | Mangrove Cay | (Statzell *et al*. 2008) |
| *K. bestiolae* | NR111373 | NG042482 | CBS 10118 | Vietnam | Litchi fruit borer | (Findley *et al*. 2009) |
| *K. pini* | NR111269 | NG042453 | VKM Y-2958 | Hungary | Pine litter | (Golubev *et al*. 2008) |
| *Cryptococcus wingfieldii* | NR111375 | NG042484 | CBS 7118 | South Africa | Scolytid beetles | (Findley *et al*. 2009) |
| *C. neoformans* | NR130682 | NG058766 | CBS 8710 | USA | Patient | (Findley *et al*. 2009) |
| *C. neoformans* | NR171785 | - | ATCC 32045 | USA | Culture collection | Unpublished |
| *C. gattii* | NR165941 | - | CBS 6289 | China | Culture collection | Unpublished |
| *C. gattii* | JN944027 | - | WM 05.414 | Brazil | - | (Schoch *et al.* 2012) |
| *C. gattii* | - | KC171326 | ATCC MYA-4873 | USA | Culture collection | Unpublished |
| *C. gattii* | - | KC171325 | ATCC MYA-4871 | USA | Culture collection | Unpublished |
| *Genolevuria tibetensis* | NR159814 | - | CBS 10456 | China | Plant | (Wang *et al*. 2007) |
| *G. tibetensis* | - | NG068728 | CGMCC AS 2.2653 | China | Plant | (Wang *et al*. 2007) |

**Table 1 (contd)**

| | | | | | | |
|---|---|---|---|---|---|---|
| *G. amylolytica* | NR137810 | - | CBS 10048 | China | Plant | (Liu *et al*. 2015) |
| *G. bromeliarum* | NR144815 | KY107746 | CBS 10424 | Brazil | Bromelia | (Landell *et al*. 2009) |
| *Pseudotremella lacticolor* | NR158875 | NG060058 | CBS 10915 | Japan | Culture collection | (Satoh *et al*. 2013) |
| *P. moriformis* | NR155685 | NG058379 | CBS 7810 | Canada | Plant | (Vu *et al*. 2016) |
| *Bullera hannae* | KY101788 | KY106238 | CBS 8286 | New Zealand | Plant | (Vu *et al*. 2016) |
| *B. penniseticola* | KY101792 | KY106242 | CBS 8623 | Thailand | Plant | (Vu *et al*. 2016) |
| *B. unica* | KY101799 | KY106248 | CBS 8290 | New Zealand | Plant | (Vu *et al*. 2016) |
| *B. alba* | NR111083 | AF444759 | CBS 500 | USA | Culture collection | (Scorzetti *et al*. 2002) |
| *B. alba* | - | KU179840 | RKAT232 | Canada | Sea-ice | Unpublished |
| *Candida tropicalis* | NR111250 | - | CBS 94 | Australia | Culture collection | Unpublished |
| *C. tropicalis* | - | NG054834 | ATCC 750 | Germany | Culture collection | Unpublished |

## Results

- Morphological identification of isolate

On the PDA medium, young colonies of strain IRAN 18507F appeared cream-colored, changing to yellow with extended growth (Fig. 1 a-d). Colonies exhibited a mucoid texture, a smooth raised surface, and a complete edge. Under light microscopy, single cells were spherical (Fig. 1 e, f).

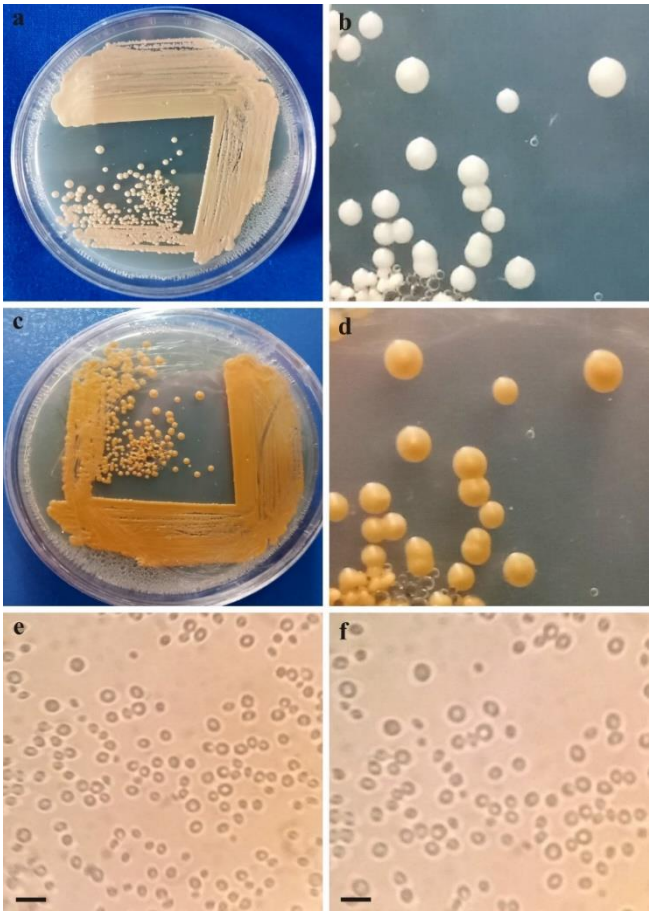


**Fig. 1.** Morphology of *Fonsecazyma quercina* sp. nov. strain (IRAN 18507F) isolated from Persian oak tree bark in Kermanshah Province (Iran): a, b. Young colony, c, d. Colony after 30 days at 25 °C, e, f. Coccus cells under the light microscope (Bar = 5 µm).

- Molecular phylogenetic

Based on a BLASTn search of the NCBI GenBank nucleotide database, the closest sequence to *Fonsecazyma quercina* (GenBank accession numbers: ITS; OR286121 and D1/D2; OR286124) was *Fonsecazyma* sp. isolate KT301 from the Czech Republic (GenBank accession number KY558358; identities = 97.58%, (Mašínová *et al*. 2018). The phylogenetic reconstruction of 27 ITS and D1/D2 sequences was inferred using distance and cladistic methods. The final aligned data matrix contained 593 characters, including alignment gaps, of which 86 characters were parsimony-informative, 171 were variable, and 419 were conserved. The phylogenetic trees inferred for both ITS and D1/D2 sequences showed the same topology, although there were differences in bootstrap values between equivalent branches (Figs 2–5). In the maximum likelihood method, the Kimura 2-parameter model was determined as the best-fitting nucleotide substitution model of evolution for each dataset based on the Bayesian Information Criterion (BIC) of MEGA X. The tree with the highest log likelihood (-1981.33) is shown in figure 2. In the maximum parsimony analysis, the tree length was 272 with a consistency index (CI) of 0.53, a retention index (RI) 0.84, a composite index = 0.57 for all sites, and 0.45 for parsimony informative sites. One of the most parsimonious trees is shown in figure 3. Phylogenetic analysis revealed that, the sequences of *F. quercina* were clustered and formed an independent branch with strong statistical support. The species described here as *F. quercina* Ghobadi & Jamali sp. nov. formed a sister lineage to the other *Fonsecazyma* species from other countries with high statistical support.

**Taxonomy**

***Fonsecazyma quercina*** Ghobadi & Jamali sp. nov.

Etymology: The specific epithet quercina refers to the oak genus *Quercus* from which this species was isolated.

Holotype: IRAN: Kermanshah Province, Chahar Zebar-e-Oliya, 34°13'58.8" N, 46°40'58.3" E, 1562 m, 2.11.2022, in oak forests on a branch of *Quercus brantii* Lind., A. Ghobadi & S. Jamali (IRAN 18507F, GenBank ITS: OR286121 and D1/D2: OR286124).

After three days of growth on PDA medium at 25 °C, the young colonies appeared creamy at first but became yellow as they grew. The colonies had a mucoid and mucilaginous texture, a smooth raised surface, and a complete edge. Under light microscopy, individual cells appeared spherical or coccoid. The isolate had a high ability to utilize and assimilate glucose and trehalose as carbon sources; as well, this isolate was able to utilize sucrose, mannose, maltose, and fructose as carbon sources, but its utilization of cellobiose, sorbitol, and rhamnose was weak. This isolate could not utilize and assimilate galactose, sorbose, lactose, melibiose, arabinose, ribose, and glycine. In addition, the isolate can produce catalase, oxidase, urease, and esterase enzymes but was unable to produce amylase, lipase, lecithinase, protease, and xylanase enzymes. This isolate can grow in the presence of a maximum of 1 M NaCl. It is psychrotolerant (in the range of 6–25 °C) and can grow well at pH 4, 8, and 10.

The type strain was deposited in the culture collection of the Herbarium Ministerii Iranici Agriculturae, under number IRAN 18507F. The name was registered in MycoBank under the number MB852634.

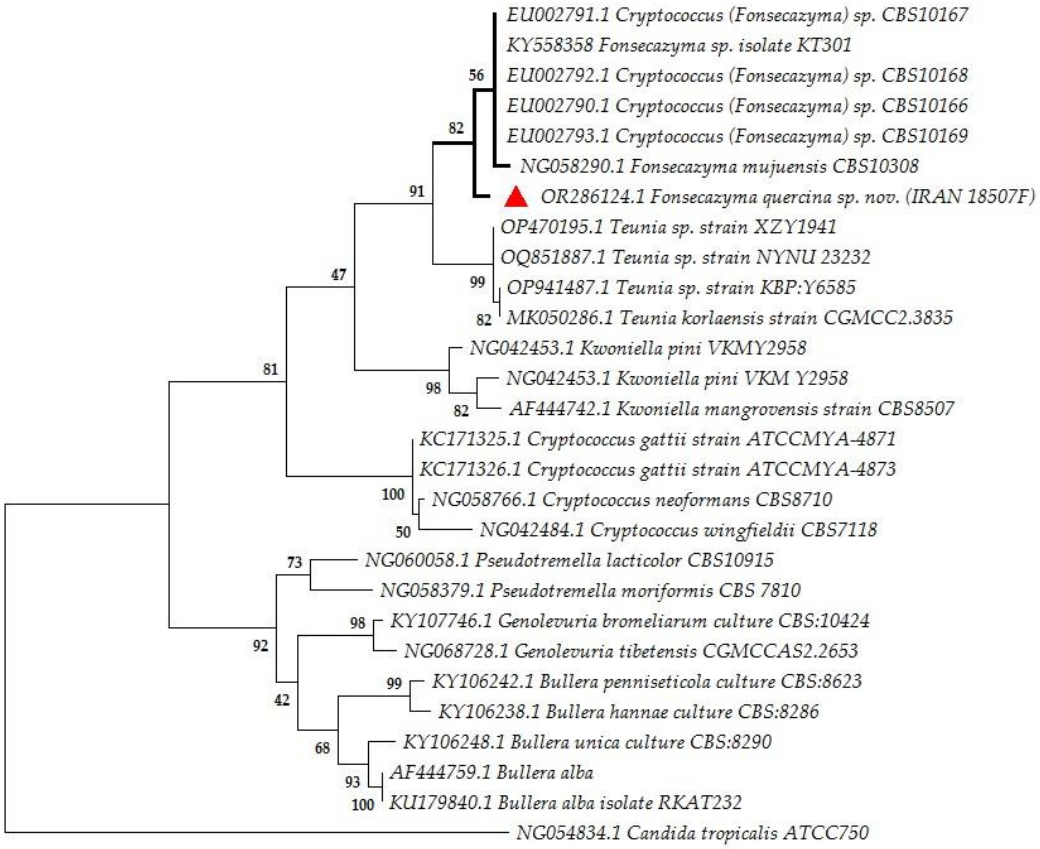


**Fig. 2.** Phylogenetic tree constructed from D1/D2 domains of the large subunit (LSU or 26S) rRNA gene sequences of *Fonsecazyma quercina* sp. nov. strain (IRAN 18507F) isolated from Persian oak tree bark in Kermanshah Province (Iran) and some sequences from the GenBank with maximum likelihood method in MEGA X software. *Candida tropicalis* ATCC750 was included as an outgroup. Bootstrap percentages are 1000 replicates.

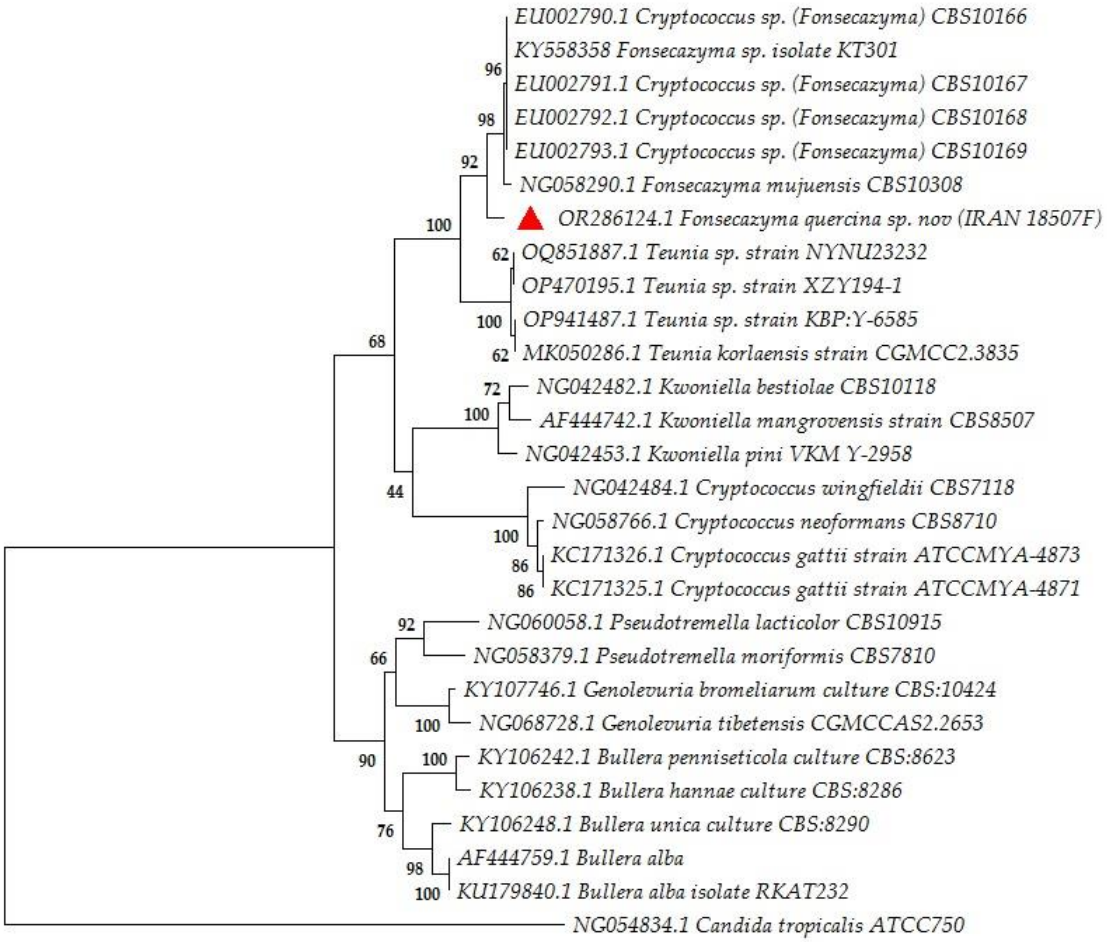


**Fig. 3.** Phylogenetic tree constructed from D1/D2 domains of the large subunit (LSU or 26S) rRNA gene sequences of *Fonsecazyma quercina* sp. nov. strain (IRAN 18507F) isolated from Persian oak tree bark in Kermanshah Province (Iran) and some sequences from the GenBank with maximum parsimony method in MEGA X software. *Candida tropicalis* ATCC750 was included as an outgroup. Bootstrap percentages are 1000 replicates.

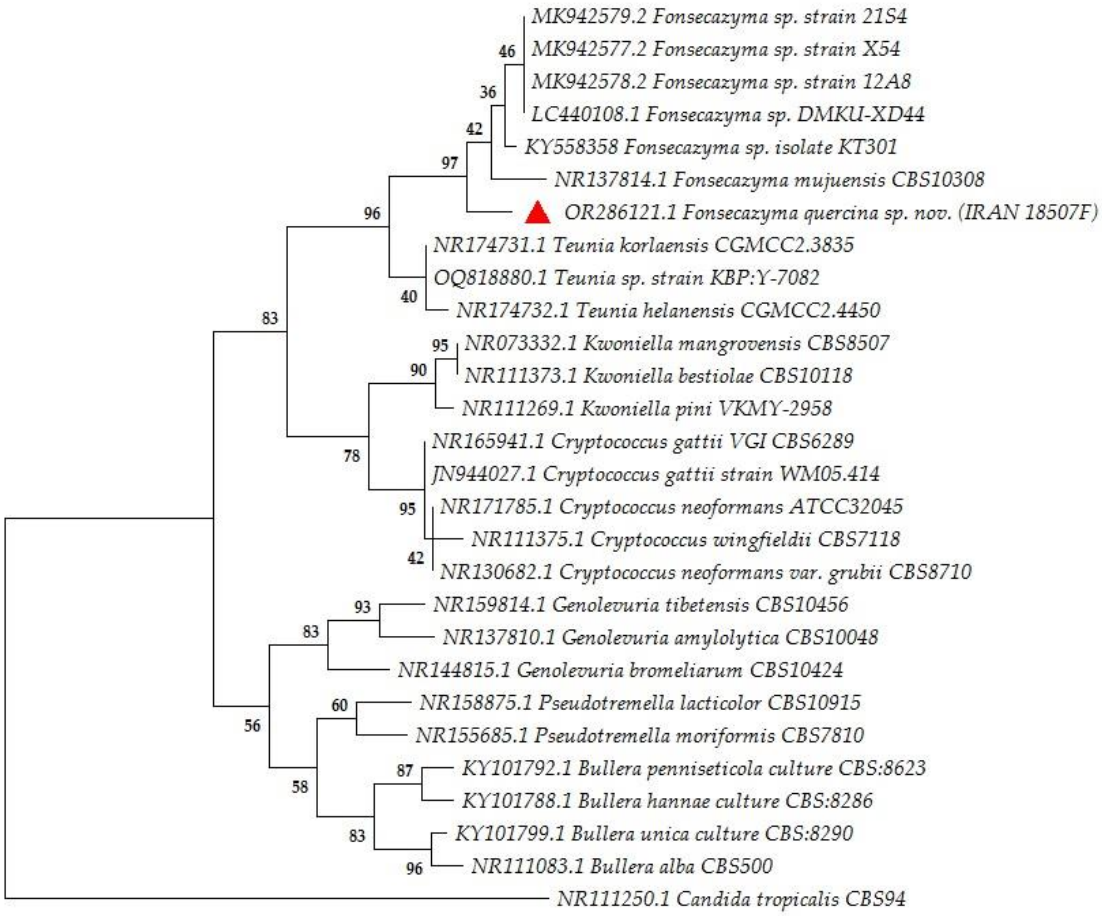


**Fig. 4.** Phylogenetic tree constructed from the internal transcribed spacer (ITS) gene sequences of *Fonsecazyma quercina* sp. nov. strain (IRAN 18507F) isolated from Persian oak tree bark in Kermanshah Province (Iran), and some sequences from the GenBank with maximum parsimony method in MEGA X software. *Candida tropicalis* ATCC750 was included as an outgroup. Bootstrap percentages are 1000 replicates.

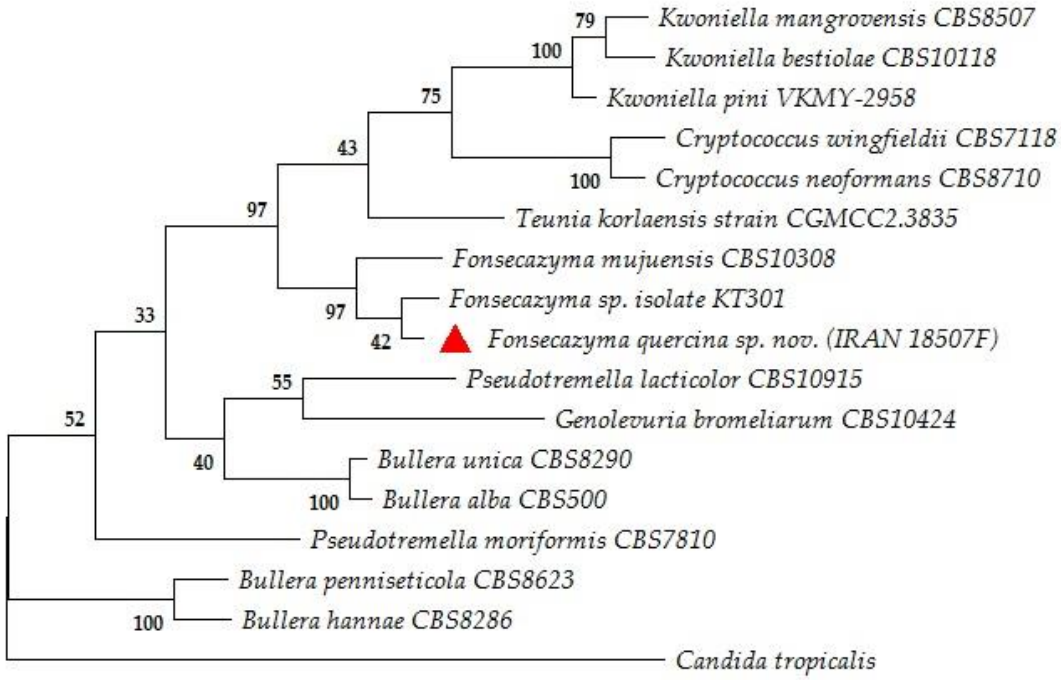


**Fig. 5.** Phylogenetic tree constructed from concatenated ITS and D1-D2 sequences of *Fonsecazyma quercina* sp. nov. strain (IRAN 18507F) isolated from Persian oak tree bark in Kermanshah Province (Iran), and some sequences from the GenBank with maximum parsimony method in MEGA X software. *Candida tropicalis* was included as an outgroup. Bootstrap percentages are 1000 replicates.

- Characteristics of isolate

The ability of the yeast isolate to assimilate and utilize different carbon sources was investigated. For this purpose, 16 different carbon sources were utilized by the yeast isolate. The results showed that, the considered isolate had a high ability to utilize and assimilate glucose and trehalose as carbon sources, which strongly changed the color of the medium from green to yellow in the test tubes by producing acid from these sources. In addition, this isolate used sucrose, mannose, maltose, and fructose as carbon sources but weakly utilized cellobiose, sorbitol, and rhamnose. This isolate was not able to utilize and assimilate galactose, sorbose, lactose, melibiose, arabinose, ribose, and glycine as the only carbon source. In this study, there were no changes in the control treatments used (Table 2).

The ability of the yeast isolate to produce extracellular enzymes was investigated, and the results showed that, the isolate could produce catalase, oxidase, urease, and esterase enzymes but was unable to produce amylase, lipase, lecithinase, protease, and xylanase enzymes. In this study, the clear halo or sediment around the growth site of the yeast isolate on PDA media supplemented with starch for amylase enzyme, Tween 80 for lipase enzyme, lecithin in egg yolk for lecithinase, casein in skim milk for protease, and xylan for xylanase, failed, which indicates the lack of production of the enzymes to hydrolyze the used substrates; but the yeast isolate immediately after being in the 3% hydrogen peroxide solution was able to produce water and oxygen from the degradation of hydrogen peroxide and cause foaming. Additionally, the isolate could oxidize Tetramethyl-p-phenylenediamine dihydrochloride reagent by producing an oxidase enzyme, which appeared in dark blue. By producing the urease enzyme in less than six hr, this isolate was able to alkalinize the medium by breaking down the urea in the medium into ammonia and changing the color of the medium from orange to red. Unlike Tween 80, this isolate created crystal precipitation by hydrolyzing Tween 20 on the surface of the medium by producing an esterase enzyme (Table 2).

The physiological characteristics of the isolate, such as different temperatures, salinity, and acidity were investigated. The results showed that, the studied yeast

isolate could grow in the presence of a maximum of 1 M NaCl (58.44 g/L) and could not grow at concentrations higher than 1 M. The yeast isolate showed its highest growth in neutral acidity (pH = 6). Additionally, the isolate could grow at pH 4, 8, and 10, but it could not grow at acidic pH 2. The studied yeast isolate is psychrotolerant and can grow well in the range of 6–25 °C, but it was unable to grow at temperatures below and above this range. The results of the effect of physiological characteristics are shown in table 2.

**Table 2.** Physiological and biochemical characteristics, as well as extracellular enzymatic activity of *Fonsecazyma quercina* sp. nov. strain (IRAN 18507F) isolated from bark samples of a Persian oak tree (Kermanshah Province, Iran)

| Characteristic | Reaction** | Characteristic | Reaction** | Characteristic | Reaction** |
|---|---|---|---|---|---|
| **Extracellular enzyme** | | Mannose | + | 3M | - |
| Oxidase | + | Maltose | + | **Growth temperature** (°C) | |
| Catalase | + | Cellobiose | w+ | 2 | - |
| Urease | +* | Trehalose | ++ | 6 | w+ |
| Amylase | - | Lactose | - | 10 | + |
| Lipase | - | Melibiose | - | 15 | ++ |
| Esterase | + | Sorbitol | w+ | 20 | ++ |
| Lecithinase | - | Fructose | + | 25 | ++ |
| Protease | - | Arabinose | - | 28 | w+ |
| Xylanase | - | Ribose | - | 30 | - |
| Gelatinase | - | Rhamnose | w+ | **Growth on pH** | |
| **Assimilation of** | | Glycine | - | 2 | - |
| Glucose | ++ | **Growth on NaCl** | | 4 | + |
| Galactose | - | 0.5 M | ++ | 6 | ++ |
| Sucrose | + | 1 M | + | 8 | ++ |
| Sorbose | - | 2 M | - | 10 | + |

[+] Positive; [-] Negative; [++] Strong positive; [w+] Weak positive; M, Molar. * Positive result after six hr; **Result obtained from three independent experiments.

## Discussion

Many parts of the world, including threatened habitats such as natural forests, have not yet been sampled for yeasts, and many others have only been superficially studied. Since forests represent hotspots for yeast biodiversity, therefore, it is imperative to study these environments in places where they may soon disappear due to deforestation, conversion, and anthropogenic climate change (Boekhout *et al*. 2022). In this study, branches of Persian oaks were collected as samples during a field survey of Persian oaks in the Zagros Forest in Kermanshah Province (Iran). After the process of isolating the microorganisms, among the various fungal and yeast isolates that were obtained, one particular yeast isolate (IRAN 18507F) was identified. Upon further analysis of its morphological, biochemical, and physiological characteristics and molecular analysis, it was determined that, this particular isolate represents a new and distinct species. Therefore, the name *Fonsecazyma quercina* sp. nov. for this novel yeast species is proposed here.

Identification of yeast species is currently performed routinely through the analysis of DNA sequences. The comparisons most frequently employed involve the assessment of nucleotide sequence divergence in D1/D2 domains of the nuclear large subunit rRNA gene and ITS, which is situated between the SSU and LSU rRNA genes (Kurtzman & Robnett 1998, Fell *et al*. 2000, Scorzetti *et al*. 2002). In this research, the ITS and D1/D2 regions were subjected to sequencing to determine the yeast isolate species obtained from Persian oak tree bark. The results show that, the closest sequence of the present investigation yeast isolate was *Fonsecazyma* sp. isolate KT301 from the Czech Republic, with 97.58% identity. Moreover, the phylogenetic analysis revealed that, sequences of the isolate in the present study were clustered and formed an independent branch with strong statistical support. Consequently, an analysis of sequences and phylogenetic trees indicates the novelty of the present investigation strain (IRAN 18507F).

Extreme environments have a variety of stressful and changing environmental conditions that affect the growth of microorganisms (Alcaíno *et al*. 2015). Therefore, by evolving various mechanisms, they adapt to these environmental conditions and play critical ecological roles in the ecosystem (Margesin *et al*. 2007). In the present study, the new yeast isolate grew at temperatures between 6–25 °C, suggesting that, it is a psychrotolerant species. Psychrophilic and psychrotolerant bacteria were the main focus of previous studies, but recently, yeasts have also been identified as psychrophilic and psychrotolerant microorganisms (Russell 2006, Thomas-Hall *et al*. 2010, Singh *et al*. 2013). Scientists have always been interested in these yeasts because of their potential in various industries (Alcaíno *et al*. 2015). Therefore, the yeast isolate of this study can also be further investigated in the field of application in various industries. After six hr, the yeast isolate showed the ability to hydrolyze urea in the medium and had a high potential to generate urease enzymes. Urease, an enzyme produced by ureolytic microorganisms, hydrolyzes urea into ammonia and carbon dioxide. Microbial urease has many applications in biotechnology, agriculture, medicine, and construction (Mekonnen *et al*. 2021). In conclusion, this study highlights the importance of investigating underexplored environments to discover novel yeast species. By isolating yeasts from the bark of Persian oak trees in Iran, *Fonsecazyma quercina* sp. nov., a previously unidentified yeast species was successfully identified and characterized here. The novelty of this species was confirmed through DNA analysis, and it was found to exhibit psychrotolerance and produce urease enzymes, indicating its potential for industrial applications. This research underscores the significance of exploring underexplored habitats to uncover new yeast species that possess distinctive and potentially valuable properties.

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