Molecular and phenotypic characterization of ascomycetous yeasts in hypersaline soils of Urmia Lake basin (NW Iran)

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Lachin Mokhtarnejad✉: PhD Student, Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran (l.mokhtarnejad@gmail.com)

Mahdi Arzanlou: Associate Prof., Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Asadollah Babai-Ahari: Prof., Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Abstract

The Urmia Lake National Park (NW Iran) represents a unique ecosystem owing to special ecological conditions prevailing the region. The Urmia Lake National Park comprises a range of normal to extreme environments, with variable range of salinity between 120-300 g/L. The present study was aimed to characterize ascomycetous yeast mycobiota inhabiting hypersaline soils in this region. Soil samples were collected from different sites in Urmia Lake basin as well as islands inside the lake. Yeast isolates were recovered using Dichloran Rose Bengal agar (DRB) + chloramphenicol culture medium. Pure cultures were established using routine microbiological techniques. Yeast isolates were identified based on the sequence data from the D1/D2 domain of nuclear ribosomal DNA. The present study reports six ascomycetous yeast species, viz., *Meyerozyma guilliermondii*, *Metschnikowia sinensis*, *Debaryomyces hansenii*, *Debaryomyces subglobosus*, *Torulaspora delbrueckii* and *Candida baotianensis* from soils of this region. A phylogeny inferred using sequence data based on the D1/D2 region clustered our isolates with the representative sequences for each of the species from GenBank. All of six species identified in this study, represent new records for the mycobiota of Iran. Additionally, *Candida baotianensis*, *Debaryomyces subglobosus*, *Metschnikowia sinensi* and *Torulaspora delbrueckii* are newly recorded from hypersaline soils throughout the world.

Keywords: D1/D2 domain, mycobiota, new records, salinity

شناسایی مولکولی و فنوتیپیکی مخمرهای آسکومیستی در خاک‌های فوق‌شور حوزه دریاچه ارومیه*

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لاچین مختارنژاد✉: دانشجوی دکتری بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، دانشگاه تبریز، تبریز، ایران

مهدی ارزنلو: دانشیار قارچ‌شناسی و بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، دانشگاه تبریز، تبریز، ایران

اسداله بابای اهری: استاد بیماری‌شناسی گیاهی، گروه گیاه‌پزشکی، دانشگاه تبریز، تبریز، ایران

خلاصه

پارک ملی دریاچه ارومیه با توجه به شرایط زیست محیطی خاص حاکم بر منطقه یک اکوسیستم منحصر به فرد محسوب می‌شود. پارک ملی دریاچه ارومیه دارای طیف وسیعی از شرایط محیطی طبیعی تا فرانرمال را با محدوده غلظت نمک بین ۱۲۰ تا ۳۰۰ گرم در لیتر را شامل می‌شود. این مطالعه به منظور شناسایی مخمرهای آسکومیستی ساکن خاک‌های فوق‌شور در این منطقه انجام پذیرفته است. نمونه‌های خاک از بخش‌های مختلف حاشیه دریاچه و همچنین جزایر داخل آن جمع‌آوری گردید. نمونه‌های مخمر با استفاده از محیط کشت رزبنگال آگار+ کلرامفنیکل (DRB) جداسازی گردیدند. جدایه‌های مخمر براساس توالی‌یابی ناحیه DNA D1/D2 ریبوزومی شناسایی گردیدند. در این بررسی، شش گونه *Meyerozyma guilliermondii*، *Metschnikowia sinensis*، *Debaryomyces hansenii*، *Torulaspora delbrueckii*، *Debaromyces subglobosus* و *Candida baotianensis* از منطقه مذکور گزارش می‌شود. هر شش گونه شناسایی شده در این مطالعه، نخستین گزارش برای میکوبیوتای ایران محسوب می‌شود. به علاوه، گونه‌های *Candida baotianensis*، *Debaryomyces subglobosus*، *Metschnikowia sinensis* و *Torulaspora delbrueckii* نخستین گزارش از وقوع این گونه‌ها از خاک‌های شور در دنیا می‌باشد.

واژه‌های کلیدی: شوری، قلمرو D1/D2، میکوبیوتا، گزارش جدید

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Introduction

Yeast species in general exhibit cosmopolitan distribution, occur in diverse ecological niches such as soil, sediments, rock, plant materials, food, insect's elementary tracts, rivers, lakes and oceans (Van Uden 1960, Connell *et al*. 2008). Many yeast species are adapted to reduced a_w conditions and are able to grow in natural habitats with low water activity. These species have been described as osmotolerant yeasts. Osmotolerant yeasts were primarily known as contaminants in the food industry; however, largely remained unknown in natural habitats with low water activity. They have been sporadically recovered from gently saline environments such as estuarine water and sediments (Soares *et al.* 1997), saline soil (Steiman *et al.* 1997), salt marshes (Pitt & Hocking 1985), deep-sea environments (Nagahama *et al.* 2001 a, b) as well as on the phylloplane of plants, especially in the Mediterranean area (Inacio *et al*. 2002). Few reports exist on the occurrence and biodiversity of yeasts in natural hypersaline environments. *Torulopsis famata, Rhodotorula rubra*, *Pichia etchelsii*, *Candida parapsilosis* and *Debaryomyces hansenii* are the most common yeast species that known for their ability to tolerate high NaCl concentrations, growing on salt concentrations above 10–15% (Samson *et al.* 2000, Lages *et al.* 1999). From ascomycetous yeasts, members of the genera *Candida*, *Debaryomyces*, *Metschnikowia* and *Pichia* are the most commonly isolated genera from hypersaline environments (Butinar *et al*. 2005). In general, high levels of Na^+ and sodium do not show an acute toxic effect on osmotolerant yeast cells, and they are able to tolerate high levels of Na^+ and sodium (Prista *et al.* 2005).

Conventional methods for the identification of yeast species mainly rely on morphological and physiological data, which is time consuming and may result in inaccurate identification for the species in question. With the advents of molecular biology, a number of molecular techniques have been developed for robust and quick identification of yeast species as well as other fungal groups (Middelhoven & Kurtzman 2003, Kurtzman & Robnett 2013, Kurtzman *et al*. 2011, Arzanlou & Khoadie 2012, Davari *et al*. 2012, Bakhshi *et al*. 2012, 2014, 2015, Mokhtarnejad *et al.* 2015). Sequence data from the D1/D2 region of large subunit ribosomal DNA (LSU-rDNA) have been widely used to delineate species boundaries in both ascomycetous and basidiomycetous yeasts with great success (Middelhoven & Kurtzman 2003). As a result, extensive phylogenetic studies on both ascomycetous and basidiomycetous yeasts have been carried out based on the D1/D2 sequence (Kurtzman & Robnett 1998, Fell *et al*. 2000, Middelhoven & Kurtzman 2003). Identification of yeast species based on the 1% substitution pattern in D1/D2 sequence is quite reliable (Kurtzman *et al*. 2011); strains showing more than 1% substitutions in the ca. 600-nucleotide D1/D2 domain, are likely to be different species and strains with 0–3 nucleotide differences are either conspecific or sister species (Kurtzman & Robnett, 1998).

The study of yeast identification in extreme environments such as deep-sea hydrothermal vents, water draining, acidic mine tailings and Polar is an active field of research (Butinar *et al.* 2005). Organisms from these habitats might play important role in nutrient cycling, metal detoxification, and food-webs in extreme environments as well as potentially provide unique biomolecules for industry and medicine (Gadanho & Sampaio 2005, Gadanho *et al*. 2006). The Urmia Lake National Park represents a unique ecosystem owing to special ecological conditions prevailing in the region. Urmia Lake basin comprises a range of normal to extreme environments, with variable range of salinity between 120–300 g/L. The present study was aimed to characterize ascomycetous yeast mycobiota inhabiting hypersaline soils in this region.

Materials and Methods

- Collection sites

Urmia Lake is located between West and East Azarbaijan provinces in uppermost northwest corner of Iran, which has been designated as one of the 59

international parks of the world heritage by the United Nations (Asem *et al.* 2014). It is located in closed basin between 37° 04' N and 38° 17' N latitude and 45° E and 46° E longitude in the northwest zone of Iran. Soil samples were collected from different localities in Urmia Lake basin. Aliquots (about 500 g) of surface and deep (from 0.1 to 10 cm) soil samples were collected using sterile shovel. Samples were aseptically collected into sterile tubes, placed at 4° C for 24–48 h, until isolation process.

- Initial culture techniques and yeast isolation.

For yeast isolation, Dichloran Rose Bengal agar (DRB) + chloramphenicol medium was used. Based on positive growth results during previous tests, selective nutrient media were also used; the media were optimized for lower water activity by addition of high concentrations of glycerol (18%) or NaCl (with 10%, 15%, 20% and 32%). Fifty mg/L filter-sterilized antibiotic solution of streptomycin was applied to prevent bacterial overgrowth. Yeast colonies were observed under a stereoscopic microscope OLYMPUS SZX9 (Tokyo, Japan) with 60 X magnification. Representative colonies were selected and isolated for further investigation. Selection was based on morphological characteristics: colony color, surface, margin and elevation. The isolates were stored in YPD agar slants (glucose 20 g/L; Yeast extract 20 g/L; peptone 10 g 1-L; agar 20 g 1-L) and kept at 4° C.

The isolated and identified strains from all sampling sites are maintained in Culture Collection of the University of Tabriz, Iran (CCTU), housed at plant protection department, and in the Industrial Yeasts Collection DBVPG of the University of Perugia, Italy (www.dbvpg.unipg.it).

- DNA extraction

Total genomic DNA was extracted following the protocol of Branda *et al*. (2010). Disruption of the cell wall was achieved by suspending three loopfuls of 48-h cultures in 500 µl of lysis buffer, 500 µl of phenol-chloroform (1:1, pH 8.0) and 150 µl glass beads (diameter = 425–600 mm). The samples were bead-beaten at maximum speed for 3 min. The supernatant (aqueous phase) was recovered and an equal volume of ice-cold ethanol 96%, was added. Samples were stored at –20° C for 1 hour. DNA was spin down by centrifugation. The supernatant was discarded and DNA was washed with ethanol twice and harvested after additional centrifugation step. The DNA samples were suspended in distilled water and stored at –20° C for future use.

- Sequence analysis and phylogeny

Representative strains from each group were chosen for sequence analysis. D1/D2 region of the large subunit the rDNA (LSU) was sequenced for each strain. Internal Transcribed Spacers (ITS-rDNA) including ITS1 + 5.8S + ITS2 2 was also sequenced for those strains which their identity could not be resolved using D1/D2 sequences. D1/D2 region was amplified using the primers RLR3R and V9 (Branda *et al.* 2010). PCR reaction mixtures followed those explained by Mokhtarnejad *et al.* (2015). The PCR amplification condition consisted of 2 min at 94° C for an initial denaturation step, and 34 cycles of denaturation step at 94° C for 30s, annealing at 55° C for 1 min and extension at 72° C for 2 min, with a final extension at 72° C for 5 min. For the amplification of the ITS region, the primers ITS1 and ITS4 (White *et al*. 1990) were applied with the following PCR conditions: 3 min at 95° C for an initial denaturation step and 35 cycles of denaturation step at 95° C for 30s, annealing at 55° C for 30s and extension at 72° C for 30s, with a final extension at 72° C for 5 min.

Amplicons were sequenced by a commercial sequencing facility (Macrogen, Amsterdam, The Netherlands). Raw sequence files were edited manually by using SeqMan™II (DNASTAR, Madison, Wisconsin, USA) and a consensus sequence was generated for each set of reverse and forward sequences. The sequences obtained were compared with those included in the GenBank database using the BLAST (http://www.ncbi.nlm.nih.gov) (Altschul *et al.* 1997).

Phylogenetic analysis was performed using molecular evolutionary genetics analysis (MEGA 6) software version 6, using neighbour-joining method with program default settings (Tamura *et al.* 2013). Bootstrap analysis (1000 replicates) was performed using full heuristic search. The alignment file included 13 sequences (six generated in this study and seven obtained from GenBank) (Table 1).

- Phenotypic characterization of yeasts

For colony forming description we used YM (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose; adjusted to pH 4.0–4.5 with 1 M HCl). Growth in the presence of 10% and 15% NaCl, fermentation of glucose, assimilation of selected carbon and nitrogen sources and production of amyloid extracellular compounds were examined according to Kurtzman *et al*. (2011). Growth at different temperatures (4, 10, 20, 25, 30 and 40° C) was checked in YPD broth for 3 weeks (Kurtzman *et al.* 2011).

Cultures were grown on malt extract agar (MEA, Merck, Germany) for 48 h at 25° C. Suspensions of yeast were prepared in 15 ml sterile water at inoculum density transmittance level 47 ± 2%. All tubes were inoculated with 100 µl of cell suspension. The microplates containing 94 biochemical tests (Table 2) were incubated for 1, 2 and 3 weeks at 25° C. During incubation, yeast respiration in wells containing compounds would result in either as reduction in tetrazolium dye (forming a purple color), or as initiation of growth (increase in turbidity). Colorimetric or turbidity change in each well was referenced against negative control wells. Microplate wells were scored as negative (-), as positive (+) or as borderline (\). The pattern was cross-referenced to a library of species. When an adequate match was found, an identification of the isolate was made (Standard Biolog methodology). In this study the assimilation of seven major and critic carbon sources and four nitrogen sources were tested. Also urea test, amyloid 1%, amyloid 3%, glucose fermentation and growth at medium with 10% sodium chloride and growth at medium with 15% sodium chloride were tested (Kurtzman *et al.* 2011).

In addition, the possible role of yeast isolates in the biodegradation of organic macromolecules, were evaluated by testing their ability in secretion of extracellular enzymes (amylase, esterase, chitinase, pectinase, protease and urease), according to the protocols previously described (de García *et al.* 2007, Brizzio *et al.* 2007, Branda *et al.* 2010).

Results and Discussion

Number of studies on the halophile and halotolerant yeast communities through out of the world are limited (Butinar *et al.* 2005). Accordingly, this study was aimed to explore biodiversity of osmotolerant yeasts in Urmia Lake basin, which provides first attempt towards exploring biodiversity of ascomycetous yeast species in hypersaline environments in the main land of Iran. In this study, we characterized six ascomycetous yeast species from Urmia Lake basin based on a combination of biochemical tests, growth rate and sequence data.

- DNA phylogeny

Approximately, 600 bp was amplified from D1/D2 region for each of the yeast strains. The obtained sequences were aligned together with sequences downloaded from GenBank. The alignment file included 13 sequences (six generated in this study and seven obtained from GenBank) (Table 1). A phylogeny inferred based on D1/D2 sequences clustered our isolates in five species clades, namely, *Candida baotianensis*, *Torulaspora delbrueckii*, *Meyerozyma guilliermondii*, *Metschnikowia sinensis* and *Debaryomyces hansenii/Debaryomyces subglobosus* (Fig. 1).

The BLAST comparison of the D1/D2 region identified strain CCTU 2072 and CCTU 2163 as *Candida baotianensis* and *Torulaspora delbrueckii*, respectively. Both strains showed 100% similarity with the representative sequences for the type strain of each species. The strain CCTU 2114 showed 99% homology with type strain, just with one nucleotide change and was identified as *Meyerozyma guilliermondii.* Strain CCTU 2105 was identified as: *Metschnikowia sinensis* (even

with three nucleotide substitutions with respect to the sequence of the type strain (DQ367881). D1/D2 sequence could not resolve the identity of the strains CCTU and CCTU, both strains showed 100 percent similarity with *Debaryomyces hansenii* and *Debaryomyces subglobosus* in D1/D2 sequence from GenBank. As the results of biochemical testes, were not identical for these strains; sequence data for ITS-rDNA region was generated and compared with sequence data in GenBank. Based on the ITS sequence data, the identity of the strains CCTU 2164 and CCTU 2164 were confirmed as *Debaryomyces subglobosus* and *Debaryomyces hansenii*, respectively.

Table 1. A list of yeast strains and their GenBank accessions used in phylogenetic analyses

Species	Culture accession No.	GenBank accession No.
Candida baotianensis	CCTU 2072	KU307216
Debaryomyces hansenii	CCTU 2164	KU215895
Debaryomyces subglobosus	CCTU 2063	KU307225
Metschnikowia sinensis	CCTU 2105	KU307214
Meyerozyma guilliermondii	CCTU 2114	KU307212
Torulaspora delbrueckii	CCTU 2163	KU215899
Debaryomyces subglobosus	CBS792	AM261069
Debaryomyces hansenii	FJ455103.1	U45808
Pichia guilliermondii	Y-2075	U45709
Torulaspora delbrueckii	NRRL Y-866 26S	U72156
Candida baotianensis	BY-29	HM588005
Metschnikowia sinensis	JQ921015.1	DQ367881
Cryptococcus aerius	CBS 155T	AF075486

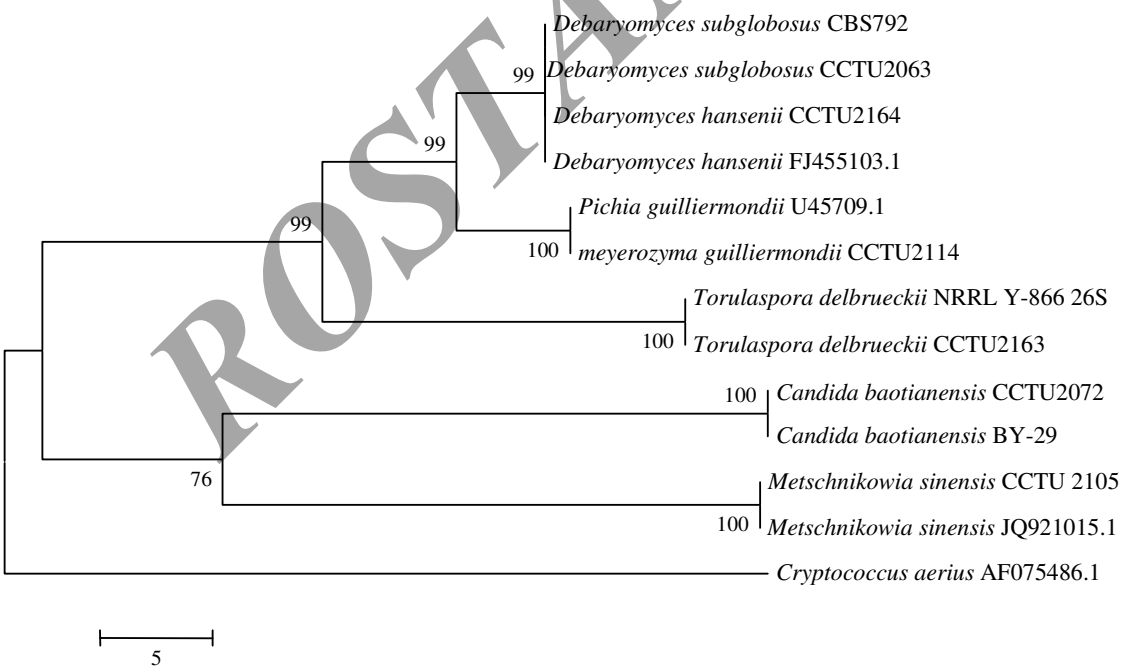

Fig. 1. A neighbour joining phylogenetic tree obtained from the D1/D2 sequence data. Bootstrap support values from 1000 replicates are indicated on the nodes. The tree was rooted to *Cryptococcus aerius* (GenBank accession No. AF075486.1). Scale bar indicates 0.02 substitutions per site.

* CCTU Collection code of Tabriz University

- Phenotypic characterization of yeasts

We performed 22 physiologic tests as shown in Table 2. Except *T. delbrueckii*, all of the strains could assimilate all carbon and nitrogen sources tested in this study. *T. delbrueckii* was not able to assimilate D-arabinose. The results of growth at high concentration of sodium chloride showed that all of the species, except *T. delbrueckii*, were able to grow at medium with 15% sodium. *Candida baotianensis* and *Torulaspora delbrueckii* did not grow at temperature above 30° C, which was in agreement with Kurtzman *et al*. (2011). All strains had negative reaction to Urea test, Amyloid 1%; Amyloid 3%; also all of them except *Debaryomyces hansenii* were able to ferment glucose (Table 2).

Table 2. Phenotypic characterization of yeasts strains isolated from soils of Urmia Lake basin in this study

Species	**Assimilation of C/N source**																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Candida baotianensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+
Debaryomyces hansenii	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Debaryomyces subglobosus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+
Meyerozyma guilliermondii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+
Metschnikowia sinensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Torulaspora delbrueckii	+	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-	+

1. Growth at 4° C, 2. Growth at 10° C, 3. Growth at 20° C, 4. Growth at 30° C, 5. Growth at 40° C, 6. Growth at medium with 10% Sodium chloride, 7. Growth at medium with 15% Sodium chloride, 8. Glocose, 9. D-xylose, 10. L-arabinose, 11. D-arabinose, 12. Celobios, 13. Glycerol, 14. Xylitol, 15. Nitrate, 16. Cadaverine, 17. Ethyilamine, 18. Lisin, 19. Urea test, 20. Amyloid 1%, 21. Amyloid 3%, 22. Glucose fermentation

Traditionally, identification and classification of yeasts has been largely based on physiological and biochemical characteristics (Kurtzman 2014). Fermentation, assimilation of different carbon and nitrogen sources, requirements for vitamins, growth at various temperatures, hydrolysis of urea, and resistance to antibiotics are considered as the key tests for species identification in yeasts (Barnett *et al*. 2000, Kurtzman & Fell 1998). The identification of yeast species based on physiological methods is difficult, time consuming and may result in inaccurate identification for the species in question. Nowadays, development of a database (barcode) of easily determined gene sequences from rRNA (D1/D2) and (ITS), make many researchers to identify species easily and more accurately without urgent need for physiological tests (Kurtzman 2014). Complementary data on physiological abilities, metabolic capacities, and physical-chemical information are essential for understanding of yeast autecology and can be used to predict yeast behavior in environment condition.

A brief description is provided for each of the species identified in this study as below:

Candida baotianensis F.-L. Hui & T. Ke (2012)

After three days of growth in YM broth at 25° C; cells are ovoid to ellipsoid, occur singly or in pairs. Budding is multilateral. Pseudohyphae are formed, but true hyphae are not formed. At 25° C; on common sporulation media including D-glucose, D-galactose, maltose (week, delayed) and sucrose (week, delayed) are fermented.

This species is only known from forest soil samples in China.

Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij (1952)

After three days of growth in YM broth at 25° C; cells are spherical to short-ovoid and occur singly, in

pairs, or in short chains. Sediment is formed. Physiological properties have been summarized in Table 2; *D. hansenii* is not able to grow at Vitamin-free culture media; starch formation test and DBB test are negative. The maximum cardinal growth temperature is 30° C. *Debaryomyces hansenii* is a member of a closely related group of species that are difficult to separate using phenotypic tests, and it is likely that strains within this species group have been misidentified in the preceding studies, which might bias the ecological assessment for this species. (Price *et al*. 1978) suggested that, *D. subglobosus* as synonym of *D. hansenii* and *D. fabryi.* (Groenewald *et al*. 2008) proposed the reinstatement of *D. hansenii*, *D. fabryi* and *D. subglobosus* based on data from PCR fingerprinting, DNA re-association and partial ACT1 gene sequence analysis. We used sequence data from ITS-rDNA region to differentiate this species from *D. subglobosus*, the other closely related species.

Debaryomyces hansenii is widespread in nature and has been commonly isolated from soil, the phylloplane of certain plant species (Fonseca & Inacio 2006), the marine environment (Fell 1976), fermented soy products (Hanya & Nakadai 2003), cheeses (Frölich-Wyder 2003) and similar products, many of which are high in salt (Prista *et al*. 2005).

Debaryomyces subglobosus (Zach) Lodder & Kreger-van Rij (1952)

After three days of growth in YM broth at 25° C; cells are spherical, sub-spherical or ovoid, and occur singly or in pairs. The spores are spherical and have a warty wall. Sediment is formed. Like *D. hansenii* this species is not able to grow at Vitamin-free medium. Starch formation test and DBB tests for this species are negative. The maximum cardinal growth temperature is 40° C. *D. subglobosus* is physiologically similar to *D. hansenii* and *D. fabryi*, but it is genetically distinct from the latter two species based on DNA reassociation experiments and ITS sequence data.

Type strain has been isolated from diseased nails in Austria. Ecology of this species is unknown.

Meyerozyma guilliermondii (Wickerham) Kurtzman & M. Suzuki (2010)

After three days, growth on 5% malt extract agar at 25° C; cells are ovoid to elongate and occur singly, in pairs, or in short chains. Colonies are smooth to wrinkle in texture and tannish-white in color.

Differentiation of *M. guilliermondii* from closely related species such as *M. caribbica* and *Candida carpophila* is difficult. *Meyerozyma caribbica* and *M. guilliermondii* show no differences on standard fermentation and growth tests. However, they can be separated based on ascospore morphology which are Saturn-shaped in *M. caribbica* and hat-shaped in *M. guilliermondii*. As ascospore formation is rare in both species on sporulating culture media, their delineation mainly rely on sequence data and nuclear DNA re-association experiments (Kurtzman *et al*. 2011).

Meyerozyma guilliermondii has worldwide distribution and has been isolated from diverse types of substrates including insect frass in USA (Wickerham 1966), flowers, fruits and other food products, mud of estuaries in Brazil (Soares *et al*. 1997) as well as and as opportunistic pathogens of humans and animals (Kurtzman *et al*. 2011).

Metschnikowia sinensis Xue & Zhang (Xue *et al.* 2006)

After three days of growth in YM broth at 25° C; cells are globose to ovoid and occur singly or in pairs. Budding is multilateral. Spherical chlamydospores (i.e. pulcherrima cells) are abundant. This species has been described from jujube (*Zizyphus jujuba*, *Rhamnaceae*) fruit in China (Xue *et al*. 2006). The presence of yeast species in the *M. pulcherrima* clade on the surface of fruit is not unusual, but the selective factors favoring this distribution remain unknown (Kurtzman *et al*. 2011). The closest relative to *M. sinensis* is *M. fructicola*; however, these two species differ from each other by 15 substitutions in the sequence of the domains D1/D2 of the LSU rRNA gene.

Torulaspora delbrueckii (Lindner) Lindner (1904)

After three days growth on YM broth at 25° C; cells are spherical to ellipsoidal and occur singly or occasionally in pairs. Growth is butyrous, dull to glistening, and tannish-white in color. This species can ferment glucose and can grow on Vitamin-free medium as well.

In the past several *Torulaspora* species have been described based on their abilities to ferment and to assimilate carbon compounds (Kurtzman *et al*. 2011). However, sequence data from D1/D2 LSU rRNA gene and DNA re-association tests have shown several well-known species such as *Torulaspora rosei*, *T. fermentati* and *T. vafer* being conspecific with *T. delbrueckii* (Price *et al*. 1978). *T. delbrueckii* is widely distributed in nature and has been isolated from soil, fermenting grapes and other berry juices, agave juice (Lachance 1995), tea-beer (Teoh *et al.* 2004) and tree bark (Bhadra *et al.* 2008).

As noted above yeast isolates from hypersaline environments with ascomycetous affinity, included members of the genera *Candida*, *Debaryomyces*, *Metschnikowia* and *Pichia* (*Meyerozyma*) (Butinar *et al.* 2005). Different species of the genus *Pichia* have been found in similar habitats, with *P. membranifaciens* being the most common species (Soares *et al.* 1997). The opportunistic pathogen *P. guilliermondii* was the only species of this genus isolated from the salterns sampled in our work. *D. hansenii* also is osmotolerant yeast and able to tolerate high levels of Na^+ and sodium. But there is no report available on the occurrence of *Candida baotianensis*, *Debaryomyces subglobosus*, *Metschnikowia sinensis* and *Torulaspora delbrueckii* in hypersaline soils; hence, our study provides first records on the isolation of these species from hypersaline environments.

The ability of studied strains in assimilation of different carbon and nitrogen sources appeared to be related with their ecology: the species with an apparent global distribution exhibited a wider assimilation pattern and may be considered as facultative oligotrophs. Species with a more restricted distribution, or even endemic to the hypersaline environments, showed a narrower metabolic profile, suggesting a rather oligotrophic tendency. Similar pattern has been reported for some black meristematic fungi (e.g. *Friedmanniomyces endolithicus*) endemic of Antarctic rocks (Selbmann *et al.* 2005). They not only showed scant metabolic competences, but their incredible slow growth rate also cannot be implemented even when cultivated in rich substrates.

The synthesis of extracellular polymers is a well established property of cryptoendolithic fungi (i.e. fungi colonizing empty spaces or pores inside rocks) (Selbmann *et al.* 2002, Onofri *et al.* 2007). In our investigation, *Meyerozyma* showed extracellular enzymatic activity. *M. guilliermondii* also produces riboflavin (Boretsky *et al*. 2007, Sibirny 1996) and xylitol (Leathers 2003, Onishi & Suzuki 1969, Rodrigues *et al.* 2006). Both compounds are currently produced by other yeasts; however, *M. guilliermondii* is more pertinent for commercial production of these compounds. Riboflavin is used worldwide for human nutrition and as a supplement in animal feed. Xylitol is a sweetener which is used in sugar-free products, especially in products such as chewing gum.

Putting all of the data together, this study represents first contribution to the biodiversity of yeast species in hypersaline soils for the mainland of Iran. All six species are new to the mycobiota of Iran and four out of six species represents new records for the hypersaline soils throughout the world. Further studies are needed to understand the ecology of these species.

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