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Identification of yeast species from uncultivated soils by sequence analysis of the hypervariable D1/D2 domain of LSU–rDNA gene in Kermanshah province, Iran

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Abstract: Yeasts are a polyphyletic group of ascomycete and basidiomycete fungi characterized by having a unicellular growth phase and sexual stages that are not enclosed in fruiting bodies. An attempt was made to identify yeast species in uncultivated soils collected from different areas of Kermanshah province, Iran, by analyzing hypervariable D1/D2 domain of the large subunit (LSU) rDNA gene sequence and comparing the sequences with that available in NCBI database. In this study, 25 soil samples were analyzed and eight species including *Rhodotorula toruloides* (KP324973)*, *Trichosporon coremiiforme* (KP055040)*, *Naganishia uzbekistanensis* (KP324959), *Candida catenulata* (KP324968), *C. paracilopsis* (KP324965)*, *C. boidinii* (KP324 962)*, *Lecythophora* sp. (KP336745)* and *Meyerozyma guilliermondii* (KPKP324971, KP324978) were identified. Phylogenetic analysis based on D1/D2 regions allowed us to establish the precise taxonomic placement of each species. The Canonical correspondence analysis (CCA) indicated that elevation, EC, pH, and clay were important environmental parameters influencing basidiomycete yeast distribution in uncultivated soils. In this study, the presence of eight species is confirmed that asterisk species are the new records for the mycobiota of Iran.

Key words: Yeasts, rDNA genes, Phylogeny, Canonical correspondence analysis

INTRODUCTION

Yeasts are a polyphyletic group of ascomycete and basidiomycetous fungi characterized by having a unicellular growth phase and sexual stages that are not enclosed in fruiting bodies (Kurtzman & Fell 1998). Most yeasts are saprotrophs that assimilate plant or animal–derived organic compounds. Yeasts are usually decomposers, however, some species are pathogens of plants and animals. They facilitate production of fermented foods and alcoholic beverages, production of secondary metabolites, vitamins, organic acids, carotenoid and recombinant vaccine (Hierro et al. 2004). A large number of yeasts catabolize benzene compounds which may ease cleaning up spills of industrial chemicals and human friendly biosynthesized new compounds (Middelhoven 1993). Kurtzman and Fell (1999) reported approximately 100 genera of yeasts comprising more than 700 species have been identified which is less than 1% of the world species. Many yeasts are isolated from soils and have cosmopolitan distribution which spray them in both natural and artificial substrates (Phaff et al. 1966, Spencer & Spencer 1997).

Some species, including *Debaryomyces* (*Schwanniomyces*) *occidentalis*, *Lipomyces* spp., *Schizoblastosporion starkeyihenricii*, *Cryptococcus* and certain *Rhodotorula* and *Sporobolomyces* species, are isolated exclusively from soils (Hagler & Ahearn 1987). The occurrence of yeasts in the soil has been studied in various parts of the world (Jensen 1963, Vishniac 1996, Dmitriev et al. 1997). Slavikova and Vadkertiova (2000) showed that *Cryptococcus laurentii*, *Cystofilobasidium capitatum*, *Leucosporidium scottii*, *Rhodotorula aurantiaca*, and *Trichosporon cutaneum* were the most frequently isolated species from the samples taken in soil forest of Slovakia.

Intensive researches on diversity of yeasts in extreme environments have been conducted (Gadanho et al. 2006, Nagahama et al. 2001, Tosi et al. 2002). Mok et al. (1984) and Baublis et al. (1991) isolated yeasts from tropical and Antarctic soils respectively. Connell et al. (2006) studied the distribution and abundance of yeasts in Antarctica soil from Taylor Valley. The diversity of microorganisms is the building block of the ecological balance of the biosphere (Slavikova & Vadkertiova 2000). Monitoring fungal diversity has been fundamental to the increased interest of microbiological functions. Yeasts are important members in many ecosystems and form a significant contribution to the biodiversity (Fleet, 1998).

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The soil is the ultimate repository to store and develop of certain species of yeasts (Phaff and Starmer 1987). An accurate identification of species in the ecosystem and proper molecular tools are necessary factors to determine the validity of studies in yeast ecology (Kurtzman & Fell 2006).

The correct identification of yeasts is not always easy and morphology does not suffice to distinguish those fungal species which have very few discernible morphological traits. Identification of yeasts species is traditionally carried out by help of morphological and physiological traits (Kurtzman & Fell 1998) or by biochemical techniques (Barnett et al. 2000). These criteria are poorly sufficient for species with very similar morphological features and difficult to identify at the species level (Deak & Beuchat 1996). Conventional yeast identification based on phenotypic characteristics is often misleading and inconclusive, and usually needs to be corroborated by molecular methods. DNA–DNA hybridization (Vaughan Martini & Martini 1987, Torok et al. 1993), electrophoretic karyotyping (Vezinhet et al. 1990, Guillamon et al. 1996) and RFLPs of chromosomal DNA (Versavaud & Hallet 1995) tools have been developed to identify and characterize yeast species however have become less popular compared to other faster and easier molecular techniques. Within these molecular techniques, PCR based methods have permit both intra–species differentiation and species identification of yeast isolates (de Barros Lopes et al. 1998).

Mokhtarnejad et al (2015a) introduced 25 species of basidiomycete yeasts from soil of Iran. They also recorded six new species of ascomycete yeasts such as (*Meyerozyma guilliermondii*, *Metschnikowia sinensis*, *Debaryomyces hansenii*, *D. subglobosus*, *Torulaspora delbrueckii* and *Candida baotianensis*) that isolated in hypersaline soils of Urmia Lake basin (North West of Iran) (Mokhtarnejad et al. 2015b). In other study, Mokhtarnejad et al. (2016) showed that *Solicoccozyma aeria* is the dominant yeast species in hypersaline soils of Urmia Lake National Park (Mokhtarnejad et al. 2016). Also, twenty–one species belonging to the genera *Cystobasidium*, *Holtermanniella*, *Naganishia*, *Rhodotorula*, *Saitozyma*, *Solicoccozyma*, *Tausonia*, *Vanrija*, and *Vishniacozyma* isolated and identified from this area.

This study was aimed to identify yeast strains present in uncultivated soils using molecular methods and evaluation of environmental parameters on their distribution.

MATERIALS AND METHODS

Sample collection and soil analysis

Twenty five soil samples (five soil sub–samples from each area were collected and mixed) were collected from 5–10 cm depth, sieved 2 mm, 40 and 60 mesh sieves in Kermanshah province (Fig 2). Soil texture was analyzed using Gee and Bauder (1986) method. pH of each sample was measured after preparation of soil suspension (one gram of soil to five mL deionized water) using pH meter (Thomas 1996) and total soluble salts measured using electrical conductivity meter (Rhoades 1996).

Isolation of yeasts

According to the procedure is described in Waksman & Fred, 1922) ten g of soil samples were placed in 90 mL of 0.1% water-–agar containing 100 ppm NPX (Nonylphenyl polyethylene glycol ether containing a concentration of 10.5 moles of ethylene oxide), mixed and serially diluted to 10^{-2} to 10^{-5} and 1 mL of each solution flooded on yeast extract malt extract agar (YM agar). Yeast malt agar prepared by adding 3 g yeast extract, 3 g malt extract, 5 g peptone, and 10 g glucose to one liter of distilled water.

These media were amended with HCl (0.7 $ml.L^{-1}$) and chloramphenicol (0.1$g.L^{-1}$). Plates were incubated at 25–27 ºC for 3–5 days for a fine colony development. Morphological properties were determined for each isolate using Yamamoto et al. (1991) methods. The production of pigments and shape of colonies of yeast was examined.

Molecular identification

DNA extraction

The isolates were grown for approximately 24 h at 25 °C in YM medium. For each isolate, one loop of cells suspended in 100 µL distilled water and heated for 5 min at 99 °C (Suh et al., 2008). The DNA extraction was performed using the methods described by Suh et al. (1998). The amount of DNA obtained was estimated by a NanoDrop spectrophotometer (CARY100 scan Varion, Australia).

DNA amplification and sequencing

The D1/D2 domain at the 5' end of the LSU rRNA gene was symmetrically amplified with primers NL–1 (5'–GCATATCAATAAGCGGAGGAAAAG–3') and NL–4 (5'–GGTCCGTGTTTCAAGACGG–3') (O'Donell, 1993, Kurtzman & Robnet, 1998). Amplifications were performed in a T–Personal thermocycler (Biometra, Germany). The PCR mixture contained: 10–20 ng of template DNA, 1 µM of each primer, 100 µM of dNTPs, 0.4 U *Taq* DNA polymerase (CinnaGen, Iran), 1.5 mM of $MgCl_2$, 2.5 µL of 10X PCR buffer in a reaction volume of 25 µL. All PCRs consisted of 1 cycle of 95 °C for 5 min; 35 cycles of 95 °C for 1 min, 55.5 °C for 2 min, 72 °C for 2 min; and a final cycle of 72 °C for 10 min. Successful amplification was confirmed by gel electrophoresis (1 h at 80 Volts) on 1.0% agarose gels in 1X TBE buffer. Gels were stained using ethidium bromide and DNA fragments were visualized under UV light.

Sequencing of PCR product

The amplification products of all specimens were purified through GenJET PCR purification kit (Fermentas, UK) to remove excess primers and nucleotides. PCR products were sequenced (Tech Dragon, Hong Kong) in forward and reverse

orientation using the primers used for amplification and a dye terminator cycle sequencing kit (BigDye sequencing kit, Applied Biosystems, USA) on an ABI377–96 automated sequencer (Applied Biosystems, USA) according to the manufacturer's instruction. All sequences of the D1/D2 domain of the LSU–rDNA gene deposited at the GenBank (NCBI, http://www.ncbi.nlm.nih.gov/Entrez) (Bethesda, MD, USA).

Phylogenetic analysis

Closest matches to each sequence were determined using the BLASTN sequence similarity search tool in GenBank (Altschul et al. 1997, Thompson et al. 1997). Multiple alignments were performed with CLUSTAL W (Thompson et al. 1996) using default settings and were manually optimized with BIOEDIT v.7.0.9 (Hall 1999). Phylogenetic analyses were performed with Mega 4 (Kumar et al. 2004) using maximum parsimony (MP) and neighbor–joining (NJ) with the Kimura 2–parameter (K2P) model. The complete deletion method was employed in gap handling for all alignment sites. All sites containing alignment gaps were removed from the analysis before calculations and then treated as missing data. The confidence of branching was assessed by computing 500 bootstrap re–samplings (Felsenstein 1985). The final tree and matrix of sequences were submitted to TreeBASE (University at Buffalo, USA; http://www. treebase.org).

Statistical analysis

Canonical correspondence analysis (CCA) was used to examine the significance of soil texture, soil pH, total soluble salt and elevation on the distribution and abundance of yeast genotypes in different areas of Kermanshah province using CANOCO software 4.5 (Microcomputer Power, Ithaca, NY, USA). Soil parameters were tested for significant differences between the sites using Turkey's Post Hoc Test.

RESULTS

Soil analysis

The results of physico–chemical analyses on soil samples of different areas of Kermanshah province are shown in Table 1. Statistical analysis using Tukey's Post Hoc Test showed differences in soil structure parameters and amount of EC and pH among sites. The collected soils were mostly in sandy, loamy sand and alkaline pH. These soils were considered as basic and non–saline soils.

Environmental parameters

The relative importance of the determined environmental parameters to the distribution of yeasts in different areas of Kermanshah province is illustrated in CCA biplot (Fig. 1) where environmental parame–ters and yeasts are arranged on the basis of their scores on two axes. The relative position of the arrows reflects the relationship of the axes with the environmental parameters. Eigenvalues for axes 1 and 2 were 0.2 and 0.69, respectively.

Table 1. Sources, GPS information and physico–chemical parameters of soil samples from different areas of Kermanshah province, Iran

Location	Latitude	Longitude	Elevation (m)	Specimen code	pH	EC (dSm^{-1})	Sand (%)	Clay (%)	Silt (%)	Yeasts
Kangavar (21)	38^0 4′.12 N	38^0 10′.47 E	1481	21	7.30c	0.495b	55.44e	4.56a	40c	B
Sarab Bidsorkh	38^0 4′.12 N	38^0 9′.0 E	1493	22	7.45c	0.381b	43.44c	27.34c	29.22b	–
Sahneh (23)	38^0 4′.27 N	38^0 11′.16 E	1334	23	7.52d	0.338a	62.80e	15.20b	22b	B
Bisetum (24)	38^0 4′.20 N	38^0 4′.47 E	1303	24	7.15b	0.923c	66f	2a	32b	B
Shahrokh village (25)	38^0 3′.36 N	38^0 9′.36 E	1328	25	7.20b	0.551b	55.44e	7.44a	37.12c	A
Kani sharif village	38^0 3′.36 N	38^0 15′.· E	1327	26	7.31c	0.486b	59.50e	0.56a	39.94c	–
Ravansar	38^0 3′.36 N	38^0 23′.24 E	1391	27	7.27b	0.207a	55.50e	27.4c	1.10a	–
Biashush	38^0 3′.36 N	38^0 31′.12 E	1650	28	7.26b	0.298a	51.88d	31c	17.12b	–
Javanrood (20)	38^0 3′.36 N	38^0 30′.0 E	1272	29	7.61d	0.449b	59.44e	11.44b	29.12b	B
Chambegar (11)	38^0 3′.36 N	38^0 29′.24 E	1292	30	7.38c	0.249a	22.80a	49.20d	28b	B
Nazarcheshmeh (10)	38^0 3′.36 N	38^0 28′.48 E	1458	31	7.43c	0.271a	15.20a	48.8d	36c	B
Davaleh (9)	38^0 3′.39 N	38^0 28′.15 E	1243	32	7.04a	0.476b	54d	27.40c	18.60b	A
Salas	38^0 3′.36 N	38^0 25′.37 E	1278	34	7.50d	0.282a	52d	25c	23b	–
Chamzereshk	38^0 3′.39 N	38^0 23′.2 E	1427	35	7.37c	0.247a	42c	29.84c	28.16b	–
Palan (15)	38^0 3′.36 N	38^0 19′.1 E	1791	36	6.95a	0.415b	50d	2a	48d	A
Kapar babaei (4)	38^0 3′.38 N	38^0 19′.41 E	857	37	7.07a	0.656b	47.44d	13.44b	12.12a	B
Sarpol Zahab (17)	38^0 3′.25 N	38^0 18′.40 E	574	38	7.29b	0.434b	51.44d	30c	18.56b	A
Sarpol Zahab (13)	38^0 3′.25 N	38^0 11′.31 E	543	39	7.19b	0.422b	62e	18.72b	19.28b	A
Habibvand	38^0 3′.28 N	38^0 6′.21 E	778	40	7.29b	0.494b	58e	2a	40c	–
Karande Gharb (22)	38^0 3′.36 N	38^0 58′.33 E	1501	41	7.81d	1.104c	77.44f	3.44a	25.12b	B
Karande Gharb (16)	38^0 3′.39 N	37^0 55′.19 E	1535	42	7.28b	0.361b	34b	6a	60d	B
Eslam Abad (1)	38^0 3′.46 N	37^0 45′.46 E	1372	43	7.80d	0.349a	59.44e	18b	22.56b	B
Eslam Abad (5)	38^0 3′.50 N	37^0 46′.5 E	1401	44	7.32c	0.249a	58.66e	5.44a	36c	A
Mahin Dasht (3)	38^0 4′.1 N	37^0 57′.57	1473	46	7.54d	0.472b	26.50b	18.5b	55d	A

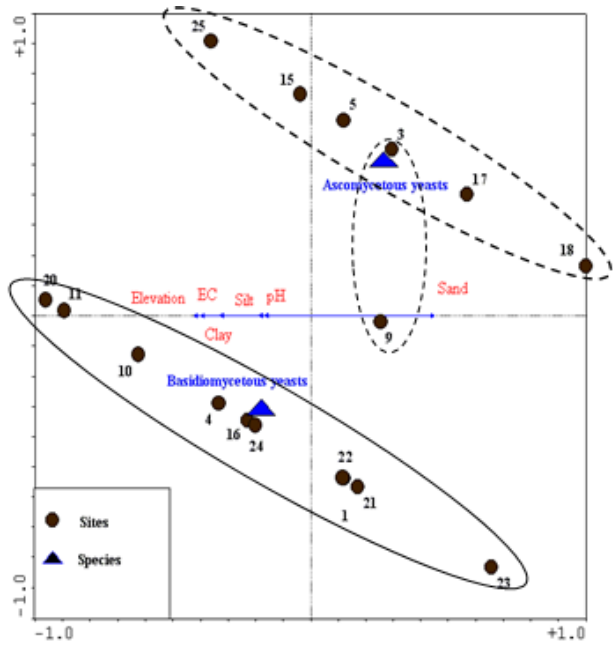

Fig 1. Correspondence analysis (CA) of the ascomycete and basidiomycete yeast communities found in uncultivated soils. The Eigenvalues of the first and second axes in the two–dimensional ordination diagrams are as: CA1 = 0.20 and CA2 = 0.69. Dash line refer to ascomycete yeasts and continuous line refer to basidiomycetous yeasts.

The yeast species and environmental variables are significantly correlated for axis one. Although pH, silt, clay, elevation and EC are negatively correlated with the first CCA axis, sand showed a positive correlation (Fig. 1). Basidiomycete yeasts on the left side of the diagram showed more prevalent in soil with high clay and silt percentage compared to ascomycete yeasts (Fig.1). Distribution of ascomycete yeasts was independent of environmental parameters (Fig.1).

Isolation of yeast colonies from soil samples

In this study, 25 soil samples of different areas of Kermanshah province were collected and studied for the prevalence of yeast species that 28 yeast isolates were obtained (Fig. 2). These isolates were identified by morphological characteristics (Fig. 3). The isolates were comprised of six genera (*Rhodosporidium*, *Trichosporon*, *Cryptococcus*, *Candida*, *Lecythophora*, *Meyerozyma*) and eight species of yeasts. Out of 28 isolated and identified species, 18 species are belonged to Basidiomycota while 10 species are belonged to ascomycete yeasts. Basidomycete species constituted 74% of the eight isolated species. *Naganishia uzbekistanensis* (35.71%) and *Candida* species (25%) were the most prevalent species followed by *Trichosporon coremiiforme* (17.85%) and *Rhodotorula toruloides* (10.71%). The remaining species were less frequently isolated. Some species including; *Rhodotorula. toruloides*, *T. coremiiforme*, *C. catenulata*, *C. boidinii* and *Lecythophora** sp. are new species for mycobiota of Iran. Voucher specimens deposited in the Culture Collection of the Ministry of Jihad–e–Agriculture (IRAN) Located at the Iranian Research Institute of Plant Protection, Tehran, Nos: IRAN 2634C (*Rhodotorula toruloides*), IRAN 2635C (*Trichosporon coremiiforme*), IRAN 2636C (*Naganishia uzbekistanensis*), IRAN 2637C (*C. catenulata*), IRAN 2638C (*C. boidinii*), IRAN 2639C (*Lecythophora* sp.) and IRAN 2640C (*Meyerozyma guilliermondii*).

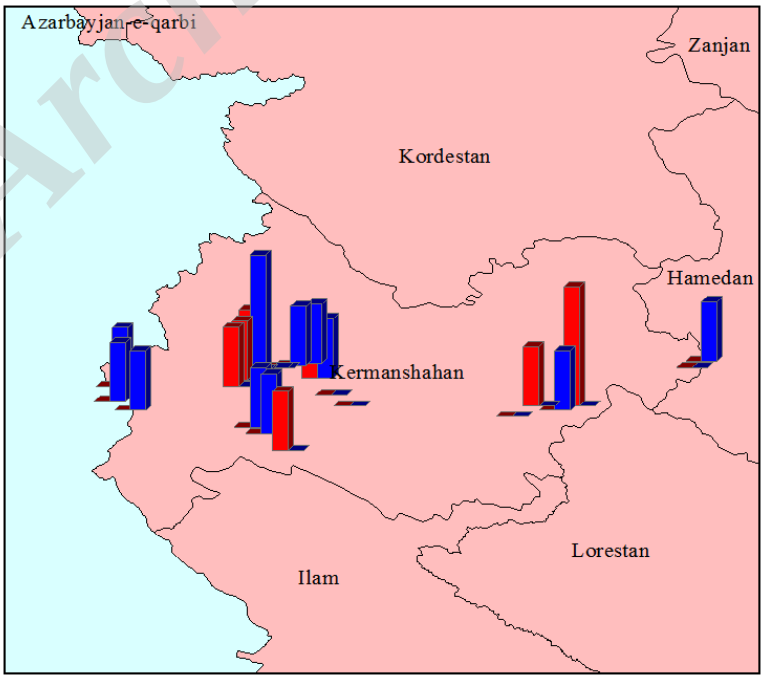

Fig. 2. Distribution of identified yeasts species in uncultivated soil in Kermanshah province (GIS Map). Blue: basidiomycete yeasts and Red: ascomycete yeasts.

Morphological and Molecular characters

According to the morphological characters, as well as the color of colonies on the 16% NaCl–agar medium, the yeast isolates were categorized into nine groups that one isolate of each group selected for the molecular analysis (Fig. 3). Search for similar sequences in the GenBank DNA database using Blast program (http://blast.ncbi.nlm.nih.gov/blast.cgi) showed 99–100 % similarity with valid sequences previously identified and deposited in GenBank: *Candida catenulata* (FSMP–Y25 GenBank FJ627977, Belloch, 2009), *Candida boidinii* (YM25345 GenBank KC442246, Lixia et al. 2012), *Candida parapsilosis* (KF214407; Ghaffari et al. 2015), *Meyerozyma guilliermondii* (HM191674; Lixia et al. 2012), *Lecythophora* sp. (YM24350 GenBank HQ220211, Zhou et al. 2011), *Rhodotorula toruloides* (GenBank AF070426, Fell et al. 1998), *Trichosporon coremiiforme* (CBS 8261, GenBank JN939454, Schoch et al. 2012) and *Naganishia uzbekistanensis* (KJ507271; Kim 2014). The determined D1/D2 domains of LSU–rDNA were deposited in the GeneBank using the NCBI database and assigned accession numbers KP324973, KP055040, KP324959, KP324968, KP324965, KP324962, KP336745 and KP324971, KP324978.

Phylogenetic analysis

An amplicon of about 600 bp was amplified for all of yeast isolates which had an almost similar size of the D1/D2 variable domain of the LSU. The D1/D2 phylogenetic trees inferred by both distance–based (data not shown) and cladistic methods showed the same topology, although there were differences in percent of bootstrapping (Fig. 4–5). Each isolate clustered in the same clade of the phylogenetic tree (with valid sequences from GeneBank with a high percentage of bootstrap support) are shown in Fig. 4–5.

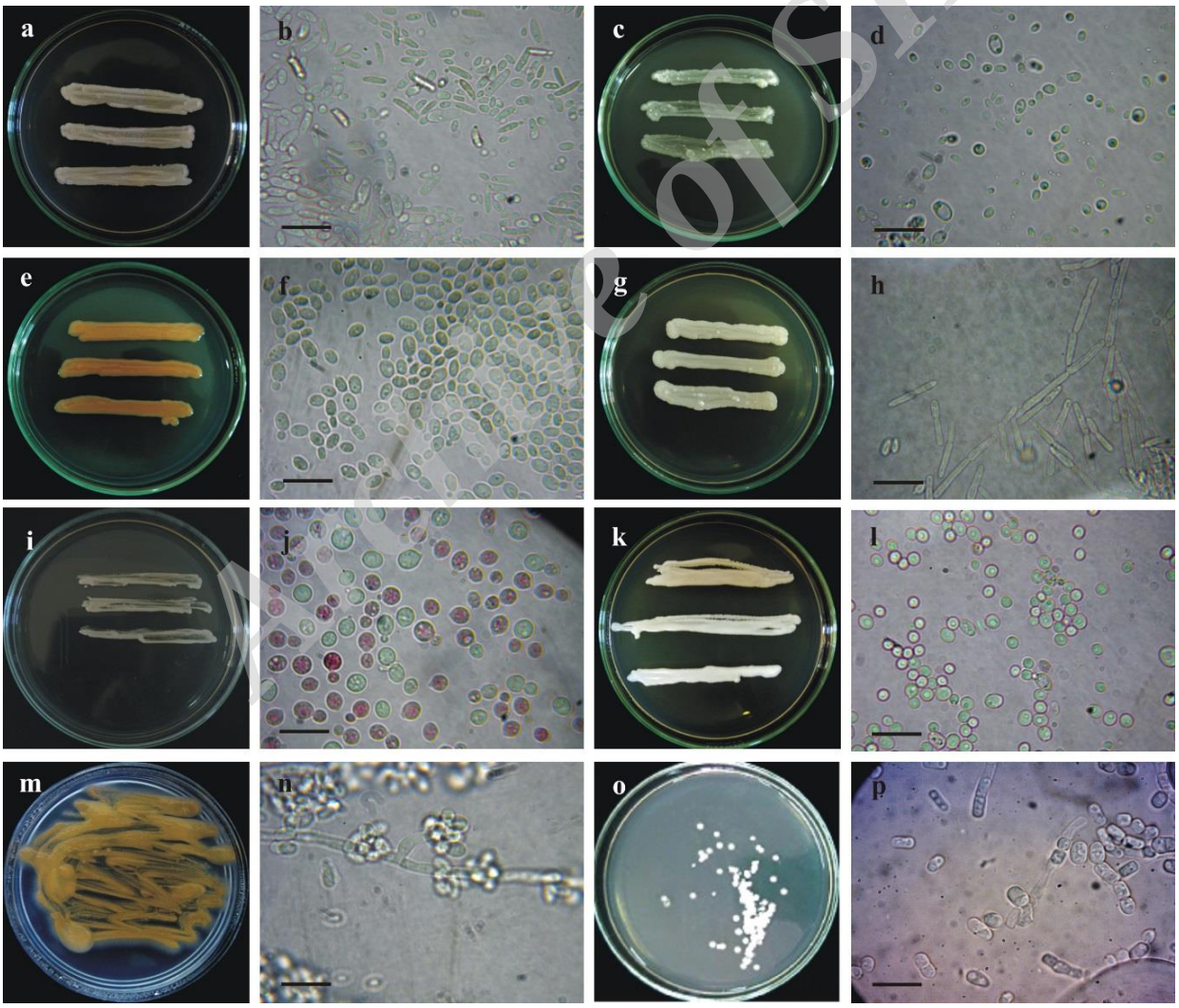

Fig. 3. Colony of yeast species on yeast extract agar medium after three days, and morphological features. a–b. *Candida boudini*; c–d. *Candida catenulate*; e–f. *Rhodotorula toruloides*; g–h. *Meyerozyma guilliermondii*; i–j. *Naganishia uzbekistanensis*; k–l. *Candida parasilopsis*; m–n. *Lecythophora* sp.; o–p. *Trichosporon coremiiforme*. – Scale bars = 10 µm.

DISCUSSION

From the 25 soil samples, a total of 28 colonies of yeast fungi were isolated from different areas of Kermanshah province. The isolated yeasts belonged to six genera and eight species. Yeasts are prone to be occurred in both arable and uncultivated lands of different geographic areas from the tropics to the arctic zones. Our results show that basidiomycete yeasts are more prominent than ascomycete species in uncultivated soils. The most frequent yeast species examined are *Naganishia uzbekistanensis* and *Trichosporon coremiforme*, which were mostly found

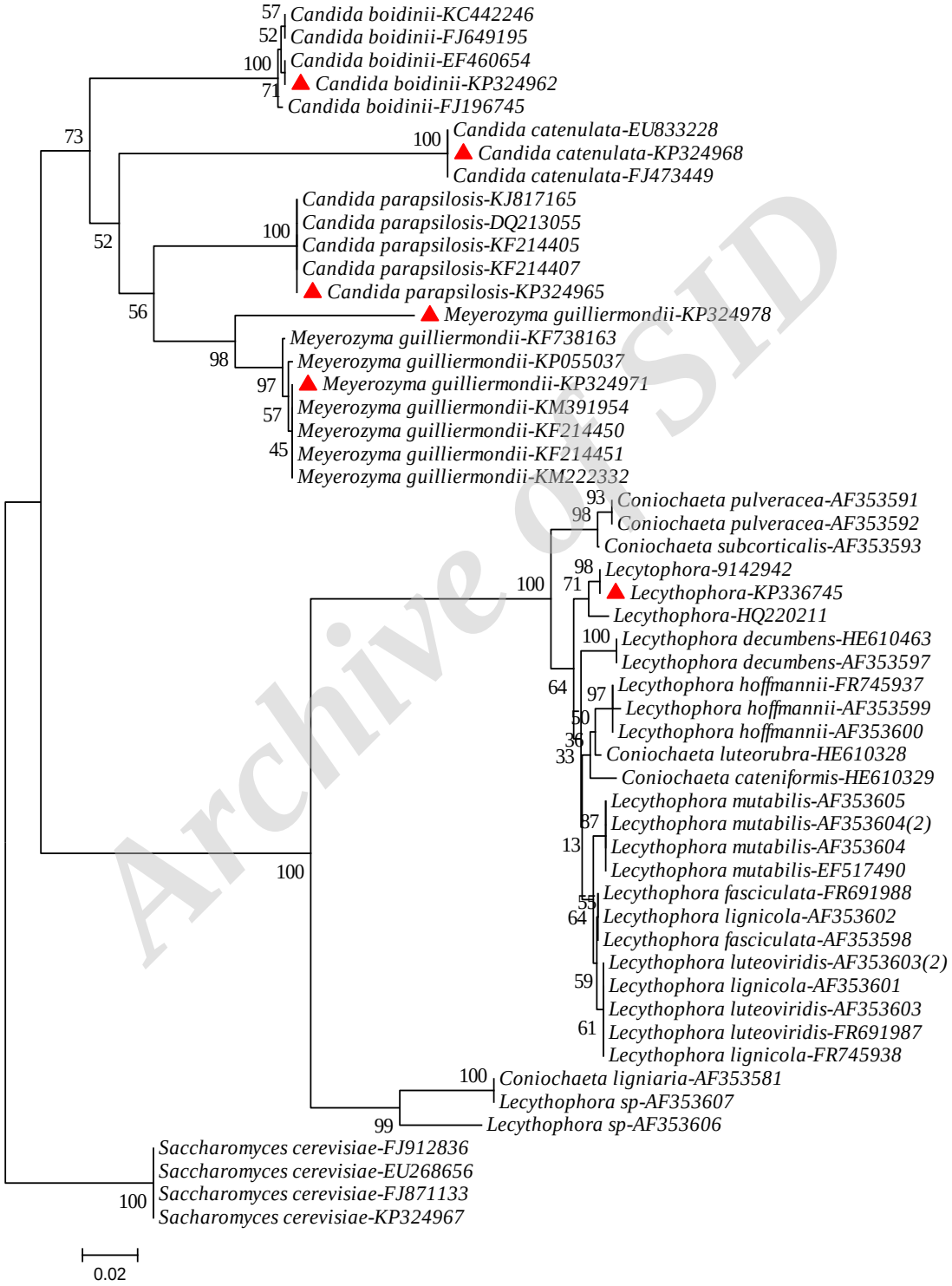

Fig. 4. Maximum parsimony tree generated in Mega 4 from the alignment of 53 combined large subunit (LSU) rDNA gene (D1/D2 region) of ascomycete yeasts with 500–replication bootstrapping. The red triangles refer to ascomycete yeasts in Iran.

in uncultivated soil–samples (Fig. 2). The most frequent ascomycete yeasts in uncultivated soils were *Candida catenulata*, *C. boidinii* and *C. parasilopsis*. Occurrence and frequency of other species did not exceed over 20%. In general, the population of yeast cells in uncultivated soil is low. Moshtaq et al. (2004) showed that occurrence of yeast in garden soil is greater than cultivated field soils which probably due to presence of different nutrients from dead and decayed plant parts. Among physiochemical factors that limit the ecology of yeasts, most important appear to be the energy sources, nutrients, temperature, pH value and water (Rose & Harrison 1987).

In Austria, Wuczkowski and Prillinger (2004) showed that the most frequent isolated genus was *Cryptococcus*. Members of this genus are protected against several physical and biological stresses which resist them to survive under harsh environment (Slavikova & Vadkertiova 2000, Spencer & Spencer 1997, Mokhtarnejad et al. 2015b). De Azeredo et al. (1998) showed that *Cryptococcus*, *Cystofilobasidium*, *Sporobolomyces*, *Rhodotorula*, and *Trichosporon* can be regularly isolated from cultivated soils. Among the ascomycete species, *Candida maltosa*, *Debaryomyces occidentalis*, *Metschnikowia pulcherrima*, and *Williopsis saturnus* are found frequently (Sláviková & Vadkertiová 2000, 2003). To date, yeasts in soils were mainly studied using culturing approaches and there are only a few reports of environmental sequences of fungi belonging to yeast lineages (Renker et al. 2004; Lynch & Thorn 2006; Buee et al. 2009). Lynch and Thorn (2006) used a cloning approach with subsequent Sanger sequencing to analyze basidiomycetes in arable soils and detected yeasts. These were already reported as soil inhabitants including; *Cryptococcus podzolicus*, *Cr. terreus*, *Cr. terricola*, *Trichosporon dulcitum* and *Guehomyces pullulans*. Similarly, 454–pyrosequencing of six forest soils showed a large number of sequences read of the yeasts *Cr. podzolicus* and *Cr. terricola* (Buee et al. 2009).

Wuczkowski & Prillinger (2004) and Botha (2006) reported that *Cryptococcus* species and some other of basidiomycetous yeasts including; *Cystofilobasidium capitatum*, *Debaryomyces occidentalis*, *Lipomyces starkeyi*, *Metschnikowia pulcherrima*, *Rhodotorula glutinis*, *Sporobolomyces roseus*, *Guehomyces pullulans* and *Williopsis saturnus* are the most frequent species of yeasts occurring in soils (Sláviková & Vadkertiová 2003; Wuczkowski & Prillinger 2004; Botha, 2006). In Iran, Mokhtarzadeh et al. (2015) obtained 25 species belonges to six genera of cultivated soils that *Cryptococcus* had high frequently among the isolated yeast (Mokhtarzadeh et al. 2015). The soil analysis showed that soils were basic and non–saline. Yeasts are happier to grow in a slightly acidic medium with an optimum pH between 4.5 and 5.5. Our results show that ascomycete yeasts are negatively correlated with the first CCA axis that agrees with other authors' results. Basidiomycetous yeasts such as *Naganishia uzbekestanensis*, *Trichosporon coremiformii* and *Rodosporidium* were able to grow at pH values 7–8. In our results, basidiomycete yeasts are especially alkali–tolerant that agree with Aono (1990) results. Macpherson et al. (2005) mentioned that the maintenance of a proton gradient across the plasma membrane against a constant intracellular pH of about 6.5 is vital for a yeast cell for optimal activity of critical metabolic processes (Macpherson et al. 2005). In our study, the patterns of ascomycete yeasts abundance are influenced negatively by pH that agree with former studies (Taylor & Francis 2008).

The CCA analysis has been used to display the inter–relationships between the environment and yeasts distribution. Elevation, EC and soil texture were variables measured in this study to produce longer arrows which strongly affect yeast distribution. In fact, the pattern of basidiomycete yeasts abundance was positively influenced by changes in elevation, EC, pH and clay and negatively by sand (Fig. 1).

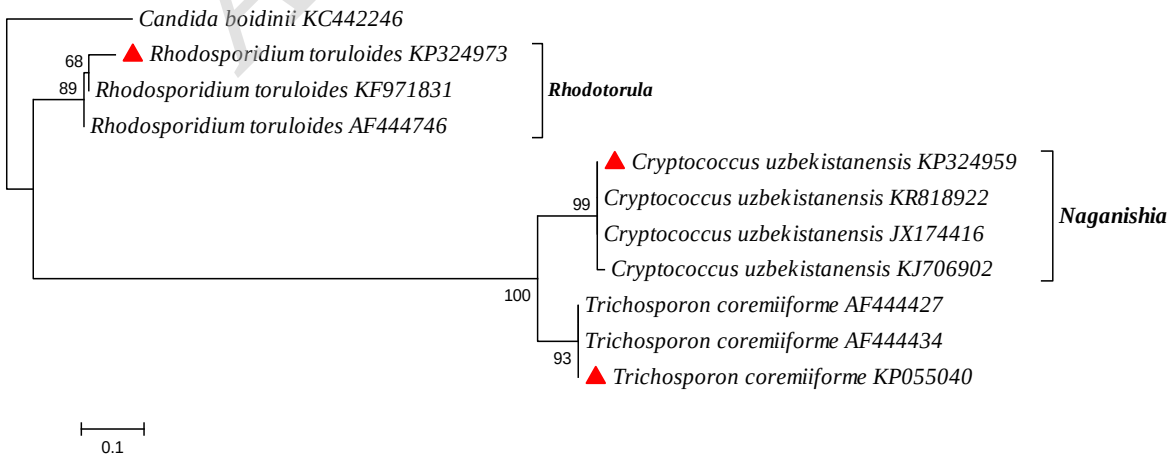

Fig. 5. Maximum parsimony tree generated in Mega 4 from the alignment of ten combined large subunit (LSU) rDNA gene (D1/D2 region) of basidiomycete yeasts with 500–replication bootstrapping. The red triangles refer to basidiomycete yeasts in Iran.

The distribution pattern of the basidiomycete yeasts seems to have a correlation with elevation, EC, pH and clay which affect their frequency. Kurtzman and Fell (1998) mentioned that correct identification of yeast species in the ecosystem is a major factor that determines the validity of studies in yeast ecology previously yeast identifications were usually based on phenotypic tests. Although, phenotype can sometimes be used to correctly identify different species, molecular comparisons have shown that those earlier identifications based on phenotype have been incorrect. Wang et al. (2015a–b) revised Pucciniomycotina and Ustilaginomycotina yeasts based on multigene sequence analyses. In this study, the yeast isolates were initially sorted based on morphology and identifications confirmed through the large subunit (LSU) rDNA gene (D1/D2 region). Based on the closest match of BLAST analysis, eight species such as *Rhodotorula toruloides*, *Trichosporon coremiiforme*, *Naganishia uzbekistanensis*, *Candida catenulata*, *C. paracilopsis*, *C. boidinii*, *Lecythophora* sp. and *Meyerozyma guilliermondii* were recovered.

This study was the first report regarding *Rhodotorula toruloides*, *Trichosporon coremiiforme*, *Candida catenulata*, *C. boidinii* and *Lecythophora* sp. on uncultivated soil of Iran. The phylogenetic trees generated using the neighbor joining and maximum parsimony methods, show that isolates from the same species are grouped in the same clade (Fig. 3 and 4). The sequencing of the D1/D2 of the large–subunit LSU rDNA is now widely accepted as a standard procedure for yeast identification (Hong et al. 2001; Scorzetti et al., 2002, Frutos et al. 2004). It was also found that the molecular methods based on the sequences of D1/D2 domain of the LSU–rDNA is rapid and precise, compared with the physiological methods for identification and typing of the yeasts species (Kurtzman and Robnett 1998, Phaff et al. 1999).

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شناسایی گونه‌های مخمر خاک‌های بکر براساس آنالیز توالی ناحیه متغییر D1/D2 از LSU–rDNA در استان کرمانشاه، ایران

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چکیده: مخمرها یک گروه چند نیایی از قارچ‌های آسکومیست و بازیدیومیست هستند که مرحله رویشی آنها به صورت تک سلولی بوده و مرحله جنسی آنها محصور در اندام بارده نیست. در این مطالعه، مخمرهای خاک‌های بکر مناطق مختلف استان کرمانشاه براساس آنالیز توالی ناحیه متغییر D1/D2 از LSU–rDNA و مقایسه با توالی‌های نوکلئوتیدی این ناحیه در پایگاه اطلاعاتی NCBI شناسایی شدند. در این بررسی، ۲۵ نمونه خاک از مناطق مختلف جمع آوری و مورد بررسی قرار گرفت و هشت گونه شامل *Naganishia uzbekistanensis* ،*Trichosporon coremiiforme* (KP055040)* ،*Rhodotorula toruloides* (KP324973)* (KP324959)، *Candida catenulata* (KP324968) ،*C. paracilopsis* (KP324965)* ،*C. boidinii* (KP324962)* *Lecythophora* sp. (KP336745)* و (KPKP324971, KP324978) *Meyerozyma guilliermondii* شناسایی شدند. آنالیز فیلوژنتیکی، صحت شناسایی گونه‌ها را با قرار دادن آنها در کنار سایر نمونه های معتبر از دیگر مناطق دنیا تائید کرد. آنالیز تطبیق متعارفی نشان داد که ارتفاع از سطح دریا، هدایت الکتریکی خاک، اسیدیته و رس خاک، پارامترهای محیطی موثر در پراکنش مخمرهای بازیدیومیستی در خاک های بکر است. در این بررسی، وجود هشت گونه تائید شد که گونه‌های ستاره دار برای اولین بار برای فلور قارچی ایران گزارش می‌شوند.

کلمات کلیدی: مخمرها، ژن های rDNA، فیلوژنی، آنالیز تطبیق متعارفی

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