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RESEARCH ARTICLE

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Mrakia terrae sp. nov. and *Mrakia soli* sp. nov., Two Novel Basidiomycetous Yeast Species Isolated from Soil in Korea

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ABSTRACT

Three strains, YP416^T, YP421^{T,} and Y422, were isolated from soil samples in Pocheon City, Gyeonggi province, South Korea. The strains belong to two novel yeast species in the genus *Mrakia*. Molecular phylogenetic analysis showed that the strain YP416^T was closely related to *Mrakia niccombsii*. Still, it differed by 9 nucleotide substitutions with no gap (1.51%) in the D1/D2 domain of the LSU rRNA gene and 14 nucleotide substitutions with 7 gaps (2.36%) in the ITS region. The strain YP421^T differed from the type strain of the most closely related species, *Mrakia aquatica*, by 5 nucleotide substitutions with no gap (0.81%) in the D1/D2 domain of the LSU rRNA gene and 9 nucleotide substitutions with one gap (1.43%) in the ITS region. The names *Mrakia terrae* sp. nov. and *Mrakia soli* sp. nov. are proposed, with type strains YP416^T (KCTC 27886^T) and YP421^T (KCTC 27890^T), respectively. MycoBank numbers of the strains YP416^T and YP421^T are MB 836844 and MB 836847, respectively.

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Novel yeast species; Mrakia; taxonomy

1. Introduction

The first *Mrakia* strains were reported by Di Menna in 1966 [1] as novel species in the genus *Candida*: *C. nivalis*, *C. gelida* and *C. frigida*. However, Fell et al. [2] reclassified *C. stokesii*, *C. nivalis*, *C. gelida* and *C. frigida* as genus *Leucosporidium*. Later, these taxa were again reclassified to a new genus, *Mrakia*, as *M. frigida* and *M. gelida*, relying on the presence of the CoQ-8 system of these species [3–5]. *Mrakia* species were isolated from a variety of cold environments: *Mrakia psychrophila* from Antarctic soil [6]; *Mrakia cryoconiti* (formerly *Mrakiella cryoconiti*) isolated from alpine glacier in Austria and a sediment sample containing mud, spring water and moss in northern Siberia [7] and *Mrakia aquatica* (formerly *Mrakiella aquatica*) [7]; *Mrakia blollopis*, *Mrakia robertii* and *Mrakia niccombsii* (formerly *Mrakiella niccombsii*) obtained from soil, lichen and snow samples collected in Antarctica, Alpine sediments and glacial melting water [8,9]; *Mrakia arctica* isolated from ice island in the Canadian high arctic [10]; *Mrakia hoshinonis* from sediment collected from front of a disappearing glacier in the Canadian Arctic [11]; *Mrakia fibulata* isolated from tree fluxes caused by tree injuries during wintertime [12]; *Mrakia stelviica* and *Mrakia montana* isolated from soil samples of nival belt and of alpine grassland, collected at Italian Alps and Apennines glaciers [4]. Here we describe three yeast strains isolated from a soil sample collected in Pocheon city during winter. Based on morphological, physiological tests, and molecular analysis of ITS and the D1/D2 domain of the LSU rRNA gene, these three strains were classified as two novel yeast species in the genus *Mrakia*, for which the names *Mrakia terrae* sp. nov. and *Mrakia soli* sp. nov. are proposed.

2. Materials and methods

2.1. Yeast isolation

Soil samples were collected in Pocheon City, Gyeonggi province, South Korea (37°47'32.2"N 127°09'54.2"E and 37°54'37.3"N 127°12'53.2"E) during winter (Table 1). The soil sample (1 *g*) was suspended in 10 ml of sterile normal saline. The soil suspension was serially diluted to make 1:10 to 1:1000 by sterile normal saline, and then 0.1 ml of each dilution was spread onto Yeast-Malt agar (YMA, Difco, Detroit, USA) plate and the plates were incubated at 25 °C for 3–4 days. Colonies were

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Supplemental data for this article can be accessed here.

Table 1. List of the yeast strains of *Mrakia terrae* sp. nov. and *Mrakia soli* sp. nov. examined in the present study and related species.

				GenBank accession number	
Species	Strain number	Isolation source	Location	D1/D2	ITS
M. terrae	YP416^T	Soil, grass field	Pocheon city, Korea	MW301660	MW301663
M. soli	YP421^T	Soil, riverside	Pocheon city, Korea	MT505693	MT505688
	YP422	Soil, river side	Pocheon city, Korea	MT505696	MT505690
M. aquatica	JCM5443^T	Scum on water	Malham tarn, UK	AF075470	AF410469
M. artica	JCM32070^T	Ice	Canadian high arctic	LC222845	.
M. blollopis	CBS8921^T	Soil	Marine plain, Antarctica	AY038814	AY038826
M. cryoconite	CBS10834^T	Glacier cryoconite	Alps	GQ911524	AJ866976
M. fibulata	DSM103931^T	Brich	Lower Saxony, Germany	MK372216	.
M. frigida	CBS5270^T	Snow and soil	Scott base, Antarctica	AF075463	AF144483
M. gelida	CBS5272^T	Soil	Scott base, Antarctica	AF189831	AF144485
M. hoshinonis	JCM32575^T	Walker glacier	Northern Ellesmere island, Canada	LC335798	.
M. montana	DBVPG 10736^T	Soil, alpine grassland	Stelvio pass, Italian Alps, Italy	MT347769	MT347765
M. niccombsii	CBS8917^T	Lichen	Vestvold hills, Antarctica	AY029345	AY029346
M. psychrophilia	AS2.1971^T	Soil	Fildes peninsula, Antarctica	EU224266	EU224267
M. robertii	CBS8912^T	Soil and lichen	Mossell lake, Antarctica	AY038811	AY038829
M. stelviica	DBVPG 10734^T	Soil, alpine grassland	Stelvio pass, Italian alps, Italy	MT347768	MT347764

isolated and purified using YM medium (Difco). As a result, the strains YP416^T, YP421^{T,} and Y422 were isolated and deposited at the Korea Collection for Type Cultures, KRIBB, Korea, and at the NITE Biological Resource Center, NITE, Japan.

2.2. DNA sequencing and phylogenetic analysis

The D1/D2 domain of the LSU rRNA gene and internal transcribed spacer (ITS) region of the three strains (YP416^T, YP421^T, and Y422) were amplified by PCR with NL1/NL4 [13] and ITS1/ITS4 primers [14], respectively. The sequences were assembled with the SeqMan program version 7.1.0. Then, pairwise sequence comparisons were made using Basic Local Alignment Search Tool (BLAST) search [15] and aligned with the sequences of related species retrieved from GenBank by using the multiple alignment program Clustal X 2.0 [16]. The phylogenetic trees based on the combined sequences of the D1/D2 domains of the LSU rRNA gene and ITS region were constructed by the maximum-likelihood (ML) and neighbor-joining (NJ) method on the MEGA X [17–19]. The evolutionary distances were calculated using the general time-reversible (GTR) and kimura two-parameter model for the ML and NJ analyses, respectively [20,21]. A bootstrap analysis was conducted with 1,000 replicates [22]. Sequence similarity and nucleotide variations in ITS and D1/D2 sequences between the strains and closely related species were calculated using the BLAST tool (https://blast.ncbi.nlm.nih.gov).

2.3. Phenotypic characterization

For the microscopy, the strains were grown on YM agar at 15 °C and observed with a phase-contrast microscope (DM500, LEICA, Wetzlar, Germany). Biochemical and physiological characteristics of the strains were examined following as described method [23]. Induction of the sexual stage and spore formation was tested by incubating single or mixed cultures of each of the two strains on cornmeal agar (CMA, Difco) at 1 and 10 °C for 2 months. Basidiospore formation was investigated by growing the individual strains on potato dextrin agar (PDA, Difco), CMA, 5 % malt extract agar (5 % malt extract and 1.5 % agar), yeast extract–peptone glucose (YPD, Difco) agar (1 % yeast extract, 2 % peptone, 2 % glucose and 1.5 % agar) and YM agar (1 % yeast extract, 2 % peptone, 2 % glucose and 1.5 % agar) at 15 and 25 °C for four weeks. Diazonium blue B (DBB, Sigma-Aldrich, Darmstadt, Germany) color reaction was performed by dropping the DBB reagent into the colonies cultured on YM agar for 3 days and observed the color after 2 min. Growth at different temperatures (4, 10, 15, 25, 30, 35, 37, 42, and 45 °C) was determined by cultivation on PDA, YPD agar, and YMA for 15 days. Growth in YM broth supplemented with different NaCl concentrations (0–10% in 1% intervals, w/v) was examined for up to five days. To observe the pseudohyphae and true hyphae formation, the cells were cultivated on YM agar in slide culture at 10 °C for up to 1 month and observed every week. The ubiquinones were extracted and analyzed as described by [24].

3. Results and Discussion

3.1. Novel species identification and delineation

A total of 472 yeast strains were isolated from 35 soil samples collected at the Pocheon city, Gyeonggi province, South Korea. Of these strains, 69 were classified as *Mrakia* (taxonomy: *Basidiomycota*, *Agaricomycotina*, *Tremellomycetes*, *Cystofilobasidiales*) by analyzing sequences of the ITS and the D1/D2 domain of the LSU rRNA gene.

Three strains (YP416^T, YP421^{T,} and YP422) were classified as the new *Mrakia* species.

The strain YP416^T was most closely related to *M. niccombsii*, against which 9 nt substitutions were observed in the D1/D2 domain (Table 2). On the other hand, the sequence of the ITS region in YP416^T contained 14 nt substitutions in comparison to that of the ITS region in *M. niccombsii*, with sequence identities of 98.4%. The phylogenetic trees obtained by maximum-likelihood and neighbor-joining methods showed that strains YP416^T, YP421^T and YP422 were grouped with members of the genus *Mrakia* (Figure 1, Figure S1).

Table 2. Nucleotide substitutions in the sequences of the D1/D2 domain of the LSU rRNA gene and ITS region of *Mrakia terrae* sp. nov. (YP416^T) and *M.* species.

	YP416^T	
	D1/D2(%)	ITS(%)
YP416^T	–	–
M. niccombsii	9 (98)	21 (97)
M. aquatic	10 (98)	16 (97)
M. cryoconiti	10 (98)	46 (92)
M. hoshinonis	11 (98)	11 (97)
M. artica	11 (98)	47 (92)
M. psychrophilia	12 (98)	50 (92)
M. blollopis	12 (98)	53 (92)
M. gelida	13 (98)	48 (92)
M. frigida	13 (98)	49 (92)
M. robertii	14 (98)	54 (91)

Values above the diagonal are a number of nucleotide substitutions in the D1/D2 domain of the LSU rRNA gene. Values below the diagonal are the number of nucleotide substitutions and sequence similarity (%, in parentheses) in the sequences of the ITS region.

Based on these results, YP416^T should be considered a new species, for which the name *Mrakia terrae* (*terrae* of the soil) is proposed. *Mrakia terrae* YP416^T produced starch, grew maximally below 25 °C, and reacted with diazonium blue B, consistent with the characteristics of the genus *Mrakia* [23,25]. In addition, the type strain YP416^T did not assimilate D-arabinose, glycerol, and Myo-inositol while *M. niccombsii* assimilate D-arabinose, glycerol, and *Myo*-inositol (Table 3). *M. terrae* YP416^T grew optimally at 15 °C but did not grow above 25 °C. True hyphae with teliospores are formed after 35 days of incubation (Figure 2). Ballistoconidia are not produced on YM and cornmeal agar.

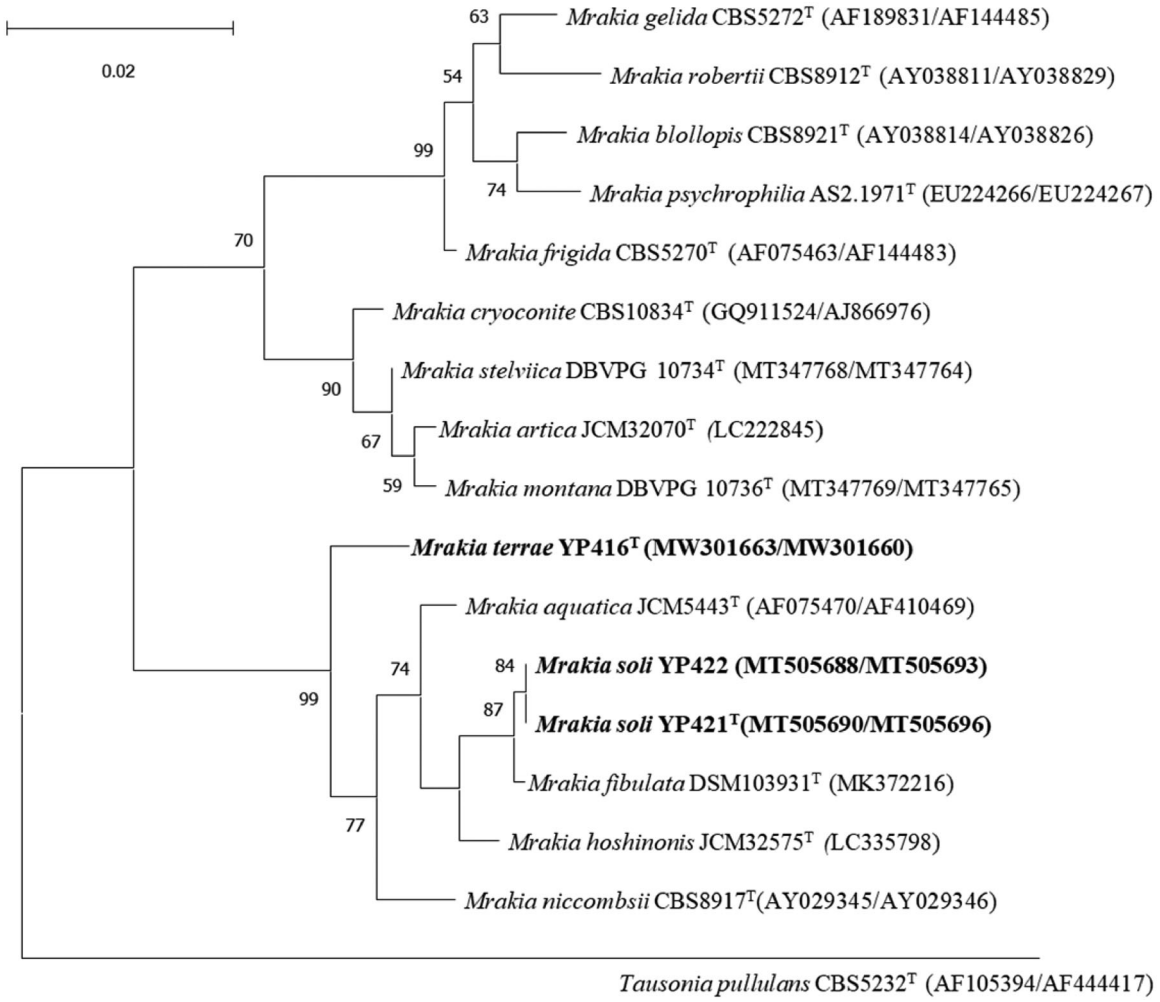

Figure 1. Phylogenetic tree based on the concatenated sequences of the D1/D2 region of the LSU rRNA gene and ITS regions and constructed by the maximum-likelihood method shows relationships between strains of a novel species (YP416^T, YP421^{T,} and YP422) and closely related species. The novel species described in this manuscript are highlighted in bold. *Tausonia pullulans* CBS 5232^T was used as an outgroup. Bootstrap values greater than 50% (% of 1,000 replications) were shown at branch points. Accession numbers were shown in parentheses. *Bar,* 0.02 substitutions per nucleotide position.

Table 3. Phenotypic characteristics that differentiate *Mrakia terrae* sp. nov. and *M. soli* sp. nov. from their related species, *M. niccombsii* and *M. aquatica*.

	1	2	3	4
Growth test				
PDA	–	+	+	ND
25 °C	–	+	–	–
NaCl 6%	+	–	+	ND
NaCl 7%	+	–	+	ND
Assimilation				
Inulin	+	+	w	–
Lactose	+	+	–	+
Maltose	+	–	+	+
Melezitose	+	–	+	+
α-methyl-D-glucoside	+	–	w,-	–
Soluble starch	–	+	w	+
Salicin	+	n	w,+	+
L-Sorbose	+	+	w	+
D-Xylose	+	+	w,+	+
L-Arabinose	+	+	w,+	+
D-Arabinose	–	v	w,-	–
D-Ribose	+	v	w	–
Methanol	+	–	–	–
Ethanol	+	+	w	+
Glycerol	–	v	–	v
Myo-Inositol	–	–	w,+	–
DL-Lactate	+	–	–	–
Citrate	+	+	w,+	w
D-Glucosamine	+	+	w,+	–
N-Acetyl-D-glucosamine	+	v	w,+	–

Strains: 1, *M. terrae* YP 416^T; 2, *M. soli* YP421^T; 3, *M. niccombsii* CBS 8917^T; 4, *M. aquatica* JCM5443^T.
All strains were positive for glucose, sucrose, raffinose, melibiose, galactose, trehalose, cellobiose, ribitol, D-mannitol, D-glucitol, on YMA, YPD, and D-guconate but negative for erythritol and galactitol.
Data for species 1–2 are from the present study, for species 3–4 are from Liu et al. [26].
Growth reactions: +, positive; w, weak positive; -, negative; v, variable; n, no data.

Table 4. Nucleotide substitutions in the sequences of the D1/D2 domain of the LSU rRNA gene and ITS region of *Mrakia soli* sp. nov. (YP421^T and YP422) and *M.* species.

	YP421^T		YP422	
	D1/D2(%)	ITS(%)	D1/D2(%)	ITS(%)
YP421^T	–	–	2 (99)	0 (100)
YP422	2 (99)	0 (100)	–	–
M. hoshinonis	5 (99)	7 (99)	6 (99)	7 (99)
M. aquatica	5 (99)	9 (98)	5 (99)	8 (98)
M. niccombsii	12 (98)	8 (98)	12 (98)	8 (98)
M. artica	13 (98)	42 (93)	13 (98)	27 (93)
M. cryoconiti	15 (97)	35 (91)	16 (97)	35 (93)
M. gelida	16 (97)	33 (92)	16 (97)	33 (92)
M. frigida	16 (97)	31 (93)	16 (97)	31 (93)
M. psychrophilia	17 (97)	34 (92)	17 (97)	34 (92)
M. blollopis	17 (97)	36 (92)	17 (97)	36 (92)
M. robertii	17 (97)	38 (92)	17 (97)	38 (92)

Values above the diagonal are number of nucleotide substitutions in the D1/D2 domain of the LSU rRNA gene. Values below the diagonal are number of nucleotide substitutions and sequence similarity (%, in parentheses) in the sequences of the ITS region.

Basidiospore formation is not observed on PDA, corn meal agar, 5% malt extract agar, YPD agar, and YM agar at 15 and 25 °C for 4 weeks. But in the case of *M. niccombsii*, no teliospores were observed in any media tested [8]. The respiratory quinone is Q-8.

The strain YP421^T was most closely related to *M. aquatica*, against which 5 nt substitutions were observed in the D1/D2 domain (Table 4). In addition, analysis of the same sequences in YP421^T showed 2 nt substitutions against YP422. On the other hand, the sequence of the ITS region in YP421^T contained 7 and 9 nt substitutions in comparison to that of the ITS region *M. hoshinonis* and *M. aquatica*, respectively, with sequence identities of 99% and 98%. Based on these results, YP421^T should be considered a new species, for which the name *Mrakia soli* (*soli* of soil) is proposed.

Mrakia soli YP421^T assimilated nitrate, produced starch, grew maximally below 25 °C, and reacted with diazonium blue B, consistent with the characteristics of the genus *Mrakia* [23,25]. In addition, the type strain YP421^T assimilated inulin and D-glucosamine but did not assimilate maltose and melezitose. In contrast, *M. aquatica* did not assimilate inulin and D-glucosamine but did assimilate maltose and melezitose. *Mrakia soli* grew optimally at 15 °C but did not grow above 25 °C. True hyphae are formed after 35 days of incubation of strain YP421^T. Ballistoconidia are not produced on PDA and cornmeal agar. Basidiospore formation is not observed on PDA, corn meal agar, 5% malt extract agar, YPD agar, and YM agar at 15 and 25 °C for 4 weeks. Teliospore is observed after 35 days of incubation. But in the case of *M. hoshinonis*, the formation of teliospores and basidiospores are not observed and pseudohyphae and true hyphae are not formed [3]. The respiratory quinone is Q-8.

3.2. Description of Mrakia terrae Park, Maeng, and Sathiyaraj sp. nov

Mrakia terrae (ter′rae. L. gen. n. *terrae* of the soil, referring to the isolation source of the type strain). Novel yeast species belonging to phylum *Basidiomycota*, subphylum *Agaricomycotina*, class *Tremellomycetes*, order *Cystofilobasidiales*, family *Mrakiaceae*.

Yeast cells after three days on YM agar at 10 °C are ovoid to the ellipsoid (6–6.5 × 1.8–2 μm). Budding is polar budding (Figure 3A). Streak culture on YM agar for 1 week at 10 °C produces colonies that are light yellow-colored, convex, round, shiny, and slimy. True hyphae with teliospores are formed after 35 days of incubation on PDA at 10 °C. Ballistoconidia are not produced on YM and cornmeal agar. Basidiospore formation is not observed on PDA, corn meal agar, 5% malt extract agar, YPD agar, and YM agar at 15 and 25 °C for 4 weeks.

Glucose, sucrose, raffinose, melibiose, galactose, lactose, trehalose, maltose, melezitose, soluble starch, cellobiose, L-rhamnose, D-xylose, D-ribose, ribitol, xylitol, D-mannitol, D-glucitol, D-gluconate, gluconolactone, D-glucosamine, *N*-acetyl-D-glucosamine,

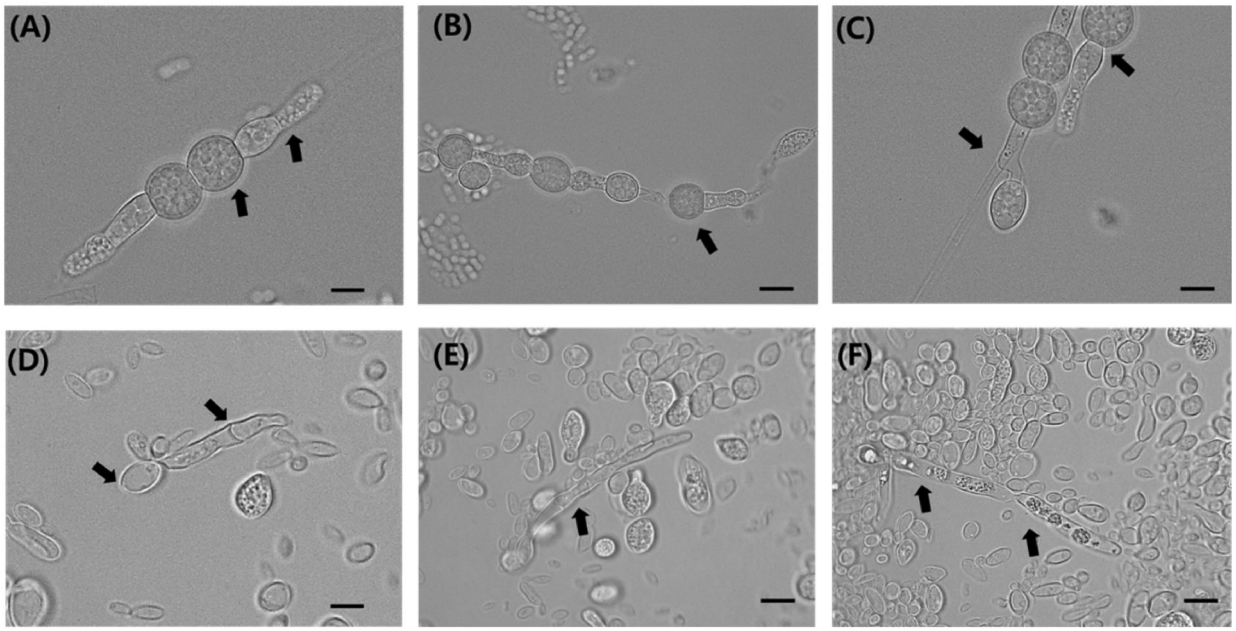

Figure 2. Light microscopic images illustrating the different stages of *Mrakia terrae* and *M. soli* sp. nov. (strains YP416^T and YP421^T) after 35 days at 10 °C on PDA: true hyphae with teliospores of YP416^T (A-C); septum of YP416^T (A-C); true hyphae with teliospores of YP421^T (D); true hyphae and septum of YP421^T(D-F), Bars, 10 µm.

potassium nitrate, sodium nitrate, cadaverine dihydrochloride, and L-lysine are assimilated. Inulin is variable. Methyl-α-D-glucoside, L-sorbose, L-arabinose, D-arabinose, methanol, ethanol, glycerol, erythritol, galactitol, Myo-inositol, DL-lactate, and citrate are not assimilated. Growth occurs at 10–25 °C (optimum 15 °C) and cells can tolerate up to 6% NaCl in YM broth. Growth occurs on YM agar, YPD agar, PDA, and 50% glucose medium. Growth in the presence of 0.01% of cycloheximide is positive. Production of starch and diazonium blue B reaction is positive while and urea hydrolysis is negative. The respiratory quinone is Q-8.

The holotype, YP416^T, was isolated from the soil sample in Pocheon City, Gyeonggi province, South Korea, and is preserved in a metabolically inactive state at the Korea Collection for Type Cultures, KRIBB, Korea as KCTC 27886^T. The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain of the LSU rRNA gene and ITS region for YP416^T are MT505691 and MT505695, respectively. The MycoBank accession number is MB 836844.

3.3. Description of Mrakia soli *Park, Maeng, and Sathiyaraj sp. nov*

Mrakia soli (so′li. L. gen. n. *soli* of soil, referring to the isolation source of the type strain). Novel yeast species belonging to phylum *Basidiomycota*, subphylum *Agaricomycotina*, class *Tremellomycetes*, order *Cystofilobasidiales*, family *Mrakiaceae*.

Yeast cells after three days on YM agar at 10 °C are ovoid to the ellipsoid (4–4.25 µm × 1.8–2 µm). Budding is polar budding (Figure 3B). Streak culture for 1 week at 10 °C are on YM agar produces colonies that are light yellow-colored, round, convex, shiny, and smooth. True hyphae with teliospores are formed after 35 days of incubation on PDA at 10 °C. Ballistoconidia are not produced on PDA and cornmeal agar. Basidiospore formation is not observed on PDA, corn meal agar, 5 % malt extract agar, YPD agar, and YM agar at 15 and 25 °C for 4 weeks.

Glucose, inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose, soluble starch, cellobiose, soluble starch, cellobiose, L-sorbose, D-xylose, L-arabinose, ethanol, ribitol, xylitol, D-mannitol, D-glucitol, citrate, D-gluconate, gluconolactone, D-glucosamine, potassium nitrate, sodium nitrate, cadaverine dihydrochloride, and L-lysine are assimilated. L-rhamnose, D-arabinose, D-ribose, glycerol, and *N*-acetyl-D-glucosamine are variable. Maltose, melezitose, methyl-α-D-glucoside, methanol, erythritol, galactitol, Myo-inositol, and D,L-lactate are not assimilated. Growth occurs at 10–25 °C (optimum 15 °C) and cells can tolerate up to 7% NaCl in a YM broth medium. Growth occurs on YM agar, YPD agar, and PDA but not on a 50% glucose medium. Growth in the presence of 0.01% of cycloheximide is positive. Production of starch and diazonium blue B reaction is positive, while and urea hydrolysis is negative. The respiratory quinone is Q-8.

The holotype, YP421^T was isolated from the soil sample in Pocheon City, Gyeonggi province, South Korea, and is preserved in a metabolically inactive state at the Korea Collection for Type Cultures, KRIBB, Korea as KCTC 27890^T. The GenBank/

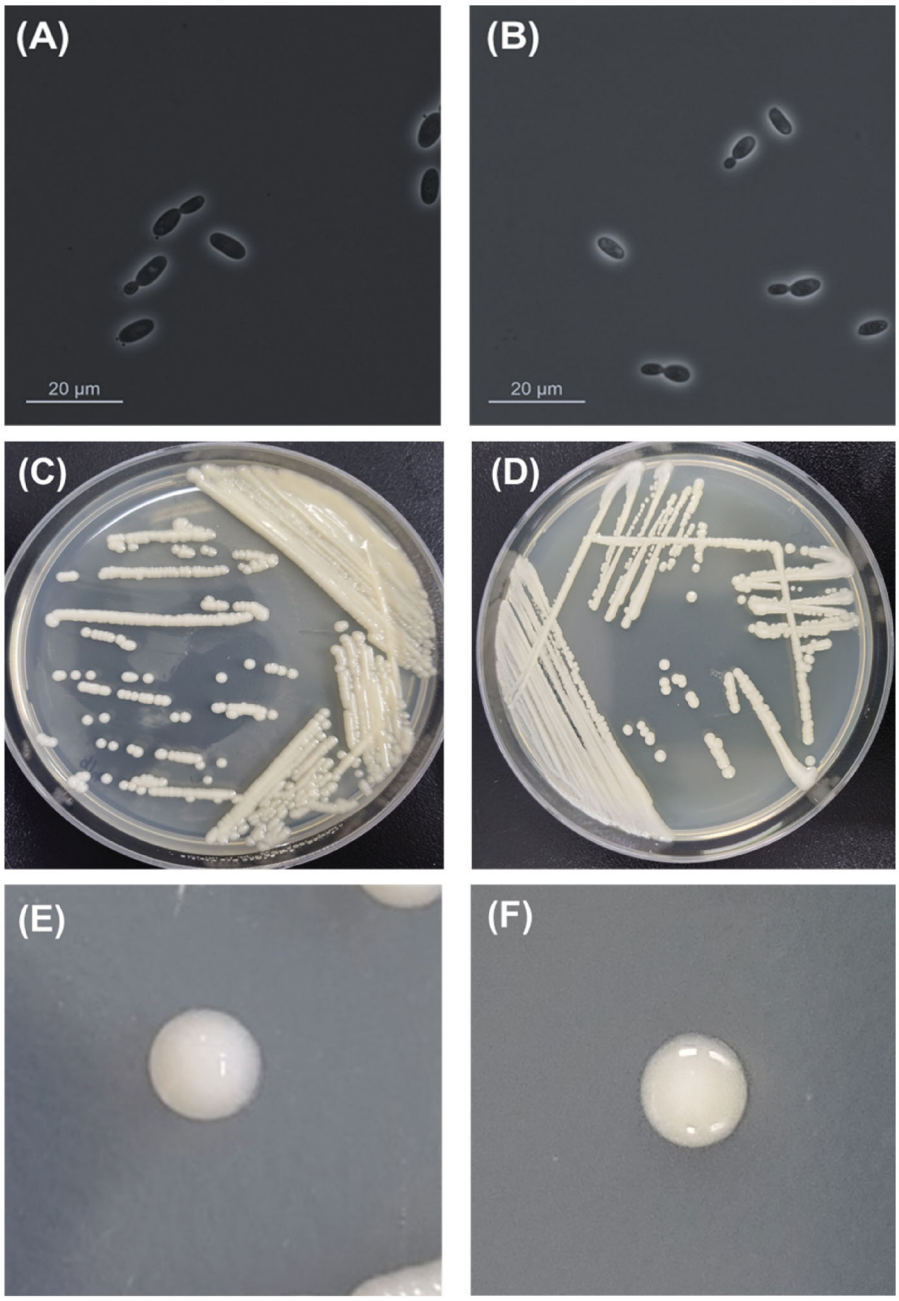

Figure 3. *Mrakia terrae* sp. nov. and *M. soli* sp. nov. (A) The polar budding cells of *M. terrae* YP416^T and (B) *M. soli* YP421^T on YM agar after three days at 10 °C; (C) and (E), colonies of YP416^T on YM agar after three days at 10 °C; (D) and (F), colonies of YP421^T on YM agar after three days at 10 °C.

EMBL/DDBJ accession numbers for the D1/D2 domain of the LSU rRNA gene and ITS region for YP421^T are MT505690 and MT505696, respectively. The MycoBank accession number is MB 836847.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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