

Description of unrecorded wild yeasts from soil in Republic of Korea under cold conditions

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The purpose of this study was to isolate and identify wild yeasts from soil collected in Daegu City and Cheongyang County, Republic of Korea. Among 11 strains isolated in this study, nine strains were previously reported and two strains were unreported in Republic of Korea. To identify wild yeast strains, pairwise sequence comparisons of the D1/D2 region of the 26S rRNA gene sequence were done using Basic Local Alignment Search Tool (BLAST). The cell morphologies were observed by phase contrast microscope and assimilation test are done using API 20C AUX kit. All strains were assigned to the phylum *Basidiomycota*. Of the two unrecorded yeast strains, CY-9-10C belongs to the genus *Mrakia* (family *Mrakiaceae*, order *Cystofilobasidiales*, class *Tremellomycetes*) and PG3-4-10C belongs to the genus *Slooffia* (family *Chrysozymaceae*, order *Microbotryomycetes incertae sedis*, class *Microbotryomycetes*). Both strains had oval-shaped and polar budding cells. This research described the morphological and biochemical properties of the two unreported yeast species that had not officially reported in Korea.

Keywords: *Mrakia*, *Slooffia*, unrecorded yeasts

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INTRODUCTION

In this study, wild yeasts were isolated from the soil samples in Daegu City and Cheongyang County, Republic of Korea. As a result of isolation and identification, many yeast strains were shown to be previously reported species and a few unrecorded species were found. The unreported species were identified to belong to the genera *Mrakia* and *Slooffia*.

The genus *Mrakia* is basidiomycetous yeast in the class *Tremellomycetes*. *Mrakia* species were isolated from snow, soil, and glaciers in cold environments (Tsuji *et al.*, 2019; Turchetti *et al.*, 2020; Yurkov *et al.*, 2020). The *Mrakia* clade consists of 20 recognized species and has type species as *Mrakia frigida* (<https://www.mycobank.org>). The species in *Mrakia* are cream colored and often mucoid, teliospore is produced at the end or in the middle of the mycelium (Li *et al.*, 2020) and the major CoQ system is CoQ-8 (Park *et al.*, 2021).

The genus *Slooffia* is basidiomycetous yeast in the class *Tremellomycetes*, phylum *Basidiomycota*. *Slooffia* species are usually isolated from soil (Tsuji *et al.*, 2019; Turchetti *et al.*, 2020; Yurkov *et al.*, 2020). The genus

Slooffia clade consists of six species, has type species as *Slooffia tsugae* (<https://www.mycobank.org>), has a mucoid colony texture on potato dextrose agar (PDA), and the major CoQ system is CoQ-10 (Wang *et al.*, 2015).

MATERIALS AND METHODS

The soil samples were collected from Daegu City and Cheongyang County, Republic of Korea and serially diluted in distilled water. The suspension was spread in a Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA) and incubated at 25°C and 10°C for 3 days. The strain PG3-4-10C is preserved in a metabolically inactive state at the Korean Agricultural Culture Collection, Korea, and strain CY-9-10C is preserved in a metabolically inactive state at Korea Collection for Type Cultures, KRIBB, Korea.

The cell morphologies of strains were done by the LEICA (DM500), using yeast strains incubated in YPD agar for 3 days. Phase contrast microscope images and the colonies of the strains GW1-3 and PG1-1-10C are shown in Fig. 1. To characterize the biochemical fea-

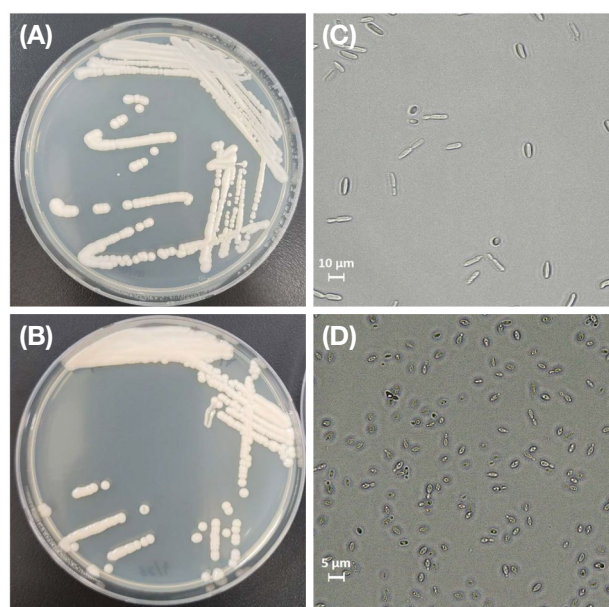


Fig. 1. Morphology of the unrecorded yeast cells incubated at 10°C. The colonies of *Mrakia frigida* CY-9-10C (A) and *Slooffia cresolica* PG3-4-10C (B). The budding cells of *Mrakia frigida* CY-9-10C (C) and *Slooffia cresolica* PG3-4-10C (D). Bars, 10 µm and 5 µm, respectively. All strains were grown after 3 days on YPD agar.

tures, API 20C AUX (bioMérieux) were tested according to the manufacturer’s instructions.

Genomic DNA was extracted after being incubated on YPD agar for 3 days. The D1/D2 region of the 26S rRNA gene sequence was amplified by PCR using NL1 (5'-GCATATCAATAAGCGGAGGA AAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') primers (Kurtzman and Robnett, 1998).

Pairwise sequence comparisons were done using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1997) and used Clustal W to align the sequences of related species retrieved from GenBank. The MYCOBANK (<https://www.mycobank.org/>) database identified each species and closely related species, and D1D2 gene sequence were obtained from the NCBI (<https://www.ncbi.nlm.nih.gov/>).

The phylogenetic trees based on the D1/D2 domain of LSU rRNA gene sequence were reconstructed by the neighboring joining algorithm of MEGA 11 program (Tamura *et al.*, 2021) The evolutionary distance was calculated using the two-parameter model of Kimura (Kimura, 1983) and bootstrap analysis are done (1,000 replicates) (Felsenstein, 1985).

RESULTS AND DISCUSSION

Eleven wild yeasts strains were isolated from soil sam-

Table 1. A list of yeast strains isolated from soil in Republic of Korea. All strains were cultured under 10°C for 3 days.

Phylum	Class	Order	Family	Strain ID	Most closely related species	26S rRNA similarity	Record in Korea
Basidiomycota	Tremellomycetes	Cystoflobasidiales	<i>Mrakiaceae</i>	CY-9-10C	<i>Mrakia frigida</i>	530/530 (100%)	Unreported
				CY-2-10C	<i>Saitozyma podzolica</i>	523/524 (99%)	Reported
		Tremellales	Trimorphomycetaceae	CY-6-10C	<i>Saitozyma podzolica</i>	522/524 (99%)	Reported
				CY-7-10C	<i>Saitozyma podzolica</i>	524/524 (100%)	Reported
				CY-8-10C	<i>Saitozyma podzolica</i>	524/524 (100%)	Reported
				CY-10-10C	<i>Saitozyma podzolica</i>	524/524 (100%)	Reported
		Filobasidiales	Piskurozymaceae	PG3-1-10C	<i>Solicozozyma terrea</i>	525/527 (99%)	Reported
				PG3-2-10C	<i>Solicozozyma terrea</i>	527/527 (100%)	Reported
				PG3-3-10C	<i>Solicozozyma terrea</i>	526/526 (100%)	Reported
		Microbotryomycetes	Microbotryomycetes	Microbotryomycetes incertae sedis	Chrysozymaceae	PG3-4-10C	<i>Slooffia cresolica</i>
PG3-5-10C	<i>Rhodotorula nothofagi</i>					516/516 (100%)	Reported

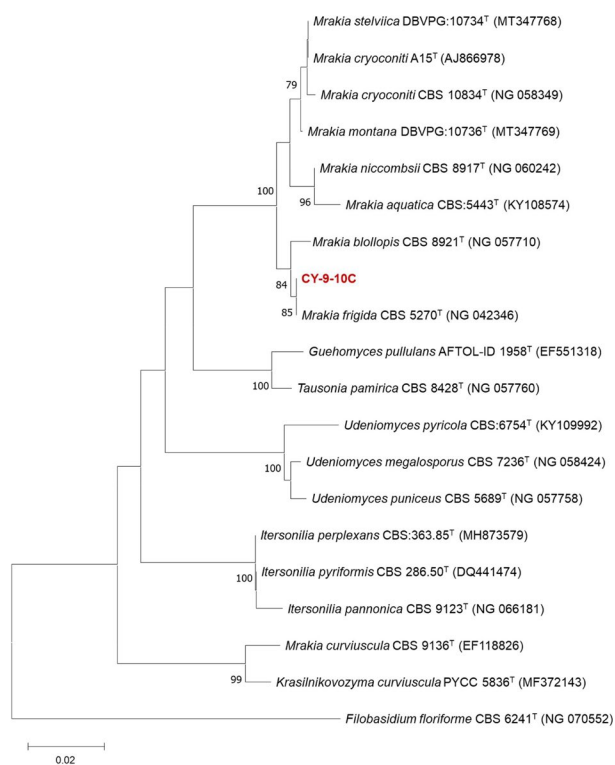


Fig. 2. A neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain CY-9-10C with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position.

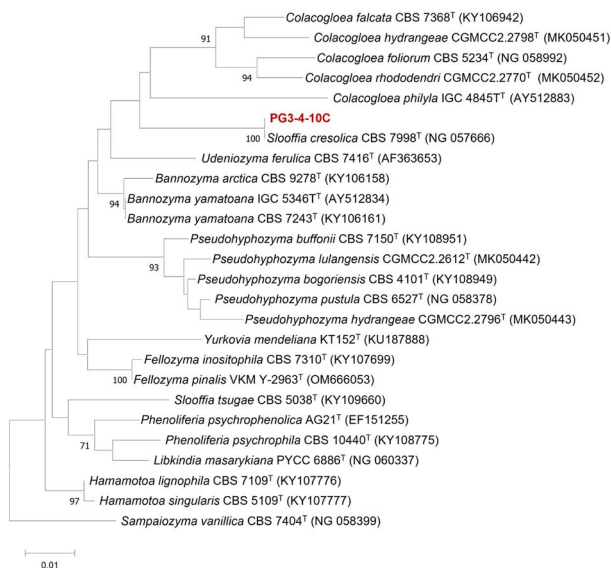


Fig. 3. A neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain PG3-4-10C with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

Table 2. Mycological characteristics and differential carbon assimilation result of *Mrakia frigida* CY-9-10C and closely related species.

Strain ID	1	2	3
Morphological characteristics			
Shape	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding
API 20C AUX			
Glycerol	–	–	v
2-keto-D-gluconate	–	+	+
L-arabinose	w	+	+
D-xylose	w	w	+
Adonitol	–	+	+
Xylitol	–	+	+
D-galactose	w	+	+
Inositol	–	w	–
D-sorbitol	–	+	+
N-methyl-D-glucoside	w	w	–
N-acetyl-D-glucosamine	+	w	–
D-lactose (bovine origin)	w	–	+
D-maltose	–	+	+
D-trehalose	w	+	+
D-melezitose	–	+	+
D-raffinose	–	+	+

Taxa: 1, *Mrakia frigida* CY-9-10C; 2, *Mrakia niccombsii* CBS 8917^T; 3, *Mrakia aquatica* JCM5443^T.

Data of reference strains were obtained from previous study (Liu *et al.*, 2015).

+ , positive; v, variable; w, weakly positive; – , negative; ND, no data.

ples collected in Daegu City and Cheongyang County in Korea. The yeast strains were identified by analyzing D1/D2 domain of 26s rRNA gene sequence similarities that were calculated by the NCBI BLAST. Result of identification based on the D1/D2 domain of 26s rRNA gene sequence, two yeast strains were identified as domestic unrecorded yeast species. The taxonomic composition and identification results are listed in Table 1. Strain CY-9-10C was closely related to the species *Mrakia frigida* with 100% D1D2 sequence similarity. Strain PG3-4-10C was closely related to the species *Slooffia cresolica* with 100% D1D2 sequence similarity.

The 11 strains were assigned to the family *Mrakiaceae* (1 strain), *Trimorphomycetaceae* (5 strains) *Piskurozymaceae* (3 strains), *Chrysozymaceae* (1 strain) and *Sporidiobolaceae* (1 strain) of the phylum *Basidiomycota*. The unrecorded yeast strains CY-9-10C and PG3-4-10C belong to the phylum *Basidiomycota* and the genera *Mrakia* and *Slooffia*, respectively.

The phylogenetic analysis shows that the strain CY-9-10C is closely related to the *Mrakia frigida* and belongs to the genus *Mrakia* (Fig. 2). The phylogenetic analysis

Table 3. Mycological characteristics and differential carbon assimilation result of *Slooffia cresolica* PG3-4-10C and closely related species.

Strain ID	1	2	3
Morphological characteristics			
Shape	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding
API 20C AUX			
Glycerol	–	+	+
2-keto-D-gluconate	–	+	ND
L-arabinose	w	–	–
D-xylose	w	+	w
Adonitol	–	+	w
Xylitol	–	+	ND
D-galactose	w	–	w
D-sorbitol	–	ND	ND
N-methyl-D-glucoside	w	+	–
N-acetyl-D-glucosamine	+	w	ND
D-cellobiose	+	w	–
D-lactose (bovine origin)	w	–	–
D-maltose	–	+	+
D-trehalose	w	+	+
D-melezitose	–	+	+

Taxa: 1, *Slooffia cresolica* PG3-4-10C; *Slooffia cresolica* G36^T; *Bannozyma yamatoana* CBS 7243^T

Data of reference strains were obtained from previous studies (Middelhoven and Spaaij, 1997 and Nakase *et al.*, 1987).

+, positive; w, weakly positive; –, negative; ND, no data.

shows that the strain PG3-4-10C is closely related to the *Slooffia cresolica* and belongs to the genus *Slooffia* (Fig. 3).

Result of carbon source assimilation test using API 20C AUX kit is described in species description. Characteristics of two unrecorded strains and related species are shown in Table 2 and Table 3.

Description of *Mrakia frigida* CY-9-10C

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, and cream colored after 3 days of incubation on YPD agar at 10°C. In the API 20C AUX test, strain CY-9-10C is positive for *N*-acetyl-D-glucosamine, glucose, D-saccharose (sucrose), and D-cellobiose; weak positive for L-arabinose, D-lactose (bovine origin), D-trehalose, 2-keto-D-gluconate, D-galactose, and D-xylose; but negative for D-melezitose D-raffinose, glycerol, L-arabinose, adonitol, inositol, D-sorbitol, *N*-methyl-D-glucoside, D-glucose, and D-maltose.

Strain CY-9-10C was isolated from the soil collected in Cheongyang County, Republic of Korea.

Description of *Slooffia cresolica* PG3-4-10C

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, and beige with red after 3 days of incubation on YPD agar at 10°C. In the API 20C AUX test, strain PG3-4-10C is positive for 2-keto-D-gluconate, L-arabinose, glucose, D-xylose, D-sorbitol, *N*-acetyl-D-glucosamine, D-cellobiose, D-lactose (bovine origin), D-maltose, D-melezitose, D-galactose, inositol, *N*-methyl-D-glucoside, D-saccharose (sucrose), and D-raffinose; weak positive for xylitol and D-trehalose; but negative for glycerol and adonitol.

Strain PG3-4-10C (KACC 410363) was isolated from the soil collected in Daegu, Republic of Korea.

CONFLICTS OF INTEREST

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

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