

Isolation and characterization of two unrecorded yeast species in the phylum *Basidiomycota*

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The purpose of this study was to isolate and identify wild yeasts from soil samples collected in Seoul and Daejeon, Republic of Korea. To identify wild yeast strains, pairwise sequence comparisons of 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 carbon source assimilation test were done using API 20C AUX kit. Among the 13 isolated strains, 11 strains were previously reported, but two strains have never been reported from Republic of Korea. The 13 strains were assigned to the phylum *Basidiomycota*. The two unrecorded yeast strains B2UV-201 and DJ1-5-B-10C belong to the genera *Rhodotorula* and *Rhodospordiobolus*, respectively. The two unrecorded yeast strains are oval shaped and polar budding cells. This research focuses on the morphological and biochemical properties of the two unreported yeast species that have not officially been reported in Korea.

Keywords: *Rhodospordiobolus*, *Rhodotorula*, Unrecorded yeasts

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INTRODUCTION

In the summer of 2023, yeasts were isolated from soil samples collected in Seoul and Daejeon, Republic of Korea. As a result of identifying the isolated yeast, this study determined two yeast strains, B2UV-201 and DJ1-5-B-10C, as unrecorded species. The strain B2UV-201 has characteristics of the genus *Rhodotorula*, and the strain DJ1-5-B-10C has characteristics of the genus *Rhodospordiobolus*.

The genus *Rhodotorula* is the member of the class *Microbotryomycetes*, and the phylum *Basidiomycota*. Species in the genus *Rhodotorula* have been isolated from subsurface water samples and gut of a xylophagous termite (Tiwari *et al.*, 2021). The genus *Rhodotorula* comprises 21 distinct species and has *Rhodotorula glutinis* as type species (<https://www.mycobank.org>).

The genus *Rhodospordiobolus* belongs to the class *Microbotryomycetes* and the phylum *Basidiomycota*. Species in *Rhodospordiobolus* have been isolated from the waste deposit of the attine ant (*Masiulionis* and Pagnocca, 2017). The genus *Rhodospordiobolus* comprises 11 distinct species and has *Rhodospordiobolus nylandii* as type species (<https://www.mycobank.org>). Sexual reproduction was observed in some *Rhodotorula* and *Rhodospordi-*

iobolus species. Both genera show clamp connections, budding cells, and pink to red and butyrous colonies. Pseudohyphae or true hyphae are variable. Ballistoconidia formed or not, ellipsoidal, allantoid or amygdaliform. Major CoQ system is Q-9 or Q-10 (Wang *et al.*, 2015).

MATERIALS AND METHODS

The soil samples were collected from Seoul and Daejeon, Republic of Korea, serially diluted in distilled water, and the suspension was spread in a Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA) and incubated at 10°C for 3 days. The strains are preserved in a metabolically inactive state at the Korean Agricultural Culture Collection (KACC), Korea.

The cell morphologies of strains were observed on a phase contrast microscope (Leica DM500), using yeast strains incubated on YPD agar for 3 days. Phase contrast microscope images and the colonies of the strains B2UV-201 and DJ1-5-B-10C are shown in Fig. 1. To characterize the biochemical features, API 20C AUX (bioMérieux) were done according to the manufacturer's instructions.

The genomic DNA were extracted after incubation on YPD agar for 3 days. The D1/D2 region of the 26S

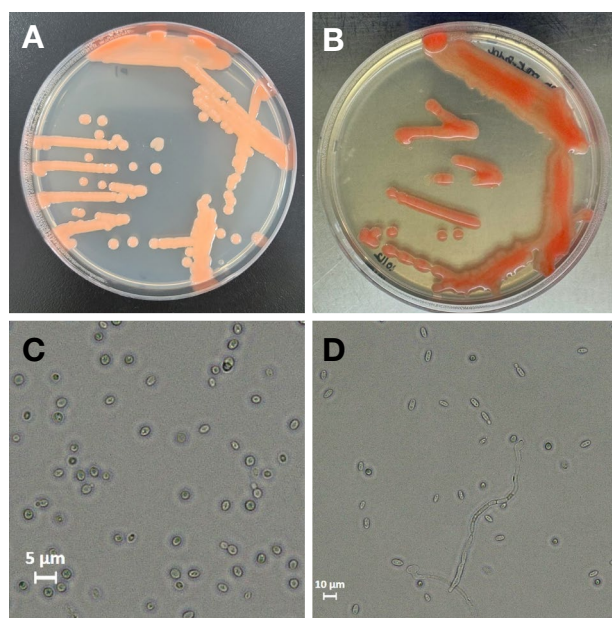


Fig. 1. Morphology of unrecorded strains incubated at 10°C. The colonies of *Rhodotorula alborubescens* B2UV-201 (A) and *Rhodosporidiobolus odoratus* DJ1-5-B-10C (B). Budding cells of *Rhodotorula alborubescens* B2UV-201 (C) and *Rhodosporidiobolus odoratus* DJ1-5-B-10C (D). All strains were grown for 3 days on YPD agar. Bars, 5 µm, and 10 µm, respectively.

rRNA gene sequence was amplified by PCR using primers NL1 (5'-GCATATCAATAAGCGGAGGA AAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') (Kurtzman and Robnett, 1998). Pairwise sequence comparisons were done using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1997) and use Clustal W to align the sequences of strains B2UV-201 and DJ1-5-B-10C with related species retrieved from GenBank.

To identify strains, Mycobank (<https://www.mycobank.org/>) database and NCBI database were used.

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 was done (1,000 replicates) (Felsenstein, 1985).

RESULTS AND DISCUSSION

Thirteen wild yeasts strains were isolated from soil samples from Seoul and Daejeon 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. Based on the result of identification using the D1/D2 domain of 26S rRNA gene sequence, two

Table 1. Yeasts isolated strains from soil in Republic of Korea. All strains were cultured at 10°C for 3 days.

Phylum	Class	Order	Family	Strain	Most closely related species	26S rRNA similarity	Record in Korea
Basidiomycota	<i>Microbotryomycetes</i>	<i>Microbotryomycetes</i>	<i>Sporidiobolaceae</i>	B2UV-201	<i>Rhodotorula alborubescens</i>	497/498 (99.80%)	Unreported
				B2UV-202	<i>Rhodotorula nothofagi</i>	515/515 (100%)	Reported
				DJ1-5-B-10C	<i>Rhodosporidiobolus odoratus</i>	501/501 (100%)	Unreported
	<i>Tremellomycetes</i>	<i>Tremellales</i>	<i>Piskurozymaceae</i>	DJ1-1-10C	<i>Solicozozyma terreia</i>	526/527 (99.00%)	Reported
				DJ1-2	<i>Solicozozyma terreia</i>	526/527 (99.00%)	Reported
				DJ1-6	<i>Solicozozyma terreia</i>	527/527 (100%)	Reported
				PG3-2-10C	<i>Solicozozyma terreia</i>	527/527 (100%)	Reported
				DJ1-3	<i>Solicozozyma terreia</i>	525/527 (99.62%)	Reported
				PG3-1-10C	<i>Solicozozyma terreia</i>	525/527 (99.62%)	Reported
	<i>Rhynchogastremaceae</i>	<i>Rhynchogastremaceae</i>	<i>Rhynchogastremaceae</i>	B2UV-206	<i>Papillotrema flavescens</i>	631/631 (100%)	Reported
				B2UV-207	<i>Papillotrema flavescens</i>	631/631 (100%)	Reported
	<i>Trimorphomycetaceae</i>	<i>Trimorphomycetaceae</i>	<i>Trimorphomycetaceae</i>	DJ1-4	<i>Saitozyma podzolica</i>	520/524 (99.00%)	Reported
				PG3-7UV	<i>Saitozyma podzolica</i>	523/524 (99.81%)	Reported

Table 2. Mycological characteristics of the unrecorded yeasts strains and closely related species.

Strain ID	1	2	3	4
Morphological characteristics				
Shape	Oval	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding	Budding
API 20C AUX				
Glucose	+	+	+	+
Glycerol	–	W	+	+
Calcium 2-keto-gluconate	–	–	+	ND
L-Arabinose	W	–	+	W
D-Xylose	W	+	+	+
Adonitol	+	+	ND	ND
Xylitol	+	–	+	W
D-Galactose	W	W	+	–
Inositol	–	–	–	–
D-Sorbitol	W	+	+	+
Methyl- α -D-glucopyranoside	–	–	–	–
<i>N</i> -Acetyl-glucosamine	–	–	ND	–
D-Cellobiose	W	–	–	–
D-Lactose (bovine origin)	–	–	–	–
D-Maltose	+	W	+	–
D-Saccharose (sucrose)	+	+	+	ND
D-Trehalose	W	W	+	+
D-Melezitose	+	–	+	–
D-Raffinose	+	+	+	ND

Taxa: 1, *Rhodotorula alborubescens* B2UV-201; 2, *Rhodospordiobolus odoratus* DJ1-5-B-10C; 3, *Rhodotorula mucilaginosa* PYCC 5166^T; 4, *Rhodospordiobolus odoratus* CBS:9115^T.

All data were obtained in this study. +, positive; W, weakly positive; –, negative; ND, no data.

yeast strains were identified as unrecorded yeast species.

The taxonomic composition and identification results are listed in Table 1. Strain B2UV-201 was closely related to *Rhodotorula alborubescens* with 99.80% D1/D2 sequence similarity. Strain DJ1-5-B-10C was closely related to *Rhodospordiobolus odoratus* with 100% D1/D2 sequence similarity.

All strains were assigned to the families *Sporidiobolaceae* (3 strains), *Piskurozymaceae* (6 strains) *Rhynchogastremaceae* (2 strains), and *Trimorphomycetaceae* (2 strains) of the phylum *Basidiomycota*. The unrecorded yeast strains B2UV-201 and DJ1-5-B-10C belong to the genera *Rhodotorula* and *Rhodospordiobolus*, respectively. The phylogenetic tree shows that the isolated strains are closely related to *Rhodotorula* and *Rhodospordiobolus* species with the highest 26S rRNA gene sequence similarity (Figs. 2 and 3).

Results 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.

Description of *Rhodotorula alborubescens* B2UV-201

Cells are oval shaped and budding is polar (Fig. 1). Colonies are convex, smooth, and pink colored after 3 days of incubation on YPD agar at 10°C. In the API 20C AUX test, strain B2UV-201 is positive for glucose, adonitol, xylitol, D-maltose, D-saccharose (sucrose), D-melezitose, and D-raffinose; weak positive for L-arabinose, D-xylose, D-galactose, D-sorbitol, D-cellobiose, and D-trehalose; but negative for *N*-acetyl-glucosamine, D-lactose (bovine origin), glycerol, calcium 2-keto-gluconate, inositol, and methyl- α -D-glucopyranoside.

Strain B2UV-201 (KCTC 37245) was isolated from soil collected in Nowon, Seoul, Republic of Korea.

Description of *Rhodospordiobolus odoratus* DJ1-5-B-10C

Cells are oval shaped and budding is polar (Fig. 1). Colonies are convex, smooth, and red-pink colored after 3 days of incubation on YPD agar at 10°C. In the API 20C AUX test, strain DJ1-5-B-10C is positive for glucose,

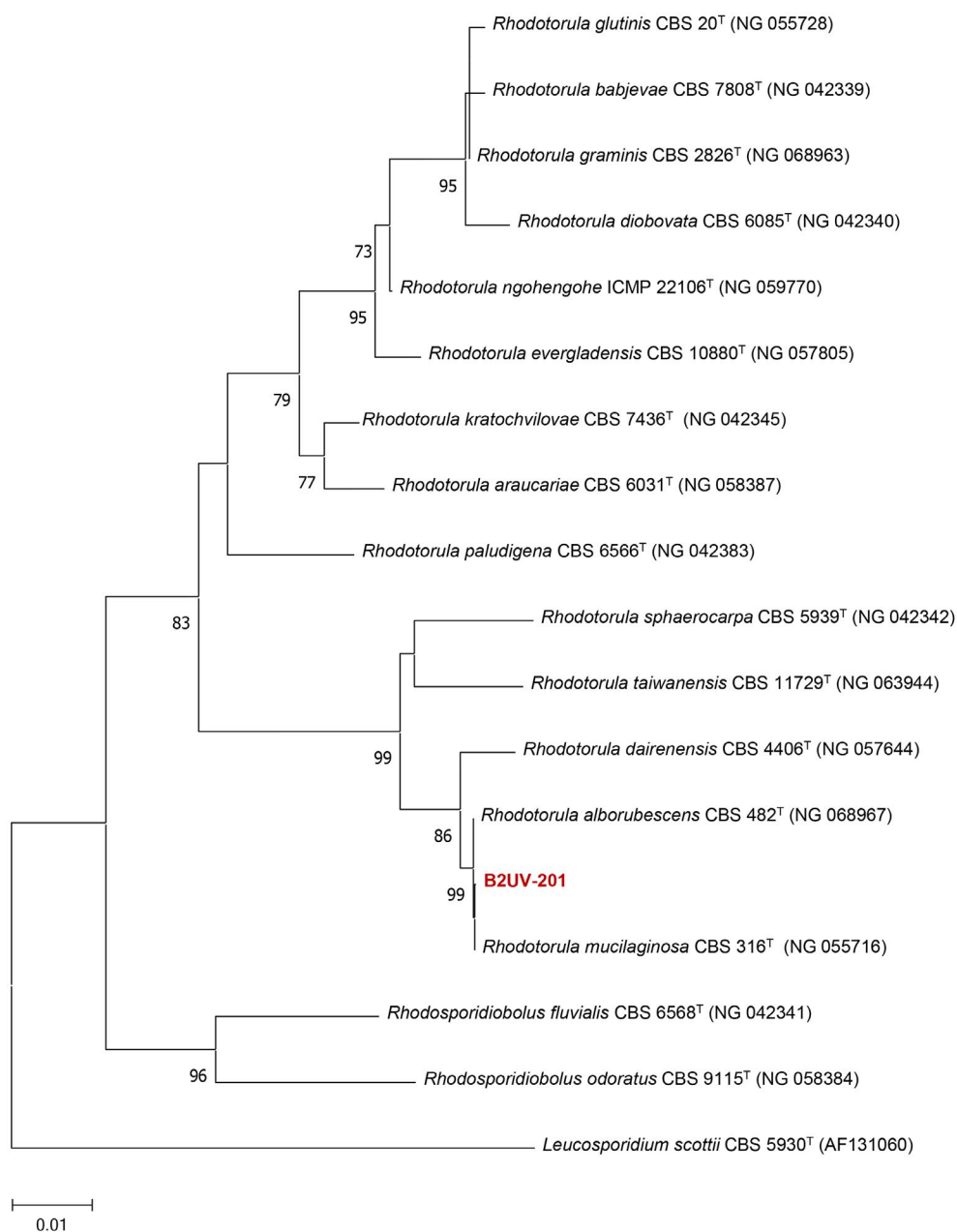


Fig. 2. A neighbor-joining phylogenetic tree constructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain B2UV-201 with closely related species. Bootstrap values (>70%) are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

D-xylose, adonitol, D-sorbitol, D-saccharose (sucrose), and D-raffinose; weak positive for glycerol, D-galactose, D-maltose, and D-trehalose; but negative for calcium 2-keto-gluconate, L-arabinose, xylitol, inositol, methyl- α -D-glucopyranoside, N-acetyl-glucosamine, D-cellobiose, D-lactose (bovine origin), and D-melezitose.

Strain DJ1-5-B-10C (KCTC XXXX) was isolated from soil collected in Daejeon, 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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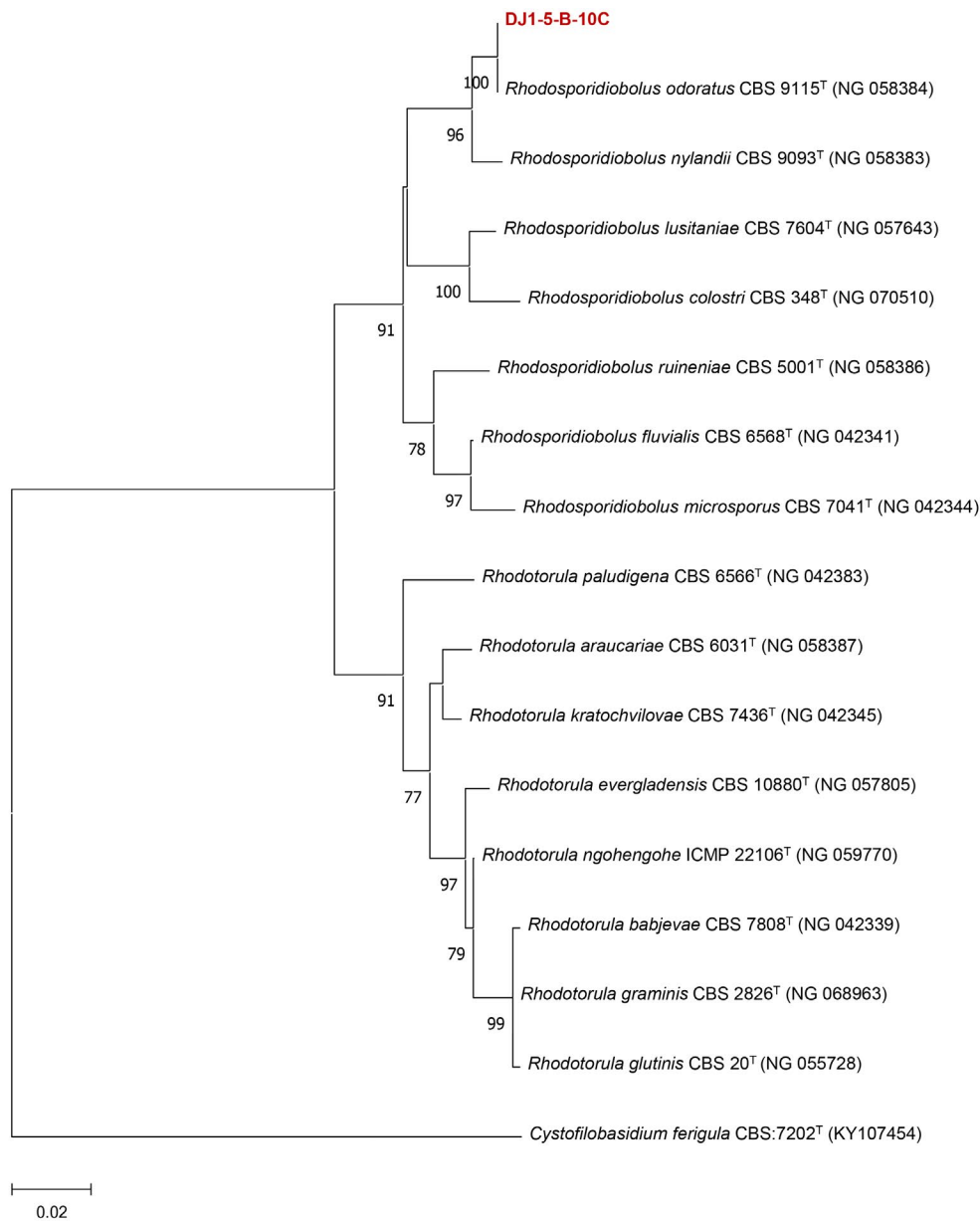


Fig. 3. A neighbor-joining phylogenetic tree constructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain DJ1-5-B-10C with closely related species. Bootstrap values (>70%) are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position.

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