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Characterization of two unrecorded yeast species in the families *Cystobasidiaceae* and *Sporidiobolaceae* isolated from *Craspedonotus tibialis* and mountain soil in Korea

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The purpose of this study was to isolate and identify wild yeasts from *Craspedonotus tibialis* samples in Taean-gun and soil samples in Uijeongbu-si, Republic of Korea. Out of the sixteen strains identified, fourteen had been previously recorded, whereas two strains were unrecorded in the Republic of Korea. Identification of wild yeast strains was performed through pairwise sequence comparisons of the D1/D2 region of the 26S rRNA gene using the Basic Local Alignment Search Tool (BLAST) (Fell *et al*., 2000). Cell morphology was examined using a phase contrast microscope, and assimilation tests were conducted with the API 20C AUX kit. All strains were classified under the phylum *Basidiomycota*. The two unrecorded yeast strains, CB9-2 and YP99, are classified under the genus *Cystobasidium* (family *Cystobasidiaceae*, order *Cystobasidiales*, class *Cystobasidiomycetes*) and the genus *Rhodosporidiobolus* (family *Sporidiobolaceae*, order *Sporidiobolales*, class *Microbotryomycetes*), respectively. These two strains exhibited morphologies consistent with their type species. Both CB9-2 and YP99 cells were oval, and both strains demonstrated polar budding. This study highlighted the morphological and biochemical characteristics of the two unrecorded yeast species that had not been previously documented in Korea.

Keywords: 26S rRNA, *Cystobasidium*, *Rhodosporidiobolus*, Unrecorded yeasts

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Introduction

Yeasts, belonging to the *Ascomycota*, *Basidiomycota*, and *Deuteromycota* phyla, have been traditionally utilized for centuries in the fermentation of foods and beverages (Lee *et al*., 1997). Among them, basidiomycetous yeasts are particularly important due to their economic, agricultural, and medical significance. Despite this, only a small fraction (1−5%) of these species has been identified, leaving the major of their diversity unexplored (Choudhary and Johri, 2009). These yeasts play a valuable role in industries, such as producing astaxanthin for aquaculture and synthesizing enzymes like xylanases and cellulases (Fell *et al*., 2001). This underlines the need for thorough classification and research to better understand their ecological functions and fully utilize their potential.

Within the Basidiomycota phylum, the genera *Cystobasidium* and *Rhodosporidiobolus* have emerged as significant contributors to both ecological balance and potential biotechnological applications. Species in the *Cystobasidium* genus, within the *Cystobasidiaceae* family, thrive in diverse environments, including cold habitats, demonstrating metabolic versatility. They produce bioactive compounds like carotenoids with antioxidant properties, which have potential industrial applications in food technology and pharmaceuticals. Their ability to adapt to lower temperatures further highlights their potential for biotechnological use in challenging environments. Similarly, the *Rhodosporidiobolus* genus, part of the *Sporidiobolaceae* family, holds substantial ecological and biotechnological importance. Members of this genus are known for their production of pigments like torulene and β-carotene, which contribute to their potential use in various industries, including food, pharmaceuticals, and cosmetics (Chreptowicz *et al*., 2019).

Historically, yeast isolation efforts have focused on fermented foods and raw materials, with some strains being applied in the fermentation industry (Kim *et al*., 1999).

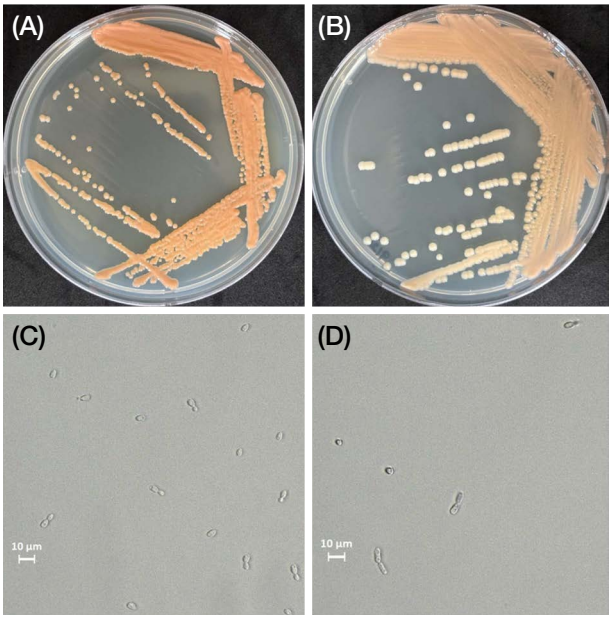


Fig. 1. Morphology of cells from the unreported strains CB9-2 and YP99 incubated at 25°C. All strains were grown on YPD agar for 3 days. The colonies of *Cystobasidium slooffiae* CB9-2 (A) and *Rhodosporidiobolus oreadorum* YP99 (B). The budding cells of *Cystobasidium slooffiae* CB9-2 (C) and *Rhodosporidiobolus oreadorum* YP99 (D). Bars, 10 μm.

However, the study of yeasts from natural habitats and their potential industrial applications remains relatively limited.

In previous work, we isolated yeast strains from various soil environments, including cold winter soils (Park *et al*., 2011), urban soils (Park *et al*., 2021; Maeng *et al*., 2022), and mountainous soils (Seok *et al*., 2024), identifying them through molecular biological tools. In this current study, yeast strains were isolated from *Craspedonotus tibialis* samples in Taean-gun and soil samples in Uijeongbu-si, Republic of Korea. Two previously unrecorded yeast strains were discovered, and their phenotypic characteristics were investigated.

Materials and Methods

Craspedonotus tibialis samples were collected in Taean-gun, and soil samples were collected in Uijeongbu-si, Republic of Korea. The samples were serially diluted in distilled water, and the dilutions were spread on Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA) plates and incubated at 25°C for 3 days. The strains have been preserved in a metabolically inactive state at the Korean Agricultural Culture Collection, Korea.

Cell morphologies of the strains were examined using a LEICA DM500 microscope after 3 days of incubation

Table 1. Yeasts isolated strains from soil in Republic of Korea. All strains were cultured under 25°C and 10°C for 3 days.

Phylum	Class	Order	Family	Strain ID	Most closely related species	26S rRNA similarity	Record in Korea
Basidiomycota	*Cystobasidiomycetes*	*Cystobasidiales*	*Cystobasidiaceae*	CB9-2	*Cystobasidium slooffiae*	621/622 (99.84%)	Unrecorded
	Microbotryomycetes	*Leucosporidiales*	*Leucosporidiaceae*	CB9-3	*Leucosporidium golubevii*	589/589 (100.00%)	Recorded
		Sporidiobolales	*Sporidiobolaceae*	YP99	*Rhodosporidiobolus oreadorum*	504/507 (99.41%)	Unrecorded
				YP62	*Rhodotorula mucilaginosa*	592/592 (100.00%)	Recorded
				YP63	*Rhodotorula mucilaginosa*	592/592 (100.00%)	Recorded
		Filobasidiales	*Filobasidiaceae*	YP64	*Filobasidium magnum*	620/620 (100.00%)	Recorded
				YP88	*Filobasidium magnum*	620/620 (100.00%)	Recorded
		Cystofilobasidiales	*Cystofilobasidiaceae*	CB9-5	*Cystofilobasidium capitatum*	618/618 (100.00%)	Recorded
				CB9-4	*Cystofilobasidium macerans*	609/611 (99.67%)	Recorded
	Tremellomycetes	*Tremellales*	*Bulleribasidiaceae*	YP73	*Dioszegia ovata*	614/616 (99.68%)	Recorded
				YP75	*Dioszegia zsoltii*	608/610 (99.67%)	Recorded
				YP91	*Dioszegia zsoltii*	620/620 (100.00%)	Recorded
				YP84	*Vishniacozyma tephrensis*	604/606 (99.67%)	Recorded
				YP85	*Vishniacozyma tephrensis*	604/606 (99.67%)	Recorded
				YP82	*Vishniacozyma victoriae*	613/615 (99.67%)	Recorded
	Ustilaginomycetes	*Ustilaginales*	*Ustilaginaceae*	YP83	*Moesziomyces aphidis*	618/618 (100.00%)	Recorded

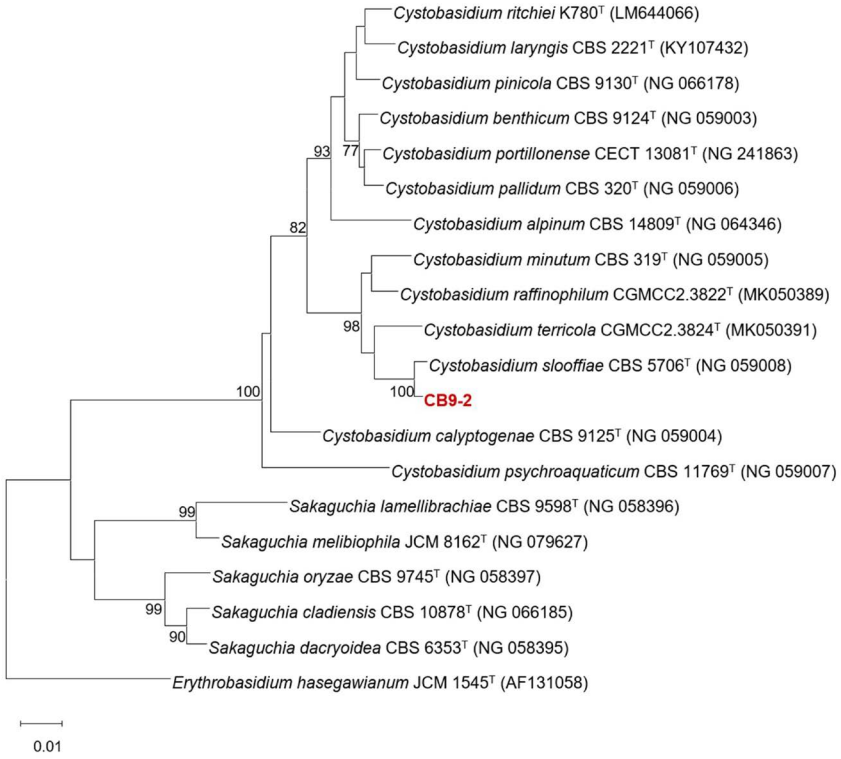


Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains CB9-2 with closely related species. *Erythrobasidium hasegawianum* JCM 1545^{T} was used as the outgroup. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

on YPD agar. Phase contrast microscope images and colony morphologies of strains MFL-1 and NYB3-16 are presented in Fig. 1. For biochemical characterization, the API 20C AUX system (bioMérieux) was used according to the manufacturer's instructions.

Genomic DNA was extracted after 3 days of incubation at 25°C on YPD agar. The D1/D2 region of the 26S rRNA gene was amplified by PCR using the primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′).

Pairwise sequence comparisons were conducted using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) to align with sequences of related species retrieved from GenBank. The MYCOBANK database (https://www.mycobank.org/) was used to identify strain types for each species, and the gene sequences of closely related strains for the 26S rDNA region were obtained from NCBI (https://www.ncbi.nlm.nih.gov/).

Phylogenetic trees based on the D1/D2 domain of the LSU rRNA gene were reconstructed using the neighbor-joining method (Saitou and Nei, 1987) with the MEGA 11 program (Tamura *et al*., 2021). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983) for the neighbor-joining analysis, and the confidence levels of the clades were assessed through bootstrap analysis with 1,000 replicates (Felsenstein, 1985). Outgroup species for the phylogenetic tree were selected based on established yeast taxonomy practices, ensuring the inclusion of distantly related species to appropriately root the tree (Guarro *et al*., 1999; Rainey and Oren, 2011).

Results

Sixteen wild yeast strains were collected yeasts from *Craspedonotus tibialis* samples in Taean-gun and soil samples in Uijeongbu-si, Korea. The yeast strains were identified by sequence analysis of the D1/D2 domain of the 26S rRNA gene using NCBI BLAST for similarity calculation. Based on this analysis, two yeast strains were identified as previously unrecorded species in Korea. All strains were classified into the *Cystobasidiaceae* (1 strain), *Leucosporidiaceae* (1 strain), *Sporidiobolaceae* (3 strains), *Filobasidiaceae* (2 strains), *Cystofilobasidiaceae* (2 strains), *Bulleribasidiaceae* (6 strains), and *Ustilaginaceae* (1 strain) within the phylum *Basidiomycota*.

Among the yeast strains collected from *Craspedonotus tibialis* samples in Taean-gun, one unrecorded species and three recorded species were identified. The unrecorded species was identified as *Cystobasidium slooffiae* CB9-2, while the recorded species included *Leucosporidium gol-*

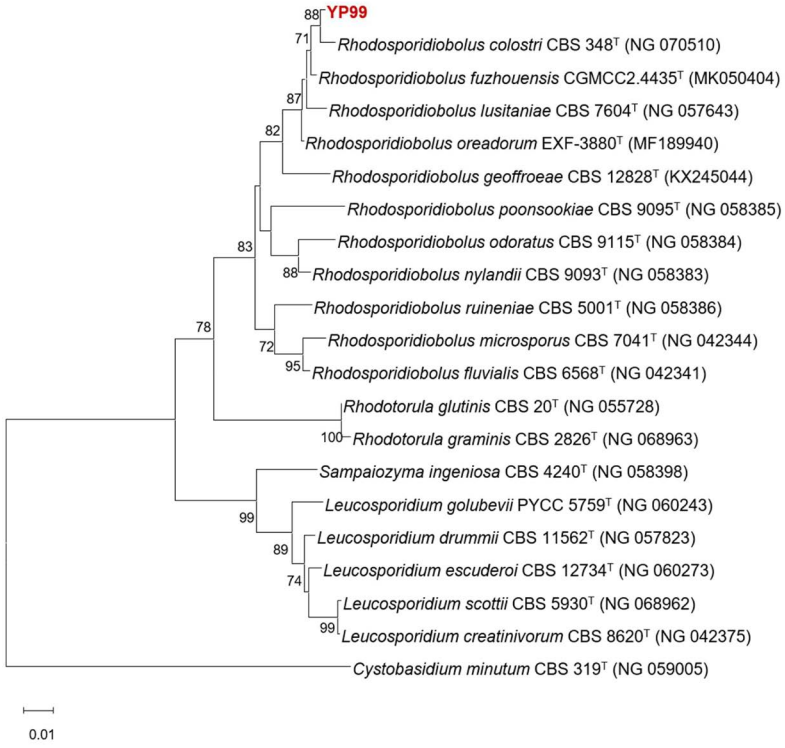


Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain YP99 with closely related species. *Cystobasidium minutum* CBS 319^{T} was used as the outgroup. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

ubevii CB9-3, *Cystofilobasidium macerans* CB9-4, and *Cystofilobasidium macerans* CB9-5.

From the soil samples collected in Uijeongbu-si, one unrecorded species and eleven recorded species were identified. The unrecorded species was identified as *Rhodosporidiobolus oreadorum* YP99. The recorded species included *Rhodotorula mucilaginosa* YP62, *Rhodotorula mucilaginosa* YP63, *Filobasidium magnum* YP64, *Filobasidium magnum* YP88, *Dioszegia ovata* YP73, *Dioszegia zsoltii* YP75, *Dioszegia zsoltii* YP91, *Vishniacozyma tephrensis* YP84, *Vishniacozyma tephrensis* YP85, *Vishniacozyma victoriae* YP82, and *Moesziomyces aphidis* YP83. The taxonomic composition and identification results are detailed in Table 1.

The unrecorded yeast strains CB9-2 and YP99 also belong to *Basidiomycota*, with CB9-2 classified in the genus *Cystobasidium* and YP99 in the genus *Rhodosporidiobolus*. The phylogenetic tree indicates that strain CB9-2 is closely related to species of *Cystobasidium*, while strain YP99 is closely related to species of *Rhodosporidiobolus*, based on the highest 26S rRNA gene sequence similarity (Figs. 2 and 3), thus supporting their close phylogenetic relationships.

Discussion

Species of *Cystobasidium* have been isolated from diverse cold environments such as glaciers, alpine regions, and the Arctic (Turchetti *et al*., 2018), while *Rhodosporidiobolus* species are frequently found in environments rich in organic matter, including birch forests where they are isolated from tree bark, sap, and slime exudates (Kot *et al*., 2020). The genus *Cystobasidium* includes sixteen species, with *Cystobasidium fimetarium* as the type species, while the genus *Rhodosporidiobolus* consists of fourteen species, with *Rhodosporidiobolus ruineniae* as the type species (https://www.theyeasts.org/).

Despite belonging to different families, *Cystobasidiaceae* and *Rhodosporidiobolus* share several characteristics typical of their respective taxonomic groups. The *Cystobasidiaceae* family, which includes *Cystobasidium*, is characterized by the presence of polar budding cells and the absence of pseudohyphae. Members of this family do not form basidiocarps in laboratory conditions and lack fermentative abilities. Additionally, they produce carotenoid pigments, such as torularhodin and torulene, resulting in pink to orange colonies. They are capable of assimilating various carbon compounds but do not utilize nitrate (Yurkov *et al*., 2015).

Table 2. Phenotypic characteristics that differentiate strain CB9-2 and related species.

	1	2	3
Morphological characteristics			
Shape	Oval	Ellipsoidal	Oval
Reproduction	Budding	Budding	Budding
Colony color	Light orange	Light pink	Pink
Growth on			
Temp (°C)	10−25	19−30	19−35
Assimilation of			
Glucose	+	+	+
Glycerol	+	+	+
Calcium 2-Keto-Gluconate	+	+	ND
L-Arabinose	+	+	+
D-Xylose	+	+	+
Adonitol	−	+	+
Xylitol	−	d	ND
D-Galactose	−	−	v
Inositol	−	−	−
D-Sorbitol	−	+	+
Methyl-α-D-Glucopyranoside	−	−	−
N-Acetyl-Glucosamine	w	ND	ND
D-Cellobiose	−	+	+
D-Lactose	−	+	+
D-Maltose	−	−	−
D-Saccharose	+	+	+
D-Trehalose	+	v	+
D-Melezitose	+	+	+
D-Raffinose	−	−	v

Taxa: 1, *Cystobasidium slooffiae* CB9-2; 2, *Cystobasidium slooffiae* CBS 5706^{T}; 3, *Cystobasidium terricola* CGMCC 2.3823^{T}.
+, positive; w, weak positive; −, negative; v, variable; ND, no data.

Similarly, the *Sporidiobolaceae* family, which includes *Rhodosporidiobolus*, shares several features, including the presence of budding cells and the possible presence or absence of pseudohyphae or true hyphae under certain conditions. Some members of this genus can form minute basidiocarps under specific conditions related to sexual reproduction. They are also known for producing red to orange colonies due to the presence of carotenoid pigments, and their primary coenzyme Q system is typically CoQ-10. These yeasts metabolize a variety of carbon sources, including glucose and cellobiose (Wang *et al*., 2015).

These characteristics emphasize the taxonomic diversity within *Basidiomycota* and highlight specific ecological adaptations in both *Cystobasidium* and *Rhodosporidiobolus*. Despite their differences, both genera are notable for their pigmentation and ability to assimilate various carbon sources, suggesting roles in nutrient cycling. The discovery of these unrecorded species expands our understanding of Korean yeast biodiversity and points to their potential in biotechnological applications, including food production and environmental sustainability.

Description of *Cystobasidium slooffiae* CB9-2

Cells are circular and exhibit polar budding (Fig. 1). After 3 days of incubation on YPD agar at 25°C, colonies are light orange, mucoid, round to irregular, and raised. In the API 20C AUX test, strain CB9-2 tests positive for glucose, glycerol, calcium-2-keto-D-gluconate, L-arabinose, D-xylose, D-saccharose (sucrose), D-trehalose, and D-melezitose; weakly positive for *N*-acetyl-D-glucosamine; but negative for adonitol, xylitol, D-galactose, inositol, D-sorbitol, methyl-α-D-glucopyranoside, D-cellobiose, D-lactose (bovine origin), D-maltose, and D-raffinose.

Strain CB9-2 (KCTC 37312) was isolated from *Craspedonotus tibialis* samples collected in Taean-gun, Chungcheongnam-do, Republic of Korea.

Table 3. Phenotypic characteristics that differentiate strain YP99 and related species.

	1	2	3
Morphological characteristics			
Shape	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding
Colony color	Cream to light orange	Reddish	Deep pink
Growth on			
Temp (°C)	10−25	4−25	4−25
Assimilation of			
Glucose	−	+	+
Glycerol	−	d	+
Calcium 2-Keto-Gluconate	−	ND	v
L-Arabinose	−	+	v
D-Xylose	−	+	v
Adonitol	w	+	+
Xylitol	−	ND	v
D-Galactose	−	+	+
Inositol	−	−	v
D-Sorbitol	w	+	+
Methyl-α-D-Glucopyranoside	−	ND	v
N-Acetyl-Glucosamine	−	ND	ND
D-Cellobiose	−	−	+
D-Lactose	−	−	v
D-Maltose	−	−	−
D-Saccharose	−	+	+
D-Trehalose	−	+	+
D-Melezitose	−	−	v
D-Raffinose	−	w	v

Taxa: 1, *Rhodosporidiobolus oreadorum* YP99; 2, *Rhodosporidiobolus oreadorum* EXF-3880^{T}; 3, *Rhodosporidiobolus colostri* CBS 348^{T}.
+, positive; w, weak positive; −, negative; v, variable; ND, no data.

Description of *Rhodosporidiobolus oreadorum* YP99

Cells are oval and exhibit polar budding (Fig. 1). After 3 days of incubation on YPD agar at 25°C, colonies range in color from cream to light orange, appearing round and raised. In the API 20C AUX test, strain YP99 tests weakly positive for adonitol and D-sorbitol; but negative for glucose, glycerol, calcium-2-keto-D-gluconate, L-arabinose, D-xylose, xylitol, D-galactose, inositol, methyl-α-D-glucopyranoside, *N*-acetyl-D-glucosamine, D-cellobiose, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose, and D-raffinose.

Strain YP99 (KACC 48748) was isolated from soil samples collected in Uijeongbu-si, Gyeonggi-do, Republic of Korea.

Conflicts of Interest

These authors of this paper have no affiliation with any interests and is solely responsible for the paper.

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