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Isolation and characterization of two unreported yeast species from wildflowers and mountain soil in Korea in the families *Filobasidiaceae* and *Piskurozymaceae*

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The purpose of this study was to isolate and identify wild yeasts from wildflower samples in Okcheon-gun and soil samples in Nowon-gu, Republic of Korea. Out of the fourteen strains identified, twelve had been previously reported, whereas two strains were unreported 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 unreported yeast strains, MFL-1 and NYB3-16, are classified under the genus *Naganishia* (family *Filobasidiaceae*, order *Filobasidiales*, class *Tremellomycetes*) and the genus *Solicoccozyma* (family *Piskurozymaceae*, order *Filobasidiales*, class *Tremellomycetes*), respectively. These two strains exhibited morphologies consistent with their type species. MFL-1 cells were circular, while NYB3-16 cells were oval. Both strains demonstrated polar budding. This study highlighted the morphological and biochemical characteristics of the two unreported yeast species that had not been previously documented in Korea.

Keywords: 26S rRNA, *Naganishia*, *Solicoccozyma*, unreported yeasts

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INTRODUCTION

Yeasts, belonging to the *Ascomycota*, *Basidiomycota*, and *Deuteromycota* phyla, have been used for centuries in the fermentation of traditional foods and beverages (Lee *et al*., 1997). Among these, basidiomycetous yeasts hold particular economic, agricultural, and medical significance. However, despite their potential, only 1 to 5% of these species have been identified, leaving much of their diversity unexplored (Boekhout *et al*., 2022). These yeasts are valuable in industrial applications, such as the production of astaxanthin in aquaculture and enzymes like xylanases and cellulases (Fell *et al*., 2001). This highlights the need for systematic classification and research to fully understand their ecological roles and harness their potential.

Within the *Basidiomycota*, families like *Filobasidiaceae* and *Piskurozymaceae* are particularly noteworthy due to their adaptations to extreme environments. The *Naganishia* genus, part of *Filobasidiaceae*, includes yeasts that thrive in cold, UV-intense, and nutrient-poor habitats, supported by genetic traits for trehalose synthesis and lipid metabolism. They also produce bioactive compounds like carotenoids and mycosporines, which offer UV protection (Nizovoy *et al*., 2021). Similarly, *Solicoccozyma* species from the *Piskurozymaceae* family are often found in soils and are known for their lipid accumulation, crucial for survival in resource-limited environments and promising for industrial applications like biodiesel production (Passoth, 2017). Their metabolic versatility further enhances their ecological and biotechnological value (Fonseca *et al*., 2011). Understanding these yeast families deepens our knowledge of microbial diversity and provides pathways for sustainable biotechnologies.

Until now, yeasts have mainly been isolated from fermented foods and raw materials, with some isolates applied in the food fermentation industry (Kim *et al*., 1999). However, research on the classification of yeasts from natural habitats and their industrial applications remains limited.

We have previously isolated yeasts 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), and identified them using molecular biological tools. In this study, wild yeasts were isolated from wildflower samples collected in Okcheon-gun and soil samples from Nowon-gu, Republic of Korea. Two unreported yeast strains were discovered, and their phenotypic characteristics were investigated.

MATERIALS AND METHODS

Wildflower samples were collected from Okcheon-gun, and soil samples were collected from Nowon-gu, 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 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.

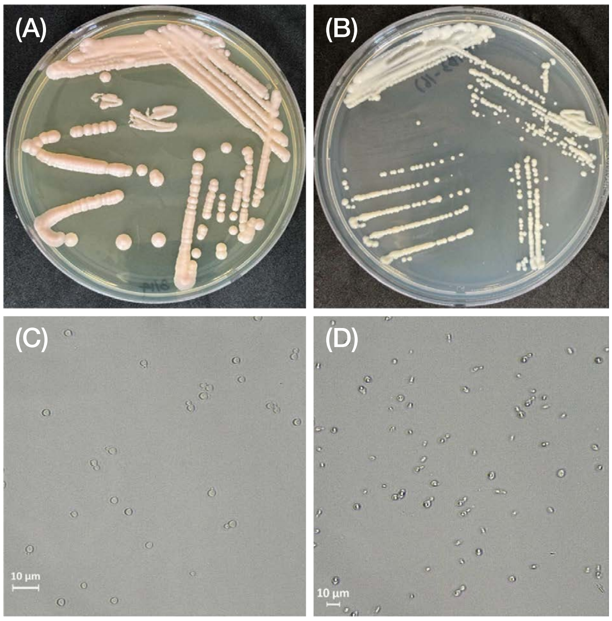

Fig. 1. Morphology of cells from the unreported strains MFL-1 and NYB3-16 incubated at 25°C. All strains were grown on YPD agar for 3 days. The colonies of *Naganishia liquefaciens* MFL-1 (A) and *Solicoccozyma gelidoterrea* NYB3-16 (B). The budding cells of *Naganishia liquefaciens* MFL-1 (C) and *Solicoccozyma gelidoterrea* NYB3-16 (D). Bars, 10 µm.

Genomic DNA was extracted after 3 days of incubation on YPD agar. The D1/D2 region of the 26S rRNA gene was amplified by PCR using the primers NL1 (5′-GCATATCA ATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGT GTTTCAAGACGG-3′) (Kurtzman and Robnett, 1998).

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

Fourteen wild yeast strains were collected from wildflower samples in Okcheon-gun and soil samples in Nowon-gu, 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 unreported species in Korea. All strains were classified into the families *Mrakiaceae* (1 strain), *Filobasidiaceae* (1 strain), *Piskurozymaceae* (6 strains), *Holtermanniella* (1 strain), and *Trimorphomycetaceae* (5 strains) within the phylum *Basidiomycota*.

Among the yeast strains collected from wildflower samples in Okcheon-gun, one strain, *Naganishia liquefaciens* MFL-1, was identified as an unreported species in Korea. From the soil samples in Nowon-gu, the isolated species included *Guehomyces pullulans* YB6-2-1, *Solicoccozyma gelidoterrea* NYB3-16, *Solicoccozyma terrea* YB1UV-4, YB1UV-6, YB1UV-3, *Solicoccozyma terricola* NYB3-14, NYB3-15, *Holtermanniella wattica* YB6-1, and *Saitozyma podzolica* YB2UV-1, YB3UV-1, YB3UV-4, NYB3-12, and NYB2-1. Additionally, *Solicoccozyma gelidoterrea* NYB3-16 was identified as another previously unreported species. The taxonomic composition and identification results are detailed in Table 1.

The unreported yeast strains MFL-1 and NYB3-16 both

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	*Tremellomycetes*	*Cystofilobasidiales*	*Mrakiaceae*	YB6-2-1	*Guehomyces pullulans*	525/529 (99.24%)	Reported
		Filobasidiales	*Filobasidiaceae*	MFL-1	*Naganishia liquefaciens*	603/603 (100.00%)	Unreported
			Piskurozymaceae	NYB3-16	*Solicoccozyma gelidoterrea*	527/527 (100.00%)	Unreported
				YB1UV-4	*Solicoccozyma terrea*	620/620 (100.00%)	Reported
				YB1UV-6	*Solicoccozyma terrea*	620/620 (100.00%)	Reported
				YB1UV-3	*Solicoccozyma terrea*	620/620 (100.00%)	Reported
				NYB3-14	*Solicoccozyma terricola*	527/527 (100.00%)	Reported
				NYB3-15	*Solicoccozyma terricola*	527/527 (100.00%)	Reported
		Holtermanniales	*Holtermanniella*	YB6-1	*Holtermanniella wattica*	524/526 (99.62%)	Reported
		Tremellales	*Trimorphomycetaceae*	YB2UV-1	*Saitozyma podzolica*	523/524 (99.81%)	Reported
				YB3UV-1	*Saitozyma podzolica*	523/524 (99.81%)	Reported
				YB3UV-4	*Saitozyma podzolica*	523/524 (99.81%)	Reported
				NYB3-12	*Saitozyma podzolica*	523/524 (99.81%)	Reported
				NYB2-1	*Saitozyma podzolica*	523/524 (99.81%)	Reported

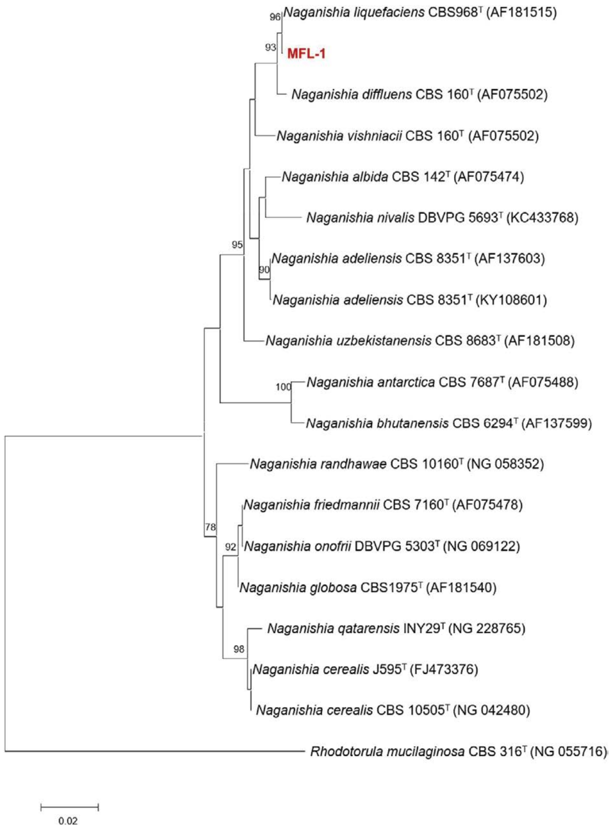

Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains MFL-1 with closely related species. *Rhodotorula mucilaginosa* CBS 316^T was used as the outgroup. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position.

belong to *Basidiomycota*, with MFL-1 classified in the genus *Naganishia* and NYB3-16 in the genus *Solicoccozyma*. Phylogenetic analysis indicates that MFL-1 is closely related to species of *Naganishia*, while NYB3-16 is closely related to species of *Solicoccozyma*, based on the highest 26S rRNA gene sequence similarity (Figs. 2 and 3), supporting their close phylogenetic relationships.

Discussion

Species of *Solicoccozyma* have been isolated from diverse environments such as soil and crater lakes (Li *et al.*, 2019), while *Naganishia* species have been found in environments like blue cheese, air, and dry moss (Luo *et al.*, 2019; Farias *et al.*, 2022). The genus *Solicoccozyma* includes eleven species, with *Solicoccozyma aeria* as the type species, while the genus *Naganishia* consists of twenty-three species, with *Naganishia globosa* as the type species (https://www.mycobank.org).

Despite belonging to different families, *Solicoccozyma* and *Naganishia* share several characteristics typical of their respective taxonomic groups. For example, the *Piskurozymaceae* family, to which *Solicoccozyma* belongs, is characterized by the possible presence or absence of pseudohyphae and true hyphae, along with budding cells and occasional clamp connections. Notably, members of this family do not form basidiocarps and often utilize nitrate, with CoQ-10 as the primary CoQ system.

Similarly, the *Filobasidiaceae* family, which includes *Naganishia*, shares features such as the absence of basidiocarps and the presence of budding cells. However, *Filobasidiaceae* members are distinguished by their tubular holobasidia with terminal, sessile basidiospores. They also have a varied CoQ system, typically CoQ-9 or CoQ-10, and frequently utilize a range of organic acids, including L-malic and p-hydroxybenzoic acids (Liu *et al*., 2015).

These shared and distinct characteristics underline the taxonomic diversity within *Basidiomycota* and suggest potential ecological adaptations worthy of further investigation. These discoveries increase our understanding of the taxonomic diversity of *Basidiomycota* and demonstrate the ecological adaptability of *Solicoccozyma* and *Naganishia* in varied environments. Their potential roles in nutrient cycling and microbial ecosystems remain underexplored but hold promise for biotechnological applications, such as in food production, waste management, and bioremediation.

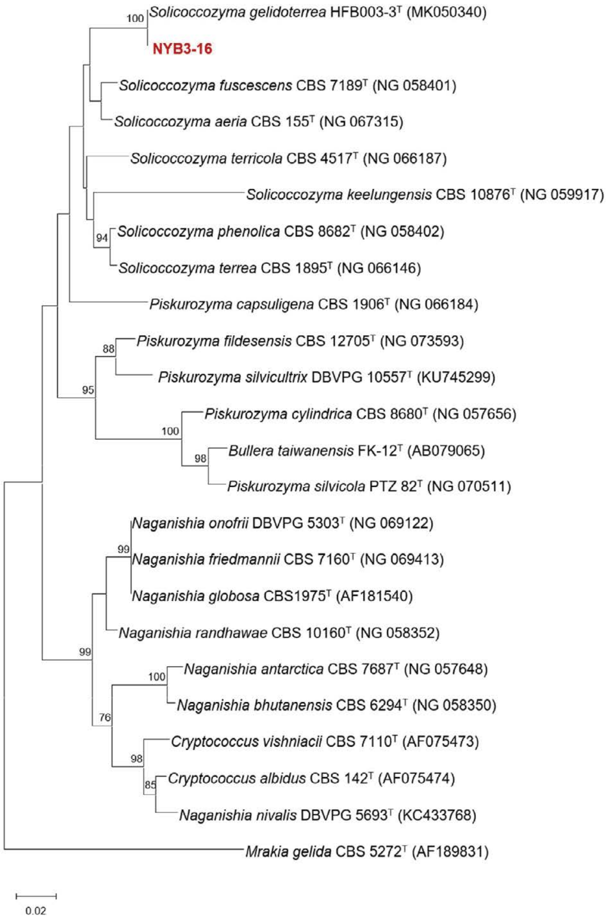

Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain NYB3-16 with closely related species. *Mrakia gelida* CBS 5272^T was used as the outgroup. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position.

By documenting these unreported species, this study contributes to a better understanding of Korean yeast biodiversity and emphasizes the need for further research into their ecological functions and environmental sustainability contributions.

Description of *Naganishia liquefaciens* MFL-1

Cells are circular and exhibit polar budding (Fig. 1). After 3 days of incubation on YPD agar at 25°C, colonies appear convex, glistening, and light pink. In the API 20C AUX test, strain MFL-1 tests positive for glucose, calcium-2-keto-D-gluconate, L-arabinose, D-xylose, D-sorbitol, methyl-α-D-glucopyranoside, D-cellobiose, D-maltose,

Table 2. Phenotypic characteristics that differentiate strain MFL-1 and related species.

	1	2	3	4
Growth on				
Temp (°C)	15–30	19–30	4–30	4–21
Assimilation of				
Glucose	+	+	+	+
Glycerol	−	w	−	−
Calcium 2-Keto-Gluconate	+	+	+	+
L-Arabinose	+	+	+	+
D-Xylose	+	+	+	v
Adonitol	w	−	+	−
Xylitol	−	w	+	−
D-Galactose	w	−	−	−
Inositol	−	−	+	−
D-Sorbitol	+	−	v	−
Methyl-α-D-Glucopyranoside	+	−	v	v
N-Acetyl-Glucosamine	−	−	ND	ND
D-Cellobiose	+	+	+	v
D-Lactose	w	+	−	−
D-Maltose	+	+	+	+
D-Saccharose	+	+	v	v
D-Trehalose	+	+	+	+
D-Melezitose	+	+	+	+
D-Raffinose	+	+	+	v

Taxa: 1, *Naganishia liquefaciens* MFL-1; 2, *Naganishia liquefaciens* CBS 968^T; 3, *Naganishia diffluens* CBS 160^T; 4, *Naganishia vishniacii* CBS 7110^T. +, positive; w, weak positive; −, negative; v, variable; ND, no data.

Table 3. Phenotypic characteristics that differentiate strain NYB3-16 and related species.

	1	2	3	4
Growth on				
Temp (°C)	15–30	19–30	4–30	19–25
Assimilation of				
Glucose	+	+	+	+
Glycerol	−	v	−	−
Calcium 2-Keto-Gluconate	+	ND	+	+
L-Arabinose	+	+	v	+
D-Xylose	+	+	+	+
Adonitol	w	+	+	−
Xylitol	−	ND	v	−
D-Galactose	+	+	+	−
Inositol	w	+	v	+
D-Sorbitol	w	+	v	−
Methyl-α-D-Glucopyranoside	+	+	+	−
N-Acetyl-Glucosamine	w	+	ND	ND
D-Cellobiose	+	+	v	+
D-Lactose	−	+	v	−
D-Maltose	+	+	+	−
D-Saccharose	w	+	v	−
D-Trehalose	−	v	+	−
D-Melezitose	+	+	+	−
D-Raffinose	−	+	+	−

Taxa: 1, *Solicoccozyma gelidoterrea* NYB3-16; 2, *Solicoccozyma gelidoterrea* CGMCC 2.5814^{T}; 3, *Solicoccozyma aeria* CBS 155^{T}; 4, *Solicoccozyma fuscescens* CBS 7189^{T}.

+, positive; w, weak positive; −, negative; v, variable; ND, no data.

D-saccharose (sucrose), D-trehalose, D-melezitose, and D-raffinose; weakly positive for adonitol, D-galactose, and D-lactose (bovine origin); but negative for glycerol, xylitol, inositol, and *N*-acetyl-D-glucosamine.

Strain MFL-1 (KCTC 37299) was isolated from wildflowers collected in Okcheon-gun, North Chungcheong Province, Republic of Korea.

Description of *Solicoccozyma gelidoterrea* NYB3-16

Cells are oval and show polar budding (Fig. 1). After 3 days of incubation on YPD agar at 25°C, colonies are convex, glistening, and light-yellow. In the API 20C AUX test, strain NYB3-16 tests positive for glucose, calcium-2-keto-D-gluconate, L-arabinose, D-xylose, D-galactose, methyl-α-D-glucopyranoside, D-cellobiose, D-maltose, and D-melezitose; weakly positive for adonitol, inositol, D-sorbitol, *N*-acetyl-D-glucosamine, and D-saccharose (sucrose); but negative for glycerol, xylitol, D-lactose (bovine origin), D-trehalose, and D-raffinose.

Strain NYB3-16 (KCTC 37289) was isolated from soil collected in Nowon-gu, Seoul, 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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