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Isolation and characterization of four unrecorded wild yeasts from the soils of Republic of Korea in winter

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The purpose of this study was to isolate and identify wild yeasts from the soil collected in Gwangju and Pocheon City, Gyeonggi Province, Republic of Korea. Among 10 strains, six strains were already reported, but four strains were unrecorded in Republic of Korea. To identify wild yeast strains, pairwise sequence comparisons of the D1/D2 region of the 26S rRNA gene sequence were performed using Basic Local Alignment Search Tool (BLAST). The cell morphologies were observed by phase contrast microscope and assimilation tests were carried out using API 20C AUX kit. The 10 strains were assigned to the phyla *Basidiomycota* (8 strains) and *Ascomycota* (2strains). The unrecorded four yeast strains, NH33, NH19, NH20, and YP416, belong to the phylum *Basidiomycota* and the genera *Buckleyzyma*, *Leucosporidium*, *Holtermanniales*, and *Mrakia*, respectively. All strains had oval-shaped and polar budding cells. In this research, the morphological and biochemical properties of four unreported yeast species were characterized intensively, which were not officially reported in Korea.

Keywords: *Buckleyzyma*, *Holtermanniales*, *Leucosporidium*, *Mrakia*, unrecorded yeasts

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Introduction

In the winter of 2020, yeasts were separated from the soil in Gwangju and Pocheon City, Gyeonggi Province, Republic of Korea. As a result of confirming the isolated yeast strains, this study determined them as an unrecorded species, and for the purpose of classification, they identified to the genera *Buckleyzyma*, *Leucosporidium*, *Holtermanniella*, and *Mrakia*.

The genus *Buckleyzyma* is a *Basidiomycetous* yeast in the class *Cystobasidiomycetes*, and the phylum *Basidiomycota*. The genus *Buckleyzyma* clade consists of five species with *B. aurantiaca* as the type species (https://www.mycobank.org). *Buckleyzyma aurantiaca* was originally described as *Torula aurantiaca* in Japan in 1922. *Buckleyzyma* has no observed pellicle formation on media. Species in *Buckleyzyma* have been isolated from plant leaves, air sampling, lake water, mangrove sediments, and litter (Wang *et al*., 2015).

The genus *Leucosporidium* is a *Basidiomycetous* yeast in the class *Microbotryomycetes*, and the phylum *Basidiomycota*. The *Leucosporidium* clade consists of 22 recognized species and has *L. scottii* as the type species (https://www.mycobank.org). This yeast reproduces by budding and are oval, ellipsoid, or extension shaped. When the colony grows in solid media, the color changes from white to cream and often to mucous (Fell *et al*., 2000; Sampaio *et al*., 2003). Species in the genus *Leucosporidium* do not ferment glucose and assimilat nitrate (Sampaio 2011a; 2011b).

The genus *Holtermanniella* is a *Basidiomycetous* yeast in the class *Tremellomycetes*, and the phylum *Basidiomycota*. Species in the clade *Holtermanniella* have been isolated from various sources such as agricultural field, soil, and leaves (Wuczkowski *et al*., 2011). The *Holtermanniella* clade consists of five recognized species and has *H. takashimae* as the type species.

The genus *Mrakia* is a *Basidiomycetous* yeast in the class *Tremellomycetes*, and the phylum *Basidiomycota*. *Mrakia* species have been isolated from snow, soil, and glaciers in cold environments (Tsuji *et al*., 2019; Turchetti *et al*., 2020; Yurkov *et al*., 2020). The species in *Mrakia* are cream-colored and often mucoid, and teliospores are produced at the end or in the middle of the mycelium (Tsuji *et al*., 2019; Turchetti *et al*., 2020; Yurkov *et al*., 2020). The *Mrakia* clade consists of 18 recognized species and

has *M. frigida* as the type species (https://www.mycobank.org).

Materials and Methods

The soil samples were collected from Gwangju and Pocheon City in Gyeonggi Province, Republic of Korea and serially diluted in distilled water and the suspension was spread on a YM agar (Difco, USA) and incubated at 10°C for 3−4 days, cultured at 25°C. The strains are preserved in a metabolically inactive state at the Korea Collection for Type Cultures, KRIBB, Korea.

The cell morphologies of strains were observed using a LEICA (DM500), using yeast strains incubated in YM agar for 3 days. Phase contrast microscope images and the colonies of the strains NH19, NH20, NH33, and YP416 are shown in Fig. 1. Biochemical features were characterized using a API 20C AUX (bioMérieux) kit according to the manufacturer's instructions.

The genomic DNA was extracted after incubation on YM agar for 3−4 days. The D1/D2 region of the 26S rRNA gene sequence was amplified by PCR using NL1 and NL4 primers (Kurtzman and Robnett, 1998).

Pairwise sequence comparisons were made using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) and aligned with the sequences of related species retrieved from GenBank. The MYCOBANK (https://www.mycobank.org/) database identified strain types for each species and close strains gene sequence were obtained from NCBI (https://www.ncbi.nlm.nih.gov/) for 26S rRNA.

The phylogenetic trees based on the D1/D2 domain of LSU rRNA gene sequence were reconstructed with the neighboring joining algorithm of MEGA 7.0 program (Kumar *et al*., 2016). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983) for neighborhood joining analysis and the confidence level of the class was estimated through bootstrap analysis (1,000 replicates) (Felsenstein, 1985).

Results and Discussion

Ten wild yeasts strains were collected from soil samples in Gyeonggi Province 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, four yeast strains were identified as domestic unrecorded yeast species. The taxonomic composition and identification results are listed in Table 1.

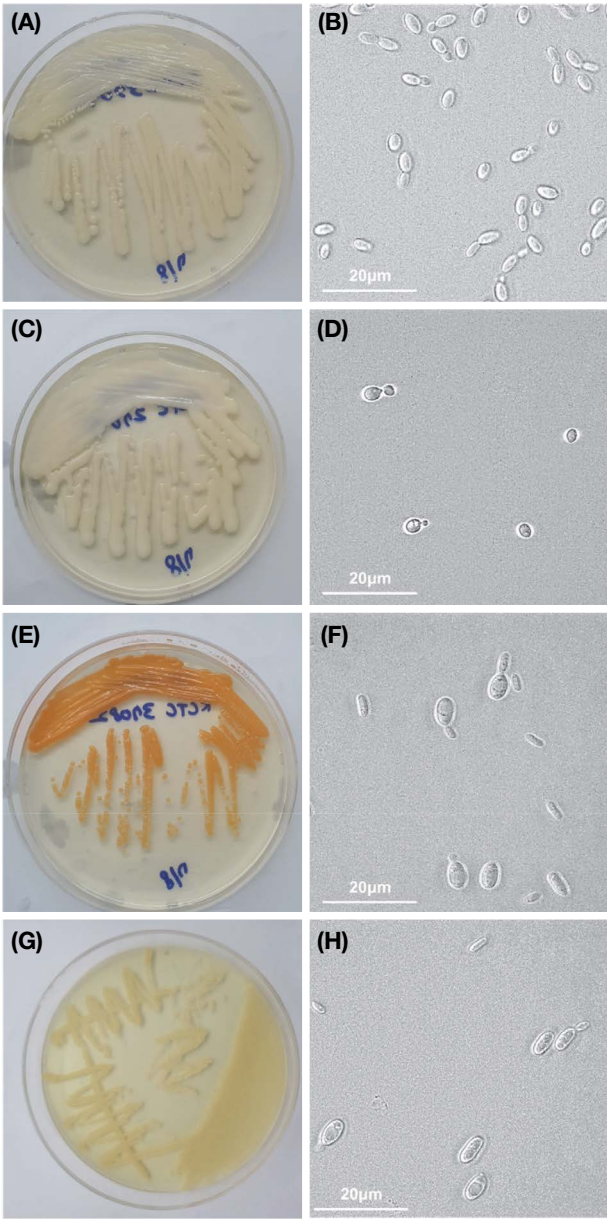

Fig. 1. Morphology of cells from the unrecorded strains incubated at 10°C. The colonies of *Leucosporidium scottii* NH19 (A), *Holtermanniella wattica* NH20 (B), *Buckleyzyma aurantiaca* NH33 (C), and *Mrakia aquatica* YP416 (D). The budding cells of *Leucosporidium scottii* NH19 (F), *Holtermanniella wattica* NH20 (G), *Buckleyzyma aurantiaca* NH33 (H), and *Mrakia aquatica* YP416 (I). Bars, 20 μm. All strains were grown after 3 days on YM agar.

In total, 8 of the 10 strains were assigned to the family *Cystobasidiomycetes incertae sedis* (1 strain), *Leucosporidiaceae* (1 strain), *Holtermanniales* (1 strain), *Trimorphomycetaceae* (4 strains), and *Cystofilobasidiaceae* (1 strain) of the phylum *Basidiomycota*, while the other two strains were assigned to the family *Saccotheciaceae* of the phylum *Ascomycota*. The unrecorded yeast strains NH33,

Table 1. Yeasts isolated strains from soil in Republic of Korea.

Phylum	Class	Order	Family	Strain	Most closely related species	26S rRNA similarity	Record in Korea
Ascomycota	*Dothideomycetes*	*Dothideales*	*Saccotheciaceae*	YF37	*Aureobasidium pullulans*	578/581 (99%)	Reported
				YF42	*Aureobasidium subglaciale*	582/584 (99%)	Reported
Basidiomycota	*Cystobasidiomycetes*	*Buckleyzymales*	*Buckleyzymaceae*	NH33	*Buckleyzyma aurantiaca*	619/619 (100%)	Unreported
	Microbotryomycetes	*Leucosporidiales*	*Leucosporidiaceae*	NH19	*Leucosporidium scottii*	577/578 (99%)	Unreported
	Tremellomycetes	*Holtermanniales*	.	NH20	*Holtermanniella wattica*	619/621 (99%)	Unreported
		Tremellales	*Trimorphomycetaceae*	NH235	*Saitozyma flava*	621/621 (100%)	Reported
				NH233	*Saitozyma podzolica*	614/615 (99%)	Reported
				NH8	*Saitozyma podzolica*	631/631 (100%)	Reported
				NH9	*Saitozyma podzolica*	624/624 (100%)	Reported
	Cystofilobasidiomycetes	*Cystofilobasidiales*	*Cystofilobasidiaceae*	YP416	*Mrakia aquatica*	617/623 (99%)	Unreported

All strains were cultured at 10 °C for 3 days.

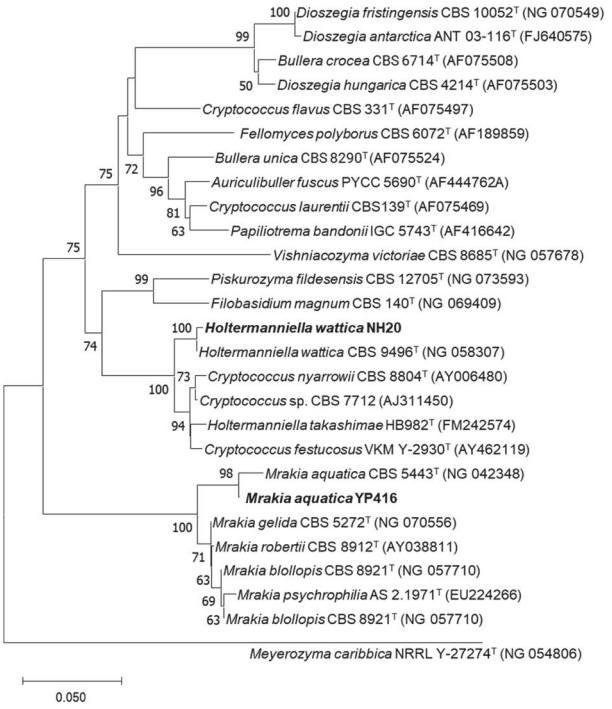

Fig. 2. Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains NH20 and YP416 with closely related species. Bootstrap values (>50%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

NH19, NH20, and, YP416 belong to the phylum *Basidiomycota* and the genera *Buckleyzyma*, *Leucosporidium*, *Holtermanniales*, and *Mrakia*, respectively. The phylogenetic agreement of the four strains shows that the isolated strain is closely related to the strains with the highest 26s rRNA gene sequence similarity (Figs. 2, 3 and 4) and thus supports close relationships. As a result of this study, four unrecorded yeast strains were found in domestic ecosystems and phenotypic characteristics of unrecorded species were investigated (Table 2).

Description of *Buckleyzyma aurantiaca* NH33

Cells are oval shaped and budding is polar (Fig. 1). Colonies are convex, smooth, and orange-colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain NH33 is positive for L-arabinose, *N*-acetyl-D-glucosamine, D-lactose (bovine origin), D-saccharose (sucrose), D-trehalose, D-melezitose, and D-raffinose; weak positive for glycerol, glucose, 2-keto-D-gluconate, L-arabinose, adonitol, D-galactose, inositol, D-sorbitol, and *N*-methyl-D-glucoside; and negative for D-glucose, D-xylose, D-cellobiose, and D-maltose.

Strain NH33 (KCTC 37082) was isolated from the soil collected in Namhansanseong Forest, Gwangju City,

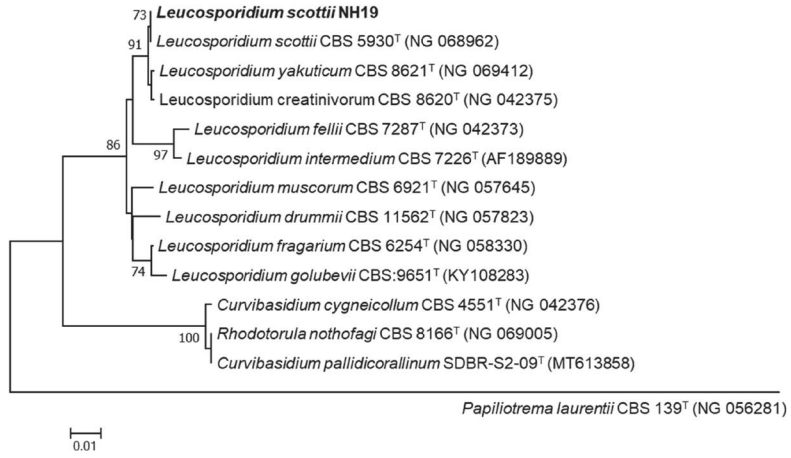

Fig. 3. Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain NH19 with closely related species. Bootstrap values (>50%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

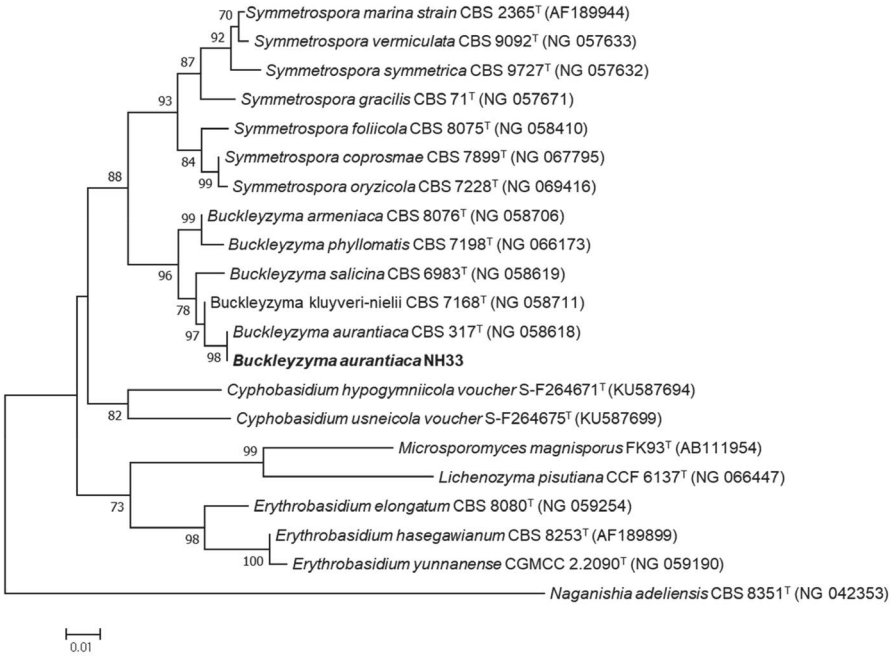

Fig. 4. Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain NH33 with closely related species. Bootstrap values (>50%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

Gyeonggi Province, Republic of Korea.

Description of *Leucosporidium scottii* NH19

Cells are oval shaped and budding is polar (Fig. 1). Colonies are convex, smooth, and beige-colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain NH19 is positive for glycerol, 2-keto-D-gluconate, L-arabinose, and adonitol; weak positive for glucose, D-xylose, D-sorbitol, *N*-acetyl-D-glucosamine, D-cellobiose, D-lactose (bovine origin), D-maltose, and D-melezitose; and negative for xylitol, D-galactose, inositol, *N*-methyl-D-glucoside, D-saccharose (sucrose), D-trehalose, and D-raffinose.

Strain NH19 (KCTC 37092) was isolated from the soil collected in Namhansanseong Forest, Gwangju City, Gyeonggi Province, Republic of Korea.

Table 2. Microbiological characteristics of the unrecorded yeasts strains.

Strain ID	1	2	3	4
Morphological characteristics				
Shape	Oval	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding	Budding
API 20C AUX				
Glucose	w	−	+	+
Glycerol	+	w	w	−
2-Keto-D-Gluconate	+	w	+	+
L-Arabinose	+	+	+	−
D-xylose	w	−	+	+
Adonitol	+	w	w	ND
Xylitol	−	−	w	+
D-Galactose	−	w	w	+
Inositol	−	w	+	−
D-Sorbitol	w	w	+	ND
N-Methyl-D-Glucoside	−	w	+	+
N-Acetyl-D-Glucosamine	w	+	−	+
D-Cellobiose	w	−	+	+
D-Lactose (bovine origin)	w	+	+	+
D-Maltose	w	−	+	+
D-Saccharose (Sucrose)	−	+	+	+
D-Trehalose	−	+	+	+
D-Melezitose	w	+	+	+
D-Raffinose	−	+	+	+

Taxa: 1, *Leucosporidium scottii* NH19; 2, *Buckleyzyma aurantiaca* NH33; 3, *Holtermanniella wattica* NH20; 4, *Mrakia aquatica* YP416.
All data were obtained in this study. +, positive; w, weakly positive; −, negative.

Description of *Holtermanniella wattica* NH20

Cells are oval shaped and budding is polar (Fig. 1). Colonies are convex, smooth, and white-colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain NH20 is positive for D-glucose, calcium, 2-keto-D-gluconate, L-arabinose, D-xylose, inositol, D-sorbitol, *N*-methyl-D-glucoside, D-cellobiose, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-trehalose, and D-raffinose; weak positive for glycerol, adonitol, xylitol, and D-galactose; and negative for *N*-acetyl-D-Glucosamine.

Strain NH20 (KCTC 37091) was isolated from the soil collected in Namhansanseong Forest, Gwangju City, Gyeonggi Province, Republic of Korea.

Description of *Mrakia terrae* YP416

Cells are oval shaped and budding is polar (Fig. 1). Colonies are convex, smooth, and orange-colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain YP416 is positive for glucose, inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose, maltose, melezitose, methyl-D-glucoside, cellobiose, salicin, L-sorbose, D-xylose, L-arabinose, D-ribose, methanol, ethanol, ribitol, xylitol, galactitol, D-mannitol, D-glucitol, D-lactate, succinate, citrate, D-gluconate, gluconolactone, D-glucosamine, *N*-acetyl-D-glucosamine, potassium nitrate, sodium nitrate, cadaverine dihydrochloride, and L-lysine; and negative for soluble starch, cellobiose, salicin, L-sorbose, L-rhamnose, D-xylose, L-arabinose, D-arabinose, D-ribose, methanol, ethanol, glycerol, erythritol, ribitol, xylitol, galactitol, D-mannitol, D-glucitol, and myo-Inositol.

Strain YP416 (KCTC 27889) was isolated from the soil collected in Pocheon City, Gyeonggi Province, Republic of Korea.

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