

# Isolation of wild yeasts from soils collected in Pochoen-si, Korea and characterization of unrecorded yeasts

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In 2019, as a subset study to discover indigenous yeast species in Korea, a total of 20 yeast species were isolated from soil samples collected in Pochoen-si. Among them, eight strains were unreported species. From the high 26S rRNA gene sequence similarity and formation of a robust phylogenetic clade with the closest species, it was determined that each strain belonged to independent and predefined yeast species. The 20 strains were assigned to the genera *Aureobasidium* (1 strain) and *Meyerozyma* (1 strain) of the phylum *Ascomycota* and *Cystofilobasidium* (2 strains), *Filobasidium* (1 strain), *Naganishia* (2 strains), *Bullera* (3 strains), *Leucosporidium* (9 strains) and *Sampaiozyma* (1 strain) of the phylum *Basidiomycota*. There is no official report of the following species in Korea: *Leucosporidium creatinivorum* (4 strains), *Leucosporidium escuderoi* (2 strains), *Leucosporidium golubevii* (1 strain) and *Leucosporidium intermedium* (2 strains). Basic biochemical characteristics, colony and cell morphology are also described in the species description section.

Keywords: 26S rRNA, *Leucosporidium*, unreported species, yeast diversity

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## INTRODUCTION

In 2019, 20 strains were isolated from diverse soil samples collected in Korea. Among them four species were unreported in Korea. The current report focuses on the description of four unreported yeast species that have not officially been reported in Korea.

The 20 strains were assigned to the genera *Aureobasidium* (1 strain) and *Meyerozyma* (1 strain) of the phylum *Ascomycota* and *Cystofilobasidium* (2 strains), *Filobasidium* (1 strain), *Naganishia* (2 strains), *Bullera* (3 strains), *Leucosporidium* (9 strains) and *Sampaiozyma* (1 strain) of the phylum *Basidiomycota*.

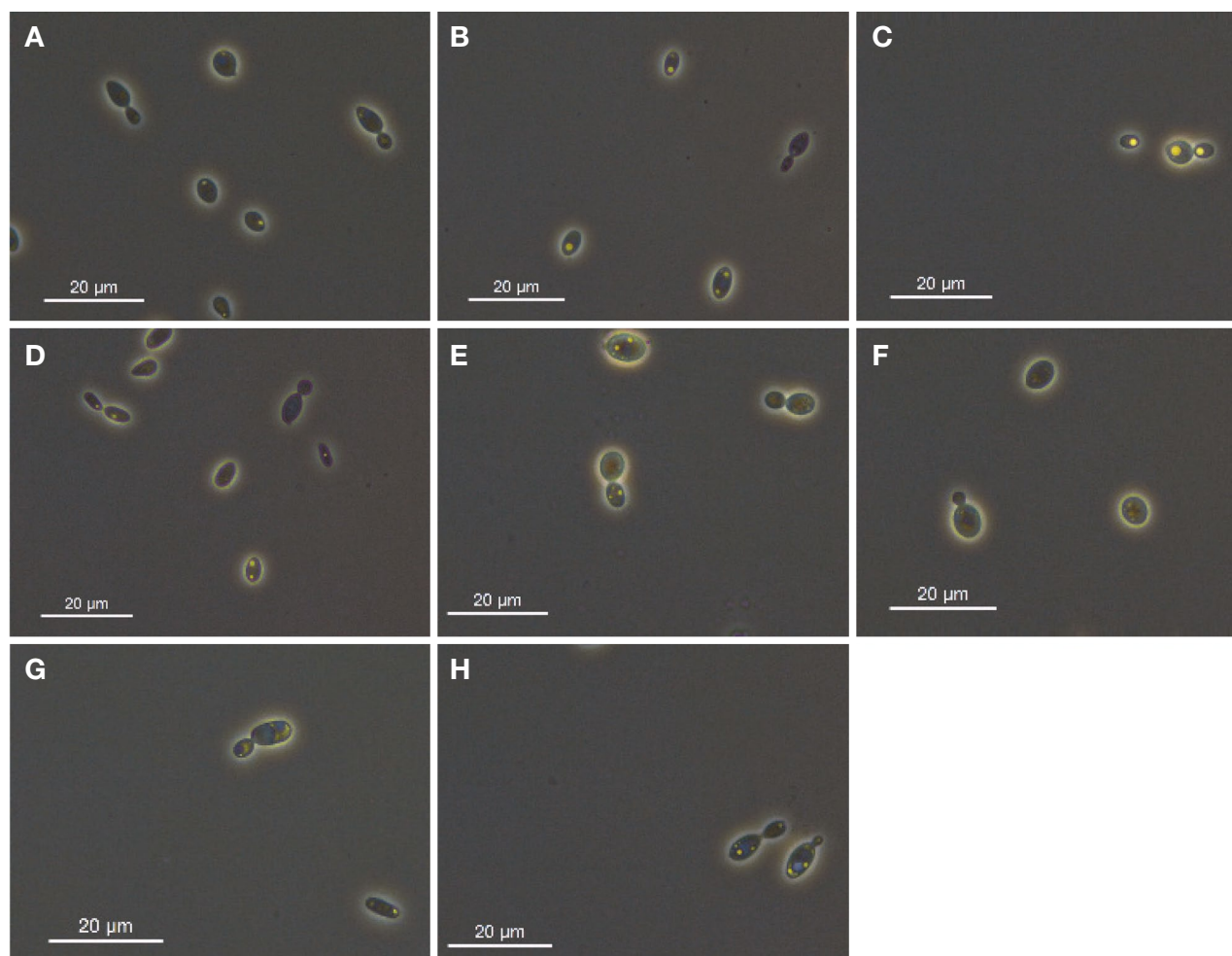
Among them eight yeast strains YP204, YP205, YP211, YP215, YP189, YP196, YP329 and YP76 are unreported strains, belong to the genus *Leucosporidium*. The species of *Leucosporidium* reproduce by budding. Cells are ovoid, ellipsoid or elongate. Pseudohyphae or true hyphae may be present. Ballistoconidia are not formed. Growth on solid media is white to cream colored and often mucous. Visible carotenoid pigments are not present (Sampaio, 2011b). The yeasts of the genera *Leucosporidium* (teleomorph) and *Leucosporidiella* (anamorph) are classified in the order Leucosporidiales (Microbotryomycetes,

Pucciniomycotina; Sampaio *et al.*, 2003; 2004; Sampaio, 2011a; 2011b). Most of the yeast species of these genera are psychrotolerant or psychrophilic organisms, and they have been isolated from rather cold environments around the world, including marine Antarctic ecosystems and terrestrial areas (Di Menna, 1960; 1966; Fell *et al.*, 1969; Goto *et al.*, 1969; Carrasco *et al.*, 2012; Duarte *et al.*, 2013; Vaca *et al.*, 2013).

## MATERIALS AND METHODS

Soil samples were collected from various places in Korea. The soil samples were serially diluted in distilled water and from the suspension, a 100 µL aliquot was spread onto YM agar, incubated at 25°C for 3–4 days. The strain IDs, sequence similarities, taxonomy and incubation conditions are summarized in Table 1. The pure culture of the strains was stored in freeze-dried ampoules.

Cell morphology and budding of strains were observed by phase contrast microscope (Leika) using cells grown for 3–4 days on YM agar. Phase contrast microscope images of the strains are shown in Fig. 1. Biochemical characteristics were established by using API



**Fig. 1.** Phase-contrast micrographs of the unrecorded strains isolated in this study. Strains: A, YP204; B, YP205; C, YP211; D, YP215; E, YP189; F, YP196; G, YP329; H, YP76.

20C AUX strips according to manufacturer's instruction (bioMérieux).

Genomic DNA was extracted and the 26S rRNA gene was amplified by PCR with NL1 and NL4 universal primers and sequenced using the same primers (Kurtzman and Robnett, 1998). The 26S rRNA gene sequences of the closely related strains were obtained from NCBI GenBank (<https://www.ncbi.nlm.nih.gov/>) and edited using the Seqman program. Using the kimura two-parameter model (Kimura, 1983), evolutionary distances were calculated. Phylogenetic trees were constructed using the neighbor-joining algorithm (Saitou and Nei, 1987) in MEGA7 (Kumar *et al.*, 2016) with 1,000 bootstrap replicates (Felsenstein, 1985).

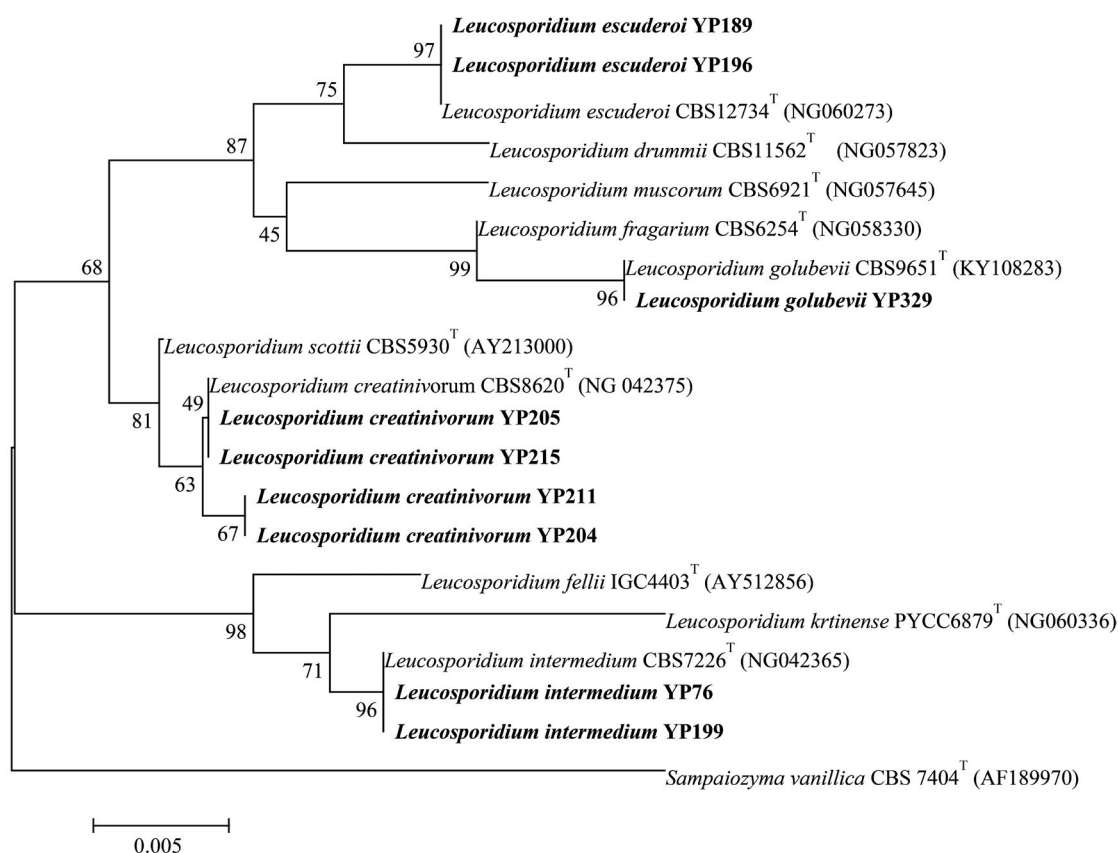
## RESULTS AND DISCUSSION

Based on the D1/D2 domain of 26S rRNA gene se-

quence similarity, 20 yeast strains unreported and reported to Korea were identified. The taxonomic composition and identification results are summarized in Table 1. The 18 strains were assigned to the families *Cystofilobasidiaceae* (2 strains), *Filobasidiaceae* (3 strains), *Bulleraceae* (3 strains), *Leucosporidiaceae* (9 strains), *Chrysozymaceae* (1 strain) of the phylum *Basidiomycota*. The remaining two strains were assigned to the families *Sacchotheciaceae* (1 strain) and *Debaryomycetaceae* (1 strain) of the phylum *Ascomycota*. At the generic level, the strains belong to six different genera: *Cystofilobasidium* (2 strains), *Filobasidium* (1 strain), *Naganishia* (2 strains), *Bullera* (3 strains), *Leucosporidium* (9 strains) and *Sampaiozyma* (1 strain). The identification of the isolates based on sequence similarity was supported by the phylogenetic tree. The neighbor-joining tree showed the closest relationship of the isolates and the type strains of validly published species. The detailed morphological and physiological characteristics were given in the strain descriptions.

Table 1. Yeasts isolated from soil samples collected in Korea.

Phylum	Class	Order	Family	Strain ID	Most closely related species	D1/D2 similarity (%)	Record in Korea	
Ascomycota	Dothideomycetes	Dothideales	Saccharomycetaceae	YP668	<i>Aureobasidium namibiae</i>	597/603 (99)	Reported	
			Debaryomycetaceae	YP551	<i>Meyerozyma caribbica</i>	589/590 (99)	Reported	
	Saccharomycetes	Cystofilobasidiales	Cystofilobasidiaceae	YP556	<i>Cystofilobasidium infirmominiatum</i>	612/612 (100)	Reported	
			Cystofilobasidiaceae	YP636	<i>Cystofilobasidium infirmominiatum</i>	609/609 (100)	Reported	
	Tremellomycetes	Filobasidiales	Filobasidiaceae	YP584	<i>Filobasidium magnum</i>	614/614 (100)	Reported	
				YP589	<i>Naganishia globosa</i>	614/614 (100)	Reported	
		Tremellales	Bulleraceae	YP537	<i>Bullera alba</i>	613/613 (100)	Reported	
				YP580	<i>Bullera alba</i>	611/611 (100)	Reported	
	Basidiomycota	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae	YP204	<i>Leucosporidium creatinivorum</i>	600/601 (99)	Unreported
					YP205	<i>Leucosporidium creatinivorum</i>	599/599 (100)	Unreported
YP211					<i>Leucosporidium creatinivorum</i>	601/602 (99)	Unreported	
Tremellales		Bulleraceae	Bulleraceae	YP215	<i>Leucosporidium creatinivorum</i>	603/603 (100)	Unreported	
				YP189	<i>Leucosporidium escuderoi</i>	592/592 (100)	Unreported	
				YP196	<i>Leucosporidium escuderoi</i>	591/591 (100)	Unreported	
				YP565	<i>Leucosporidium fragarium</i>	606/606 (100)	Reported	
				YP329	<i>Leucosporidium golubevii</i>	607/608 (99)	Unreported	
				YP76	<i>Leucosporidium intermedium</i>	607/607 (100)	Unreported	
				YP348	<i>Sampaozyma ingensosa</i>	593/593 (100)	Reported	



**Fig. 2.** Neighbor-joining phylogenetic tree based on 26S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Leucosporidium*. Bar: 0.005 substitutions per site.

As an outcome of this study, the diversity of yeast species unreported previously in Korean ecosystems were discovered. The 20 isolates were identified as unreported and reported species, and phenotypic characteristics of unreported species were examined.

#### Description of *Leucosporidium creatinivorum* YP204

Colonies are white colored after 3 days of incubation on YM at 25°C. In API 20C AUX test, strain YP204 is positive for glucose, 2-keto-D-gluconate, adonitol, xylitol, D-galactose, inositol, D-sorbitol, d-methyl-D-glucoside, D-cellobiose, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-melezitose and D-raffinose; weak positive for D-glycerol, L-arabinose, *N*-acetyl-D-glucosamine and D-trehalose; but negative for D-xylose.

Strain YP204 was isolated from a soil sample, Pocheon-si, Gyeonggi-do, Republic of Korea.

#### Description *Leucosporidium creatinivorum* YP205

Colonies are yellow colored after 2 days of incubation on YM at 25°C. In API 20C AUX test, positive for glucose, D-sorbitol and D-saccharose (sucrose); weak positive

for glycerol, 2-keto-D-gluconate, L-arabinose, adonitol, d-methyl-D-glucoside, D-cellobiose, D-maltose, D-melezitose and D-raffinose; but negative for D-xylose, xylitol, D-galactose, inositol, *N*-acetyl-D-glucosamine, D-lactose (bovine origin) and D-trehalose.

Strain YP205 was isolated from a soil sample, Pocheon-si, Gyeonggi-do, Republic of Korea.

#### Description of *Leucosporidium creatinivorum* YP211

Colonies are yellow colored after 2 days of incubation on YM agar at 25°C. In API 20C AUX test, positive for glucose, 2-keto-D-gluconate, D-sorbitol, D-saccharose (sucrose), D-melezitose and D-raffinose; weak positive for adonitol, D-cellobiose and D-maltose; but negative for glycerol, L-arabinose, D-xylose, xylitol, D-galactose, inositol, d-methyl-D-glucoside, *N*-acetyl-D-glucosamine, D-lactose (bovine origin) and D-trehalose.

Strain YP211 was isolated from a soil sample, Pocheon-si, Gyeonggi-do, Republic of Korea.

#### Description of *Leucosporidium creatinivorum* YP215

Colonies are white colored after 2 days of incubation

**Table 2.** Characteristics of the unrecorded yeasts from soil in Korea.

Strain ID	YP204	YP205	YP211	YP215	YP189	YP196	YP329	YP76
<b>Morphological characteristics</b>								
Shape	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding	Budding	Budding	Budding	Budding	Budding
<b>API 20 C AUX</b>								
Glucose	+	+	+	+	-	+	+	+
Glycerol	w	w	-	w	-	-	w	w
2-Keto-D-Gluconate	+	w	+	w	-	+	+	+
L-Arabinose	w	w	-	-	-	w	w	-
D-Xylose	-	-	-	-	-	-	-	-
Adonitol	+	w	w	w	w	w	w	w
Xylitol	+	-	-	-	-	-	w	+
D-Galactose	+	-	-	-	-	-	-	+
Inositol	+	-	-	-	w	-	-	w
D-Sorbitol	+	+	+	+	w	+	+	w
d-Methyl-D-Glucoside	+	w	-	-	-	w	w	w
N-Acetyl-D-Glucosamine	w	-	-	-	-	-	w	-
D-Cellobiose	+	w	w	-	-	w	w	+
D-Lactose (bovine origin)	+	-	-	w	-	-	-	w
D-Maltose	+	w	w	+	-	+	w	+
D-Saccharose (Sucrose)	+	+	+	+	-	+	+	-
D-Trehalose	w	-	-	w	-	-	w	-
D-Melezitose	+	w	+	+	-	+	+	+
D-Raffinose	+	w	+	+	-	w	+	-

Taxa: 1, YP204; 2, YP205; 3, YP211; 4, YP215; 5, YP189; 6, YP196; 7, YP329; 8, YP76.

All data were obtained in this study. +, positive; w, weakly positive; -, negative.

on YM agar at 25°C. In API 20C AUX test, positive for glucose, D-sorbitol, D-maltose, D-saccharose (sucrose), D-melezitose and D-raffinose; weak positive for glycerol, 2-keto-D-gluconate, adonitol, D-lactose (bovine origin) and D-trehalose, but negative for L-arabinose, D-xylose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, d-methyl-D-glucoside, N-acetyl-D-glucosamine and D-cellobiose.

Strain YP215 was isolated from a soil sample, Pocheon-si, Gyeonggi-do, Republic of Korea.

#### Description *Leucosporidium escuderoi* YP189

Colonies are yellow colored after 2 days of incubation on YM agar at 25°C. In API 20C AUX test, weakly positive for adonitol, inositol and D-sorbitol; but negative for glucose, glycerol, 2-keto-D-gluconate, L-arabinose, D-xylose, xylitol, D-galactose, d-methyl-D-glucoside, N-acetyl-D-glucosamine, D-cellobiose, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose and D-raffinose.

Strain YP189 was isolated from a soil sample, Pocheon-si, Gyeonggi-do, Republic of Korea.

#### Description *Leucosporidium escuderoi* YP196

Colonies are yellow colored after 2 days of incubation on YM agar at 25°C. In API 20C AUX test, positive for glucose, 2-keto-D-gluconate, D-sorbitol, D-maltose, D-saccharose (sucrose) and D-melezitose; weak positive for L-arabinose, adonitol, d-methyl-D-glucoside, D-cellobiose, D-raffinose; but negative for glycerol, D-xylose, xylitol, D-galactose, inositol, N-acetyl-D-glucosamine, D-lactose (bovine origin) and D-trehalose.

Strain YP196 was isolated from a soil sample, Pocheon-si, Gyeonggi-do, Republic of Korea.

#### Description *Leucosporidium golubevii* YP329

Colonies are yellow colored after 2 days of incubation on YM agar at 25°C. In API 20C AUX test, positive for glucose, 2-keto-D-gluconate, D-sorbitol, D-saccharose (sucrose), D-melezitose and D-raffinose; weak positive for glycerol, L-arabinose, adonitol, xylitol, d-methyl-D-glucoside, N-acetyl-D-glucosamine, D-cellobiose, D-maltose and D-trehalose; but negative for D-xylose, D-galactose, inositol and D-lactose (bovine origin).

Strain YP329 was isolated from a soil sample, Pocheon-si, Gyeonggi-do, Republic of Korea.

### Description *Leucosporidium intermedium* YP76

Colonies are yellow colored after 2 days of incubation on YM agar at 25°C. In API 20C AUX test, positive for glucose, 2-keto-D-gluconate, xylitol, D-galactose, D-cellobiose, D-maltose and D-melezitose); weak positive for glycerol, adonitol, inositol, D-sorbitol, d-methyl-D-glucoside and D-lactose (bovine origin); but negative for L-arabinose, D-xylose, N-acetyl-D-glucosamine, D-saccharose (sucrose), D-trehalose and D-raffinose.

Strain YP76 was isolated from a soil sample, Pocheon-si, Gyeonggi-do, Republic of Korea.

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