

Isolation of four unrecorded yeasts in the family Filobasidiaceae from soil in Korea

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In 2020, 11 Basidiomycetous yeast strains were isolated from soil samples collected from the forests of Namhansanseong in Korea. Among them, seven species were reported, but four species were unreported in Korea. To identify wild yeasts, pairwise sequence comparisons of D1/D2 domain of the 26S rRNA were performed using Basic Local Alignment Search Tool (BLAST). The cell morphologies and assimilation test are observed by phase contrast microscope and API 20C AUX kit, respectively. The 11 strains were assigned to the genera *Rhodotorula* (4 strains) of the order Sporidiobolales of the class Microbotryomycetes; and *Cryptococcus* (2 strains), *Goffeauzyma* (1 strains), *Naganishia* (2 strains) of the order Filobasidiales and *Saitozyma* (2 strains) of the order Tremellales of the class Tremellomycetes in the phylum Basidiomycota. The unreported yeast strains *Cryptococcus gastricus* 20n5-2, *Goffeauzyma gilvescens* 20n2-7, *Naganishia adeliensis* 20n8-1, and *Naganishia friedmannii* 20n24-1 belong to the family Filobasidiaceae. All strains had oval shaped cells and cream-colored colonies cultured on on YM agar for 3 days. In this study, we focus on the description of four unreported yeast species in Korea.

Keywords: 26S rRNA, Basidiomycota, Filobasidiaceae, unreported yeasts

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DOI:10.12651/JSR.2021.10.4.350

INTRODUCTION

In the winter of 2020, 11 strains were isolated from soil samples collected from the forests of Namhansanseong in Korea. Among them, seven species were reported, but four species were unreported in Korea. In this study, focuses on the description of the four unreported yeast species in Korea. The 11 strains were assigned to the genera *Rhodotorula* (4 strains) of the order Sporidiobolales of the class Microbotryomycetes; and *Cryptococcus* (2 strains), *Goffeauzyma* (1 strains), *Naganishia* (2 strains) of the order Filobasidiales and *Saitozyma* (2 strains) of the order Tremellales of the class Tremellomycetes in the phylum Basidiomycota. Among them, four yeast strains 20n5-2, 20n2-7, 20n8-1, and 20n24-1 are unreported strains, belong to the family Filobasidiaceae.

The family Filobasidiaceae was first proposed by Olive (Olive, 1968) and comprises 345 genera (<https://www.mycobank.org/>). The type species, *Filobasidium floriforme* had a pseudohyphae, thin walled basidiospores and blastopores as morphological features (Liu *et al.*, 2015). The members of the family Filobasidiaceae were often

isolated from aquatic habitats (Cannon and Kirk, 2007; Kerfahi *et al.*, 2019).

The genus *Cryptococcus* is a member of the family Filobasidiaceae, of the Basidiomycetous yeast (De Garcia *et al.*, 2009). The genus *Cryptococcus* was first described by Kützing (Kützing, 1833), and was later amended by Vuillemin (Vuillemin, 1901) and Martínez-Pérez *et al.* (Martínez-Pérez *et al.*, 2020). The genus *Cryptococcus* consists of 296 with validly published names (<https://www.mycobank.org/>) with the type species as *C. neoformans*. The genus *Cryptococcus* is resistant to Copper and Zinc (Vadkertiová and Sláviková, 2006).

The genus *Goffeauzyma* contains six recognized species and has *G. gastrica* as the type species. The members of the genus *Goffeauzyma* do not form hyphae and spore budding cells (Liu *et al.*, 2015).

The genus *Naganishia* consists of 18 recognized species and has *N. globosa* as the type species. The members of the genus *Naganishia* are asexually reproducing yeast and mostly form hyphae or pseudohyphae (Vishniac, 1985; Vishniac and Kurtzman, 1992; Turchett *et al.*, 2008; Liu *et al.*, 2015; Fotedar *et al.*, 2018).

MATERIALS AND METHODS

The soil was collected from Namhansanseong in Gwangju, Gyeonggi Province, Korea in 2020 and as a result, a total of 11 strains were isolated. After 9 mL of distilled water was subdivided, about 1 g of soil sample was added to dilute to a total volume of 10 mL. The suspension was spread on YM agar (Difco, USA) and incubated at 10°C for 3–4 days. The pure culture of the separated strains was refined by subculture, using 25% (w/v) glycerol suspension containing YM agar and maintained at –80°C deep freezer. Information on the designated strain identifications (ID), recent associations, and 26S rRNA similarities for isolated strains are described in Table 1.

The budding and cell morphology were observed through a relative harmonic microscope (Leica) using pure colony of yeast incubated on YM agar for 3–4 days. Phase contrast microscope images and the picture incubated in the plate of the strains are shown in Figure 1. The API 20C AUX yeast identification kit (BioMerius, Marcy-l'Étoile, France) was used to assimilate carbon sources by unrecorded yeast.

All genomic DNA was extracted using the cDNA Synthesis Kit (NanoHelix, Korea). The 26S rRNA gene was amplified and sequenced by PCR using NL1 and NL4 universal primers (Kurtzman and Robnett, 1998). The types of yeast closely associated with the isolated strains were collected through the MycoBank Database (<https://www.mycobank.org/>), and the 26S rRNA gene sequences of the strains were obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>). The SeqMan program was used to edit the gene sequences. Phylogenetic trees were produced using the neighbor-joining algorithm (Saitou and Nei, 1987) in the MEGA7 program (Kumar *et al.*, 2016). The statistical reliability of a phylogenetic tree topology was evaluated based on bootstrap values for 1,000 replicates (Felsenstein, 1985). The evolutionary distance was calculated using the Kimura 2-parameter model (Kimura, 1983). Using GenBank's registration number, we identified the sequence of other strains used in the trees.

RESULTS AND DISCUSSION

A total of 11 species of wild yeast were separated using the soil of Namhansanseong in Gwangju, Gyeonggi Province. Result of identification based on the D1/D2 domain of 26S rRNA gene sequence similarity, four of all isolated species were identified as unrecorded yeast in Korea. The taxonomic composition and identification results are summarized in Table 1. The 11 strains were assigned to the families Sporidiobolaceae (4 strains), Filobasidiaceae (5 strains) and Trimorphomycetaceae (2 strains) of the phylum Basidiomycota. The four unrecorded yeasts belong

Table 1. Summary of isolated strains from soil of Namhansanseong in Korea and their taxonomic affiliations.

| Phylum | Class | Order | Family | Strain ID | Most closely related species | 26S rRNA similarity | Record in Korea |
|---------------|--------------------|-----------------|---------------------|-----------|-------------------------------|---------------------|-----------------|
| Basidiomycota | Microbotryomycetes | Sporidiobolales | Sporidiobolaceae | 20n33-3 | <i>Rhodotorula nothofagi</i> | 610/610 (100%) | Reported |
| | | | | 20n35-3 | <i>Rhodotorula nothofagi</i> | 608/610 (99%) | Reported |
| | | | | 20n19-3 | <i>Rhodotorula nothofagi</i> | 609/609 (100%) | Reported |
| | | | | YF31 | <i>Rhodotorula nothofagi</i> | 604/604 (100%) | Reported |
| | Tremellomycetes | Filobasidiales | Filobasidiaceae | 20n5-2 | <i>Cryptococcus gastricus</i> | 565/565 (100%) | Unreported |
| | | | | YF34 | <i>Cryptococcus saitoi</i> | 617/617 (100%) | Reported |
| | | | | 20n2-7 | <i>Goffeauzyma gilvescens</i> | 574/577 (99%) | Unreported |
| | | Tremellales | Trimorphomycetaceae | 20n8-1 | <i>Naganishia adeliensis</i> | 619/619 (100%) | Unreported |
| | | | | 20n24-1 | <i>Naganishia friedmannii</i> | 629/631 (99%) | Unreported |
| | | | | 20n35-1 | <i>Saitozyma podzolica</i> | 614/618 (99%) | Reported |
| | | | | 20n3-2 | <i>Saitozyma podzolica</i> | 631/631 (100%) | Reported |

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

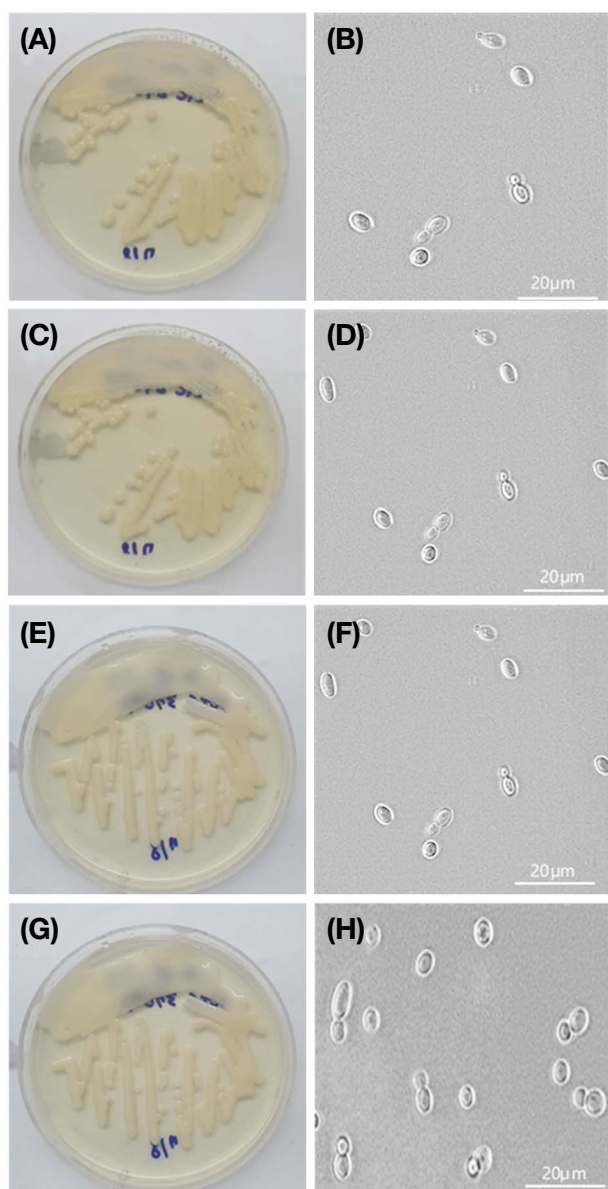


Fig. 1. Morphology of cells from the unrecorded strains incubated at 10°C. The colonies of (A) *C. gastricus* 20n5-2, (C) *G. gilvescens* 20n2-7, (E) *N. adeliensis* 20n8-1, and (G) *N. friedmannii* 20n24-1. The budding cells of (B) *C. gastricus* 20n5-2, (D) *G. gilvescens* 20n2-7, (F) *N. adeliensis* 20n8-1, and (H) *N. friedmannii* 20n24-1.

to Filobasidiaceae, and three genera have been identified: *Cryptococcus* (1 strain), *Goffeauzyma* (1 strain) and *Naganishia* (2 strains). The phylogenetic consensus trees of the four strains support a close relationship by showing that isolated strains are closely linked to strains that exhibit the highest 26S RNA gene sequence similarity (Figs. 2, 3 and 4).

As an outcome of this study, four unrecorded yeasts were found in the domestic ecosystem, and the pheno-

Table 2. Characteristics of the unrecorded strains from soil of Namhansanseong in Korea.

| Strain ID | 20n5-2 | 20n2-7 | 20n8-1 | 20n24-1 |
|--------------------------------------|---------|---------|---------|---------|
| Morphological characteristics | | | | |
| Shape | Oval | Oval | Oval | Oval |
| Vegetative reproduction | Budding | Budding | Budding | Budding |
| API 20C AUX | | | | |
| Glucose | – | + | w | – |
| Glycerol | w | w | – | – |
| 2-Keto-D-Gluconate | w | + | + | + |
| L-Arabinose | – | + | + | w |
| D-Xylose | w | + | + | – |
| Adonitol | + | w | w | w |
| Xylitol | w | w | w | w |
| D-Galactose | w | w | w | – |
| Inositol | – | w | – | – |
| D-Sorbitol | – | + | + | – |
| d-Methyl-D-Glucoside | w | + | w | – |
| N-Acetyl-D-Glucosamine | + | w | w | + |
| D-Cellobiose | w | + | w | + |
| D-Lactose (bovine origin) | – | + | w | – |
| D-Maltose | w | + | + | w |
| D-Saccharose (Sucrose) | w | + | + | + |
| D-Trehalose | w | + | + | – |
| D-Melezitose | w | + | w | w |
| D-Raffinose | w | + | + | + |

Strains: 1, 20n5-2; 2, 20n2-7; 3, 20n8-1; 4, 20n24-1.

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

typic characteristics of unrecorded species were examined.

Description of *Cryptococcus gastricus* 20n5-2

Colonies are cream colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n5-2 is positive for adonitol and N-acetyl-D-glucosamine; weakly positive for glycerol, 2-keto-D-gluconate, D-xylose, xylitol, D-galactose, d-methyl-D-glucoside, D-cellobiose, D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose, and D-raffinose. But, negative for glucose, L-arabinose, inositol, D-sorbitol, and D-lactose (bovine origin). Strain 20n5-2 (KACC 37084) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description of *Naganishia friedmannii* 20n2-7

Colonies are cream colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n2-7 is positive for glucose, 2-keto-D-gluconate,

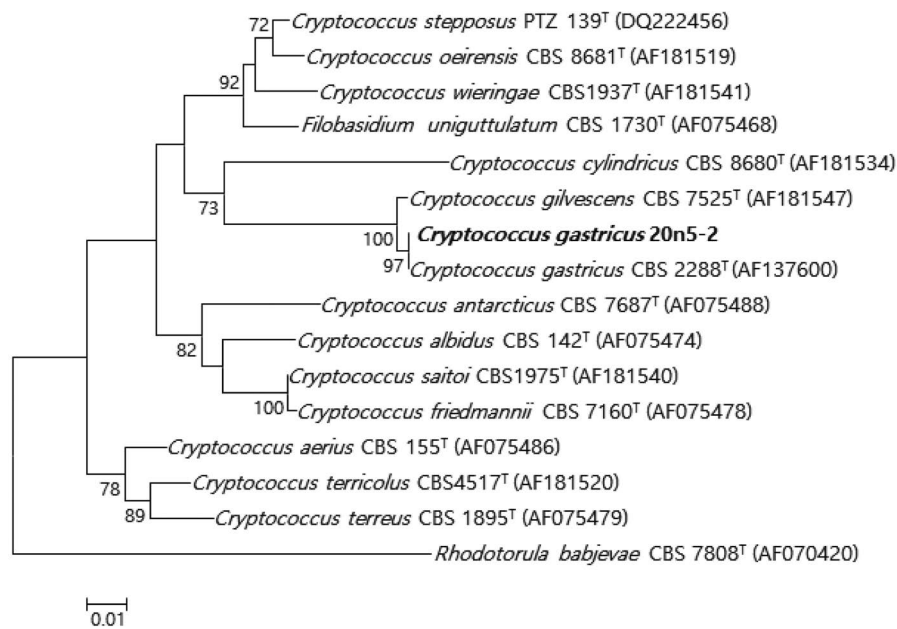


Fig. 2. Neighbor-joining phylogenetic tree based on 26S rRNA gene sequences shows the phylogenetic relationships between the isolated strains and their closest strains of the genus *Cryptococcus*. *Rhodotorula babjevae* CBS 7808^T strain was used as the outgroup. Bootstrap values (>70%) are shown at each branch. Bar, 0.01 substitutions per nucleotide position.

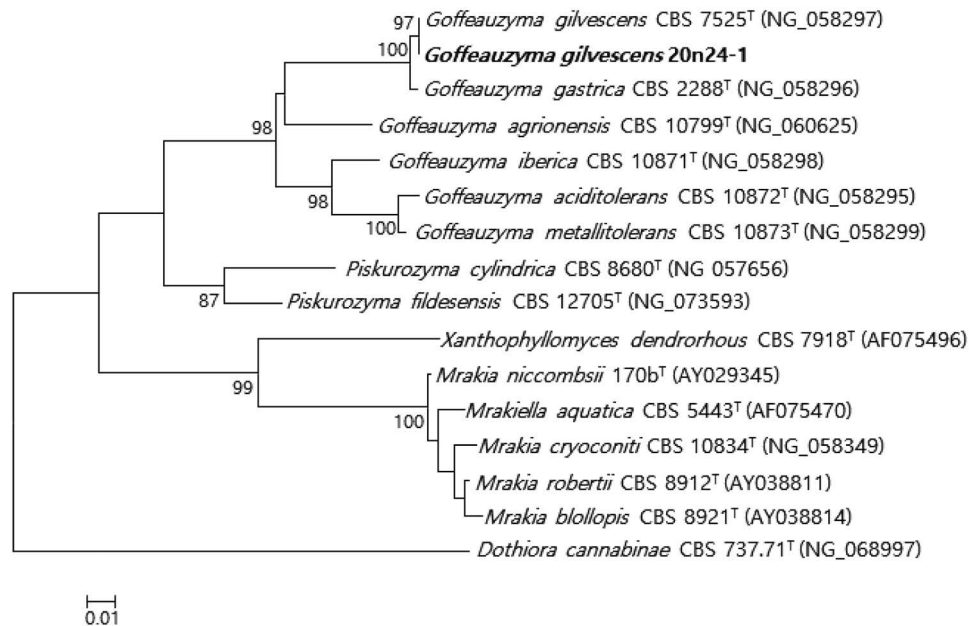


Fig. 3. Neighbor-joining phylogenetic tree based on 26S rRNA gene sequences shows the phylogenetic relationships between the isolated strains and their closest strains of the genus *Goffeauzyma*. *Dothiora cannabinae* CBS 737.71^T strain was used as the outgroup. Bootstrap values (>70%) are shown at each branch. Bar, 0.01 substitutions per nucleotide position.

L-arabinose, D-sorbitol, D-methyl-D-glucoside, D-cellobiose, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose, and D-raffinose; weak positive for glycerol, adonitol, xylitol, D-galactose,

inositol, and N-acetyl-D-glucosamine. Strain 20n2-7 (KACC 37094) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

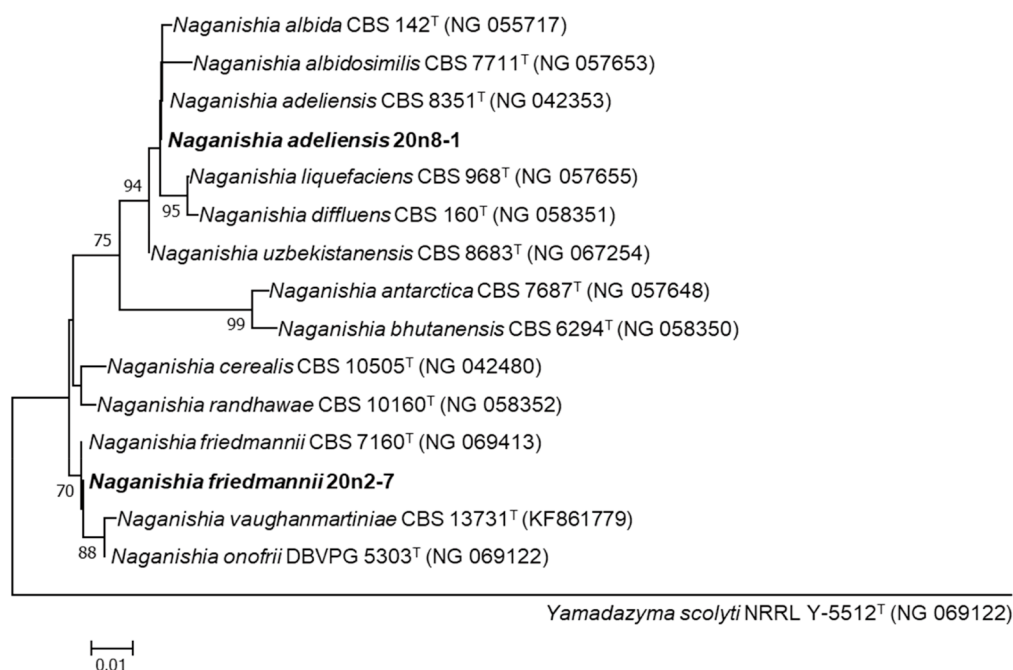


Fig. 4. Neighbor-joining phylogenetic tree based on 26S rRNA gene sequences shows the phylogenetic relationships between the isolated strains and their closest strains of the genus *Naganishia*. *Yamadazyma scolyti* NRRL Y-5512^T strain was used as the outgroup. Bootstrap values (> 70%) are shown at each branch. Bar, 0.01 substitutions per nucleotide position.

Description of *Naganishia adeliensis* 20n8-1

Colonies are cream colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n8-1 is positive for 2-keto-D-gluconate, L-arabinose, D-xylose, D-sorbitol, D-maltose, D-saccharose (sucrose), D-trehalose, and D-raffinose; weak positive for glucose, adonitol, xylitol, D-galactose, d-methyl-D-glucoside, N-acetyl-D-glucosamine, D-cellobiose, D-lactose (bovine origin) and D-melezitose; but negative for glycerol and inositol. Strain 20n8-1 (KACC 37093) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description of *Goffeazyma gilvescens* 20n24-1

Colonies are cream colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n24-1 is positive for 2-keto-D-gluconate, N-acetyl-D-glucosamine, D-cellobiose, D-saccharose (sucrose), and D-raffinose; weak positive for L-arabinose, adonitol, xylitol, D-maltose, and D-melezitose. But, negative for glucose, glycerol, D-xylose, D-galactose, inositol, D-sorbitol, d-methyl-D-glucoside, D-lactose (bovine origin) and D-trehalose. Strain 20n24-1 (KACC 37090) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

ACKNOWLEDGEMENTS

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017 R1D1A1B03035583) and supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202028201).

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Submitted: August 3, 2021

Revised: September 27, 2021

Accepted: September 27, 2021