

Isolation and characterization of two unrecorded yeast species in the order *Filobasidiales*

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The purpose of this study was to isolate and identify wild yeasts from soil samples collected in Daegu and Cheongju city, Republic of Korea. To identify the wild yeast strains, pairwise sequence comparisons of D1/D2 region of the 26S rRNA gene sequence were done using Basic Local Alignment Search Tool (BLAST). The cell morphologies were observed by phase contrast microscope and assimilation test are done using API 20C AUX kit. All strains were assigned to the phylum *Basidiomycota*. Among 13 strains, 11 strains were previously reported, but two strains were unreported from the Republic of Korea. The two unrecorded yeast strains, GW1-3 and PG1-1-10C, belong to the genus *Solicoccozyma* (family *Piskurozymaceae*, order *Filobasidiales*, class *Tremellomycetes*). The two strains had oval-shaped and polar budding cells. This research showed the morphological and biochemical properties of the two unreported yeast species that had not officially reported in Korea.

Keywords: 26s rRNA, *Solicoccozyma*, unrecorded yeasts

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DOI:10.12651/JSR.2024.13.1.100

INTRODUCTION

In this study, wild yeasts were isolated from soil samples in Daegu and Cheongju city, Republic of Korea. As a result of isolation and identification, many yeast strains were previously reported species, while a couple unrecorded species were found. The unreported species were identified to belong to the genus *Solicoccozyma* species.

The genus *Solicoccozyma* is a basidiomycetous yeast in the class *Tremellomycetes*, phylum *Basidiomycota*. *Solicoccozyma* species have been isolated from soil and crater lakes (Li *et al.*, 2019). The genus *Solicoccozyma* comprises 11 distinct species and has *Solicoccozyma aeria* as the type species (<https://www.mycobank.org>). The genus *Solicoccozyma* belongs to the family *Piskurozymaceae*, and the typical characteristics associated with the family *Piskurozymaceae* include pseudohyphae and true hyphae present or not, basidiocarps absent, budding cells present, clamp connections on dikaryotic hyphae occasionally present, no haustorial branches, and holobasidia can be formed for sexual reproduction. Additionally, fermentation is usually absent and nitrate usually utilized. The major CoQ system is CoQ-10 (Liu *et al.*, 2015).

As a result of this study, two unrecorded yeast strains

were found in domestic ecosystems and their phenotypic characteristics were investigated.

MATERIALS AND METHODS

The soil samples were collected from Daegu and Cheongju city, Republic of Korea, and serially diluted in distilled water and the suspension was spread on a Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA) and incubated at 25°C and 10°C for 3 days. The strains are preserved in a metabolically inactive state at the Korean Agricultural Culture Collection, Korea.

The cell morphologies of strains were observed on a phase contrast microscope (Leica DM500), using yeast strains incubated on YPD agar for 3 days. Phase contrast microscope images and the colonies of the strains GW1-3 and PG1-1-10C are shown in Fig. 1. To characterize the biochemical features, API 20C AUX (bioMérieux) were tested according to the manufacturer's instructions.

The genomic DNA was extracted after incubating on YPD agar for 3 days. The D1/D2 region of the 26S rRNA gene sequence was amplified by PCR using primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3')

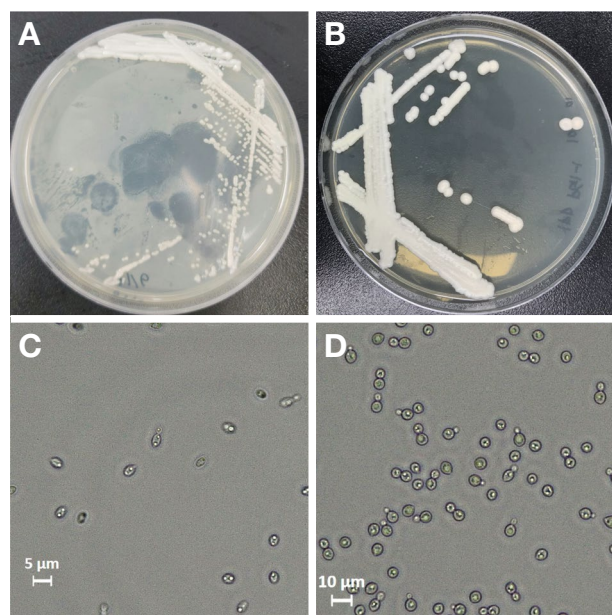


Fig. 1. Morphology of cells from the unrecorded strains GW1-3 incubated at 25°C and strain PG1-1-10C incubated at 10°C. All strains were grown after 3 days on YPD agar. The colonies of *Solicoccozyma terricola* GW1-3 (A) and *Solicoccozyma aeria* PG1-1-10C (B). The budding cells of *Solicoccozyma terricola* GW1-3 (C) and *Solicoccozyma aeria* PG1-1-10C (D). Bars, 5 μm and 10 μm, respectively.

and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') (Kurtzman and Robnett, 1998).

Pairwise sequence comparisons were made using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1997) and to align 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 the NCBI (<https://www.ncbi.nlm.nih.gov/>) for 26s rDNA in a row.

The phylogenetic trees based on the D1/D2 domain of LSU rRNA gene sequence were reconstructed using the neighboring joining algorithm of the MEGA 11 program (Tamura *et al.*, 2021) The evolutionary distance was calculated using the two-parameter model of Kimura (Kimura, 1983) for neighborhood bond analysis and the confidence level of the class was estimated through bootstrap analysis (1,000 replicates) (Felsenstein, 1985).

RESULTS AND DISCUSSION

Thirteen wild yeasts strains were isolated from soil samples from Daegu and Cheongju city in Korea. The yeast strains were identified by analyzing D1/D2 domain of 26s rRNA gene sequence similarities that were calcu-

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 | <i>Filobasidiales</i> | <i>Piskurozymaceae</i> | PG1-1-10C | <i>Solicoccozyma aeria</i> | 526/527 (99.81%) | Unreported |
| | | | | YB1UV-4 | <i>Solicoccozyma terrea</i> | 591/591 (100%) | Reported |
| | | | | YB1UV-6 | <i>Solicoccozyma terrea</i> | 603/603 (100%) | Reported |
| | | | | PG2-9 | <i>Solicoccozyma terrea</i> | 526/527 (99.81%) | Reported |
| | | | | YB1UV-3 | <i>Solicoccozyma terrea</i> | 599/599 (100%) | Reported |
| | | | | PG4-5 | <i>Solicoccozyma terrea</i> | 525/527 (99.62%) | Reported |
| | | GW1-3 | <i>Solicoccozyma terricola</i> | 527/527 (100%) | Unreported | | |
| | | YB2UV-1 | <i>Saitozyma podzolica</i> | 524/526 (99.81%) | Reported | | |
| | | YB3UV-1 | <i>Saitozyma podzolica</i> | 525/527 (99.81%) | Reported | | |
| | | YB3UV-4 | <i>Saitozyma podzolica</i> | 522/524 (99.81%) | Reported | | |
| | | PG4-1 | <i>Saitozyma podzolica</i> | 521/524 (99.62%) | Reported | | |
| | | PG4-2 | <i>Saitozyma podzolica</i> | 522/524 (99.62%) | Reported | | |
| | | PG4-4 | <i>Cryptococcaceae</i> | <i>Kwoniella bestioleae</i> | 525/526 (99.81%) | Reported | |

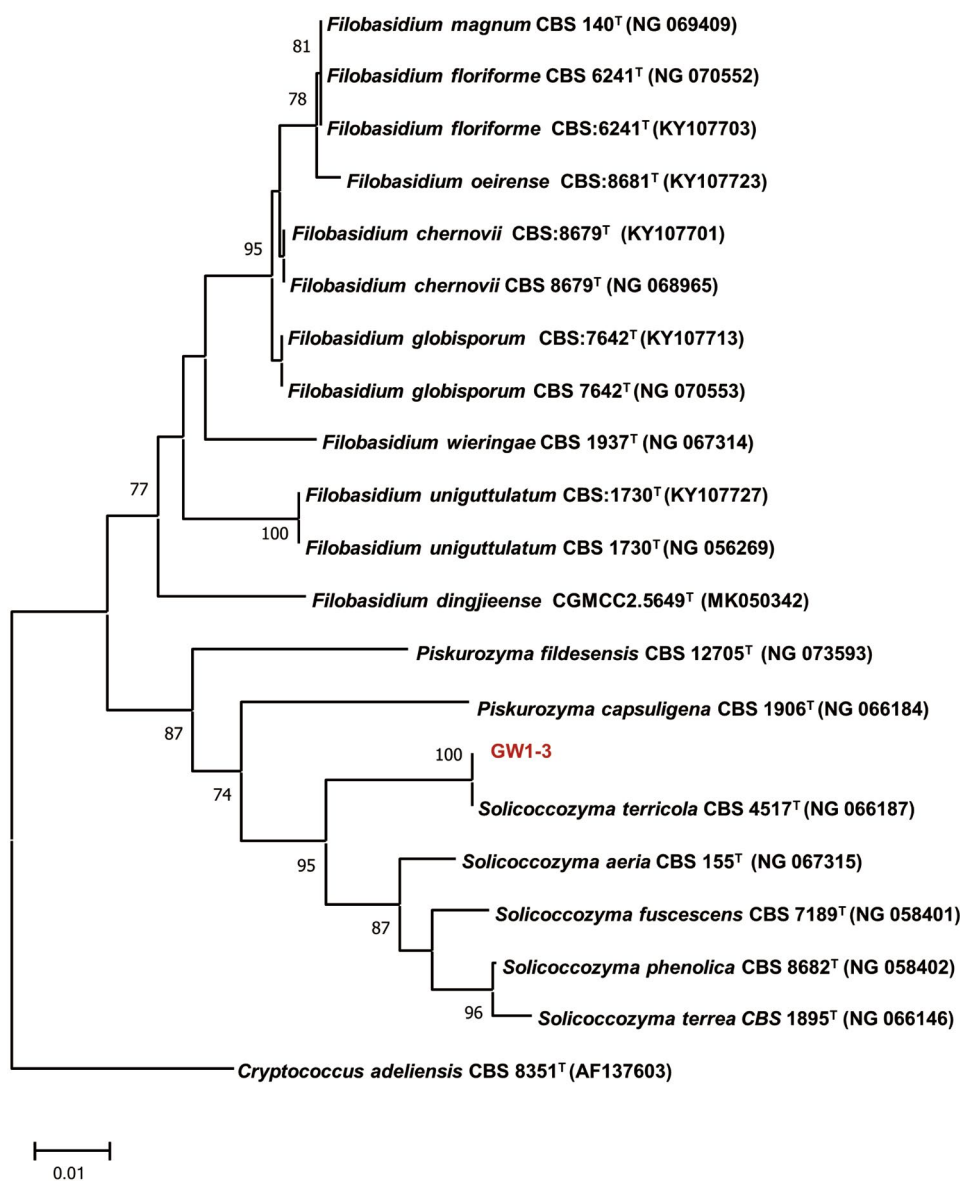


Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains GW1-3 with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

lated 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.

All strains were assigned to the families *Piskurozymaceae* (7 strains), *Trimorphomycetaceae* (5 strains) *Cryptococcaceae* (1 strain) of the phylum *Basidiomycota*. The unrecorded yeast strains GW1-3 and PG1-1-10C belong to the phylum *Basidiomycota* and the genus *Solicoccozyma*. The phylogenetic tree shows that the

isolated strains are closely related to the *Solicoccozyma* species with the highest 26S rRNA gene sequence similarity (Figs. 2 and 3) and thus supports close relationships.

Description of *Solicoccozyma terricola* GW1-3

Cells are oval shaped and budding is polar (Fig. 1). Colonies are convex, smooth, and white cream-colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain GW1-3 is positive for *N*-acetyl-D-glucosamine, D-raffinose, D-melezitose, D-sacchar-

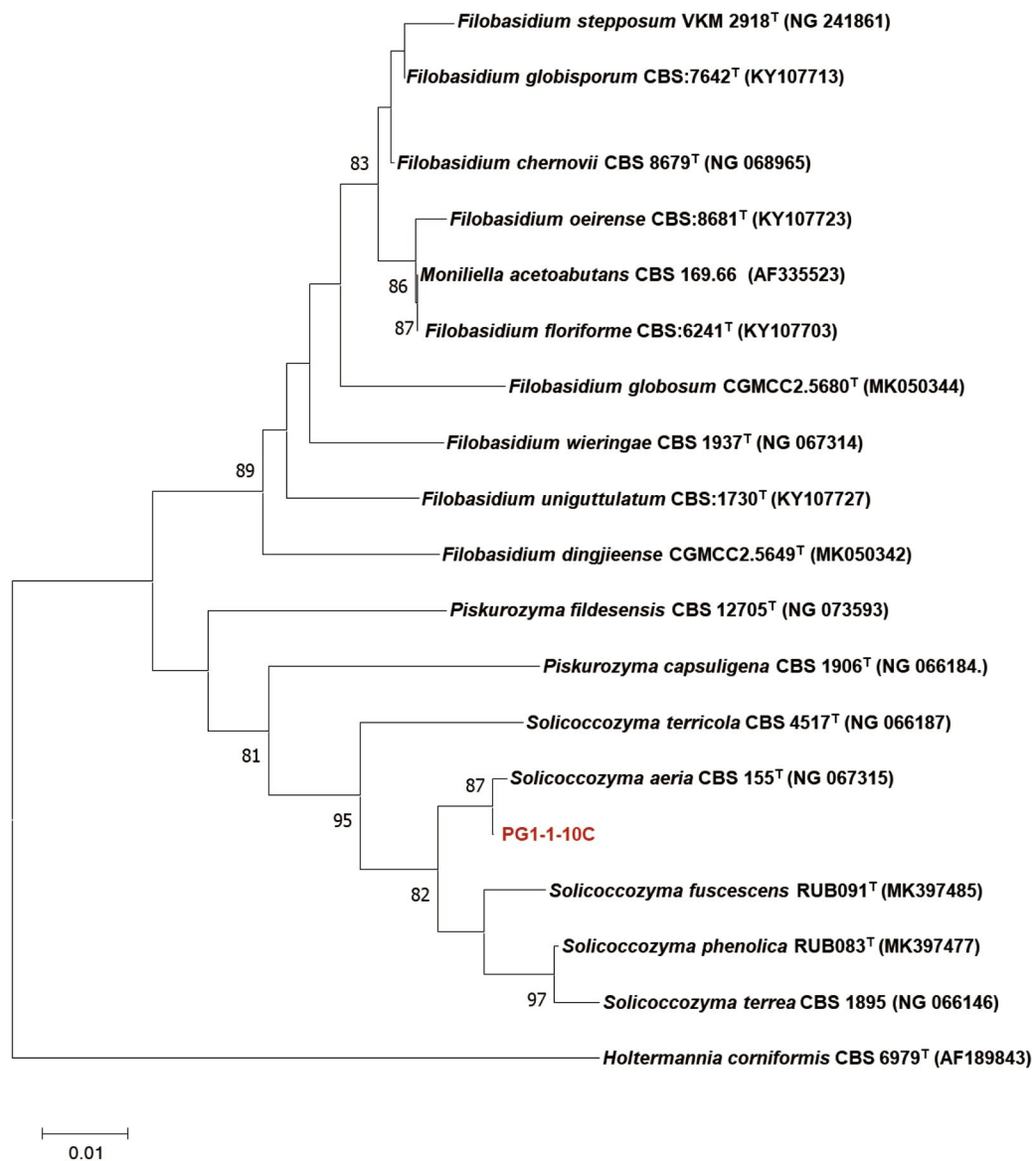


Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain PG1-1-10C with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

ose (sucrose), galactose, glucose, methyl-D-glucopyranoside, D-xylose, and D-maltose; weak positive for calcium-2-keto-D-gluconate, xylitol, and inositol; but negative for glycerol, L-arabinose, D-lactose (bovine origin), D-trehalose, adonitol, D-sorbitol, and D-cellobiose.

Strain GW1-3 (KACC 410362) was isolated from soil collected in Cheongju City, Chungcheongbuk Province, Republic of Korea.

Description of *Solicoccozyma aeria* PG1-1-10C

Cells are circular shaped and budding is polar (Fig. 1). Colonies are convex, smooth, and cream-colored after

3 days of incubation on YPD agar at 10°C. In the API 20C AUX test, strain PG1-1-10C is positive for calcium 2-keto-D-gluconate, L-arabinose, glucose, D-xylose, D-sorbitol, *N*-acetyl-D-glucosamine, D-cellobiose, D-maltose, D-melezitose, D-galactose, D-saccharose (sucrose), and D-raffinose; weak positive for D-lactose (bovine origin), inositol, and methyl- α -D-glucopyranoside; but negative for xylitol, D-trehalose, glycerol, and adonitol.

Strain PG1-1-10C (KACC 410364) was isolated from soil collected in Daegu, Republic of Korea.

Table 2. Phenotypic characteristics that differentiate *S. terricola* and related species.

| | 1 | 2 | 3 | 4 |
|------------------------------|-------|-------|-------|-------|
| Growth on | | | | |
| Temp (°C) | 15–30 | 15–30 | 15–30 | 25–28 |
| Assimilation of: | | | | |
| Sucrose | + | + | + | + |
| Raffinose | w | + | + | + |
| Galactose | w | + | w | – |
| Trehalose | w | – | + | + |
| Maltose | w | + | + | – |
| Melezitose | w | + | + | – |
| Methyl α -D-glucoside | w | w | + | + |
| Cellobiose | w | + | + | + |
| D-Xylose | w | + | + | + |
| L-Arabinose | w | + | + | + |
| Glycerol | + | – | + | – |
| N-Acetyl-D-Glucosamine | w | + | + | ND |
| 2-Keto-D-Gluconate | w | + | + | ND |
| Adonitol | w | – | ND | ND |
| Xylitol | w | – | ND | ND |
| Inositol | w | w | + | + |
| D-Sorbitol | + | + | ND | ND |
| D-Lactose (bovine origin) | w | w | + | + |

Taxa: 1, *S. terricola* GW1-3; 2, *S. aerea* PG1-1-10C; 3, *S. zizaniae* B657^T; 4, *S. aquatica* CBS 15415^T.
+, positive; w, weakly positive; –, negative.

CONFLICTS OF INTEREST

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No.2021R1F1A1061389) and was also supported in part by National Research Foundation of Korea (NRF) funded by the MSIT (2022M3H9A1082984).

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Submitted: November 14, 2023

Accepted: December 14, 2023