

Isolation of ten unrecorded yeasts from soil in Korea

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In 2020, as a subset study to discover indigenous yeast species in Korea, a total of 22 yeast species were isolated from soil samples collected in Gwangju-si, Gyeonggi-do. Among them, 10 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 independent and predefined yeast species. The 22 strains were assigned to the genera *Dothiora* (1 strain), *Sarocladium* (1 strain), *Tetrapisispora* (1 strain) and *Torulasporea* (2 strains) of the phylum *Ascomycota*; the genera *Erythrobasidium* (1 strain), *Leucosporidium* (4 strains), *Ustilentyloma* (2 strains), *Fellozyma* (1 strain), *Sampaiozyma* (2 strains), *Filobasidium* (1 strain), *Solicoccozyma* (2 strains) and *Vishniacozyma* (4 strains) of the phylum *Basidiomycota*. This is the first official report of the following species in Korea: *Dothiora cannabinae* (1 strain), *Sarocladium strictum* (1 strain), *Fellozyma inositophila* (1 strain), *Filobasidium magnum* (1 strain), *Solicoccozyma phenolicus* (1 strain), *Solicoccozyma terreus* (1 strain), *Vishniacozyma tephrensensis* (1 strain) and *Vishniacozyma victoriae* (3 strains). Cell morphology, phenotypic features and biochemical features are described in the Species Description section.

Keywords: 26S rRNA, *Ascomycota*, *Basidiomycota*, unreported yeasts

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INTRODUCTION

In 2020, 22 strains were isolated from diverse soil samples collected in Korea. Among them 10 species were unreported in Korea. This study focusses on the isolation and description of unrecorded species in the phylum *Ascomycota* and *Basidiomycota*.

The phylum *Ascomycota* consists of three subphyla: *Peizomycotina* (including 13 classes, 124 orders and 507 families), *Saccharomycotina* (including 1 class, 1 order and 13 families) and *Taphrinomycotina* (5 classes, 5 orders and 6 families). Approximately, the phylum *Ascomycota* contains 6,600 genera (Wijayawardene *et al.*, 2018). The phylum *Ascomycota* occurs in all terrestrial and aquatic ecosystems, and is isolated under extreme conditions, such as deep-sea trees (Kohlmeyer, 1977) and deposits (Raghukumar *et al.*, 2004) from rocks on the frozen plains of Antarctica (Selbmann *et al.*, 2005). They also act as reciprocalists, parasites and pathogens for animals, plants and other fungi, and act on decay of organic substrates (e.g., trees, leaves, dung) (Schoch *et al.*, 2009). The family *Sarocladiaceae* includes the genera *Parasarocladium* and

Sarocladium and has vegetative hyphae septate, hyaline, smooth- and thin-walled (Crous *et al.*, 2018). The features of the family *Dothioraceae* include either medium to large, pulvinate ascostromata with one wide or several locules (Barr, 2001).

The phylum *Basidiomycota* is the second largest phylum in the kingdom Fungi. The phylum *Basidiomycota* consists of 16 classes, 52 orders, 177 families, 1,589 genera and more than 30,000 species and it is estimated that approximately 32% of the described fungal taxa belong to this phylum. *Basidiomycota* can be a factor in wood and garbage decomposition, producing biochemical compounds used as traditional medicines to treat diseases such as cancer and diabetes (Zhao *et al.*, 2017). and *Basidiomycota* is commonly the presence of basidia (singular: basidium), which is characteristic to the total intracellular cells of hyphae, those with sexual spores (Hongliang and Hao, 2019). The family *Chrysozymaceae* is based on the description of the diagnosis of the genus *Chrysozyma* (Wang *et al.*, 2015). *Filobasidiaceae* produce pseudohyphae or true hyphae, and present budding cells, but has no ballistococidia and fermentation. The feature of *Piskurozymace-*

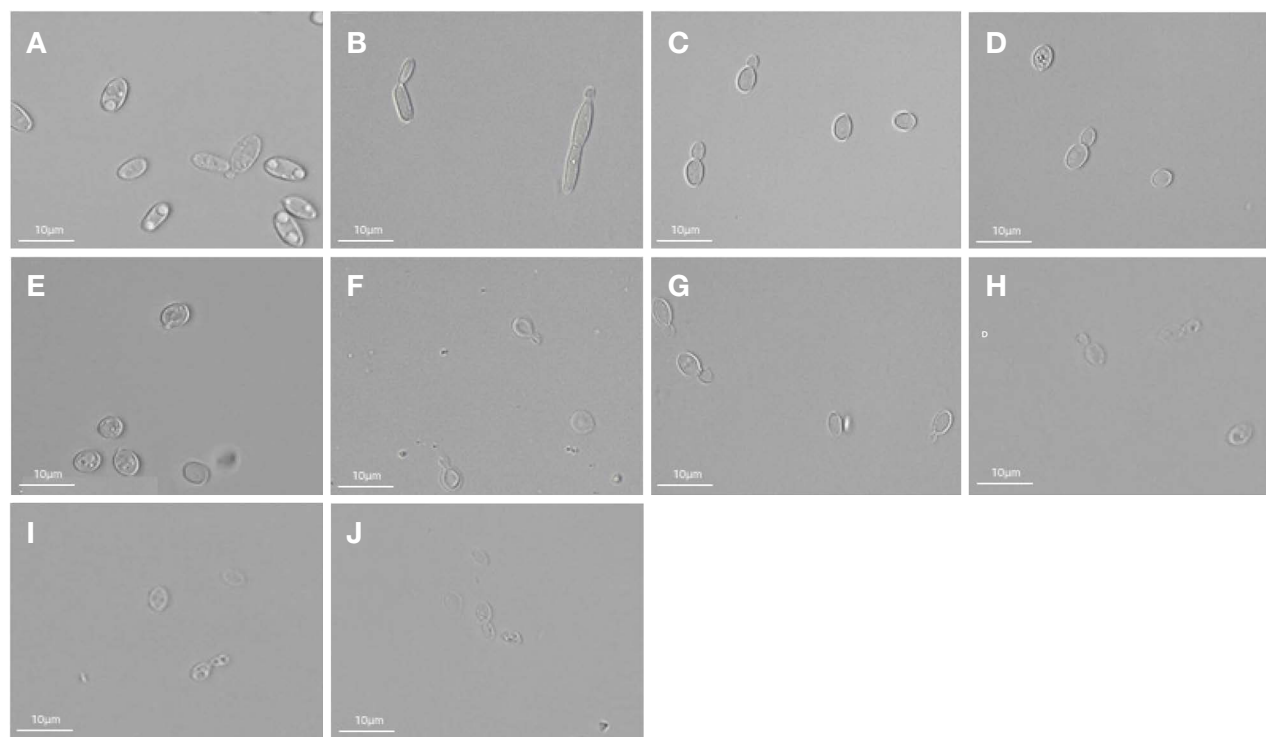


Fig. 1. Phase-contrast micrographs of cells from the unrecorded strains isolated in this study. Strains: A, 20n15-2; B, 20n6-6; C, 20n8-2; D, 20n32-3; E, 20n33-5; F, 20n26-1; G, 20n23-1; H, 20n29-2; I, 20n29-4; J, 20n29-5.

ae is that it occasionally presents clamp connections on dikaryotic hyphae and budding cells (Liu *et al.*, 2015).

The current report focuses on the description of 10 unreported yeast species that have not officially reported in Korea.

MATERIALS AND METHODS

A total of 22 strains were isolated from soil in Namhansanseong, Gwangju city, Gyeonggi-do, Korea. The soil samples (1 g) were serially diluted with distilled water (9 mL) and the suspensions (10 mL) were spread on YM agar, and they were incubated at 10°C for 3–4 days. The designated strain identifications (IDs), 26S rRNA similarities, taxonomy, isolation sources and incubation conditions are described in Table 1. All strains were purified into the pure cultures by sub-culturing, and the isolates were maintained at –80°C in nutrient broth (NB) containing 20% (v/v) glycerol suspension.

After incubating yeast in YM agar for 3–4 days, the budding and cell morphology of strains were observed using relative harmonic microscopy (Leika). Phase contrast microscope images of the strains are shown in Fig. 1. Biochemical characteristics were established by using API 20C AUX strips according to manufacturer's instruction (bioMérieux).

Genomic DNA was extracted and the 26S rRNA gene was amplified by PCR and sequenced with NL1 and NL4 universal primers (Kurtzman and Robnett, 1998). The type strains of closely related yeast were identified through the MycoBank Database (<https://www.mycobank.org/>). The 26S rRNA gene sequences of the closely related strains were obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>) and edited using the SeqMan program. The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed using the neighbor-joining algorithm (Saitou and Nei, 1987) in the MEGA7 program (Kumar *et al.*, 2016) with bootstrap values based on 1000 replicates (Felsenstein, 1985). The sequence of other strains used in the tree was searched by GenBank's accession number.

RESULTS AND DISCUSSION

Based on D1/D2 domain of 26S rRNA gene sequence similarity, 22 yeast strains were identified. The taxonomic composition and identification results are summarized in Table 1. The 10 unreported yeast strains were distributed into two phyla: *Ascomycota* and *Basidiomycota*. Two strains were assigned to the family *Dothioraceae* (1 strain) and *Sarocladiaceae* (1 strain) of the phylum *Ascomycota*, while eight strains were assigned to the family

Table 1. Summary of isolated strains from soil in Korea and their taxonomic affiliations. All strains were cultured at 10°C for 3 days.

| Phylum | Class | Order | Family | Strain ID | Most closely related species | 26S rRNA similarity | Isolation source | Record in Korea |
|-----------------|---------------------|--------------------|---------------------|---------------------------------|-------------------------------------|---------------------|------------------|-----------------|
| Ascomycota | Dothideomycetes | Dothideales | Dothioraceae | 20n15-2 | <i>Dothiora cannabinae</i> | 590/592 (99%) | Soil | Unreported |
| | | | | 20n6-6 | <i>Sarocladium strictum</i> | 592/593 (99%) | Soil | Unreported |
| | Sordariomycetes | Saccharomycetales | Saccharomycetaceae | 20n23-9 | <i>Tetrapisispora iriomotensis</i> | 577/577 (100%) | Soil | Reported |
| | | | | 20n36-2 | <i>Torulasporea delbrueckii</i> | 595/596 (99%) | Soil | Reported |
| | | | | 20n36-4 | <i>Torulasporea delbrueckii</i> | 583/585 (99%) | Soil | Reported |
| | | | | 20n6-5 | <i>Erythrobasidium hasegawianum</i> | 591/592 (99%) | Soil | Reported |
| Basidiomycota | Cystobasidiomycetes | Erythrobasidiales | Erythrobasidiaceae | 20n21-1 | <i>Leucosporidium golubevii</i> | 607/608 (99%) | Soil | Reported |
| | | | | 20n21-4 | <i>Leucosporidium golubevii</i> | 607/608 (99%) | Soil | Reported |
| | | | | 20n22-2 | <i>Leucosporidium golubevii</i> | 608/609 (99%) | Soil | Reported |
| | | | | 20n21-3 | <i>Leucosporidium golubevii</i> | 608/609 (99%) | Soil | Reported |
| | | | | 20n6-1 | <i>Ustilentyloma graminis</i> | 604/604 (100%) | Soil | Reported |
| | | | | 20n6-2 | <i>Ustilentyloma graminis</i> | 628/628 (100%) | Soil | Reported |
| | Microbotryomycetes | Microbotryales | Ustilentylomataceae | 20n8-2 | <i>Fellozyma inosiphila</i> | 599/601 (99%) | Soil | Unreported |
| | | | | 20n2-4 | <i>Sampaiozyma ingentosa</i> | 607/607 (100%) | Soil | Reported |
| | | | | 20n2-9 | <i>Sampaiozyma ingentosa</i> | 610/611 (99%) | Soil | Reported |
| | | | | 20n32-3 | <i>Filobasidium magnum</i> | 616/616 (100%) | Soil | Unreported |
| | | | | 20n33-5 | <i>Solicoccozyma phenolicus</i> | 622/622 (100%) | Soil | Unreported |
| | | | | 20n26-1 | <i>Solicoccozyma terreus</i> | 638/639 (99%) | Soil | Unreported |
| Tremellomycetes | Tremellales | Bulleribasidiaceae | 20n23-1 | <i>Vishniacozyma tephrensii</i> | 600/602 (99%) | Soil | Unreported | |
| | | | 20n29-2 | <i>Vishniacozyma victoriae</i> | 616/616 (100%) | Soil | Unreported | |
| | | | 20n29-4 | <i>Vishniacozyma victoriae</i> | 613/613 (100%) | Soil | Unreported | |
| | | | 20n29-5 | <i>Vishniacozyma victoriae</i> | 616/616 (100%) | Soil | Unreported | |
| | | | 20n29-5 | <i>Vishniacozyma victoriae</i> | 616/616 (100%) | Soil | Unreported | |

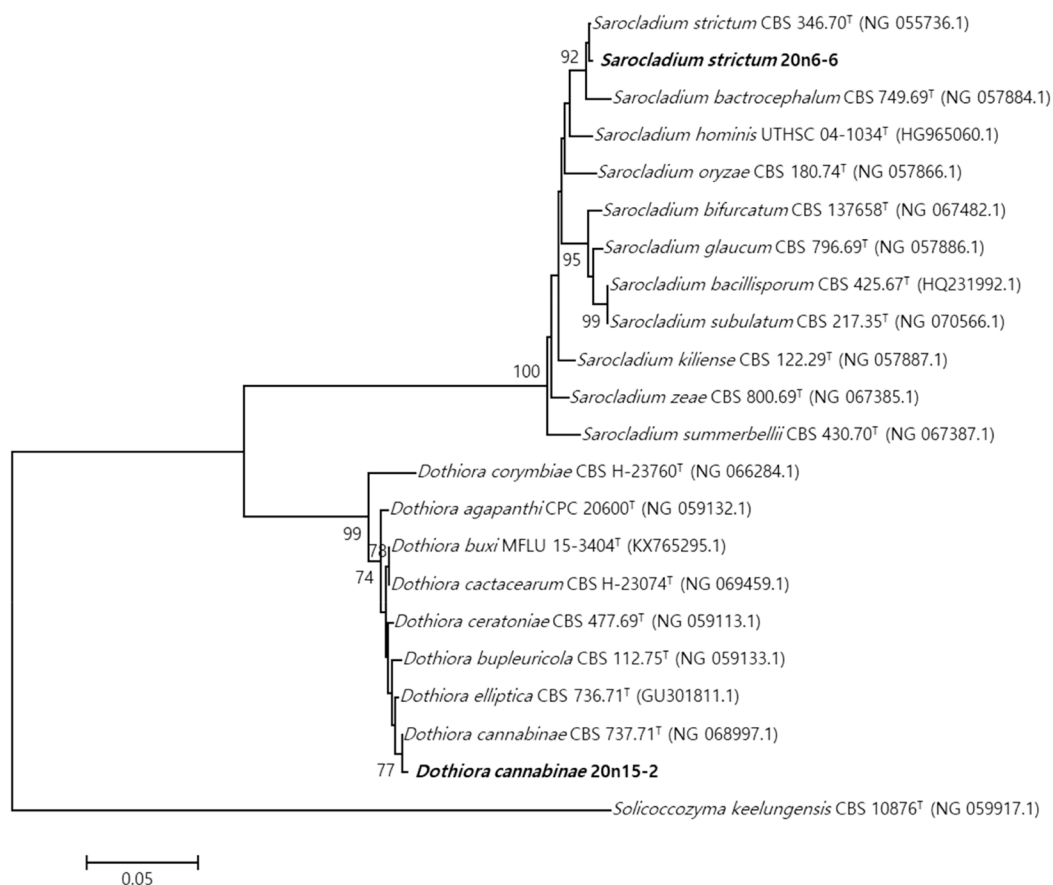


Fig. 2. Neighbor-joining phylogenetic tree based on 26S rRNA gene sequences shows the phylogenetic relationships between the isolated strains and their closest relatives. The sequence of *Solicoccozyma keelungensis* CBS 10876^T was used as the outgroup. Bootstrap values (>70%) are shown at each branch. Bar, 0.05 substitutions per nucleotide position.

Chrysozymaceae (1 strains), *Filobasidiaceae* (1 strain), *Piskurozymaceae* (2 strains) and *Bulleribasidiaceae* (4 strains) of the phylum *Basidiomycota*. Ten unrecorded yeasts belong to six different genera: *Dothiora* (1 strain), *Sarocladium* (1 strain), *Fellozyma* (1 strain), *Filobasidium* (1 strain), *Solicoccozyma* (2 strains) and *Vishniacozyma* (4 strains). The identification of the isolates based on sequence similarity was supported by the phylogenetic tree (Figs. 2 and 3). The neighbor-joining tree showed the closest relationship of the isolated strains and the type strains of validly published species. The detailed morphological and physiological characteristics were presented in the strain descriptions.

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

Description of *Dothiora cannabinae* 20n15-2

Colonies are cream colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain

20n15-2 is positive for glucose, D-xylose, D-sorbitol, D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose and D-raffinose; weak positive for glycerol, adonitol, xylitol, inositol and D-cellobiose; but negative for 2-keto-D-gluconate, D-galactose, d-methyl-D-glucoside, D-lactose (bovine origin), L-arabinose and N-acetyl-D-glucosamine.

Strain 20n15-2 (KACC 37087, OK560110) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description of *Sarocladium strictum* 20n6-6

Colonies are white colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n6-6 is positive for glucose, glycerol, L-arabinose, D-xylose, xylitol, D-galactose, D-sorbitol, N-acetyl-D-glucosamine, D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose and D-raffinose; weak positive for 2-keto-D-gluconate, inositol and D-cellobiose; but negative for adonitol, d-methyl-D-glucoside and D-lactose (bovine origin).

Strain 20n6-6 (KACC 37096, OK560100) was isolated

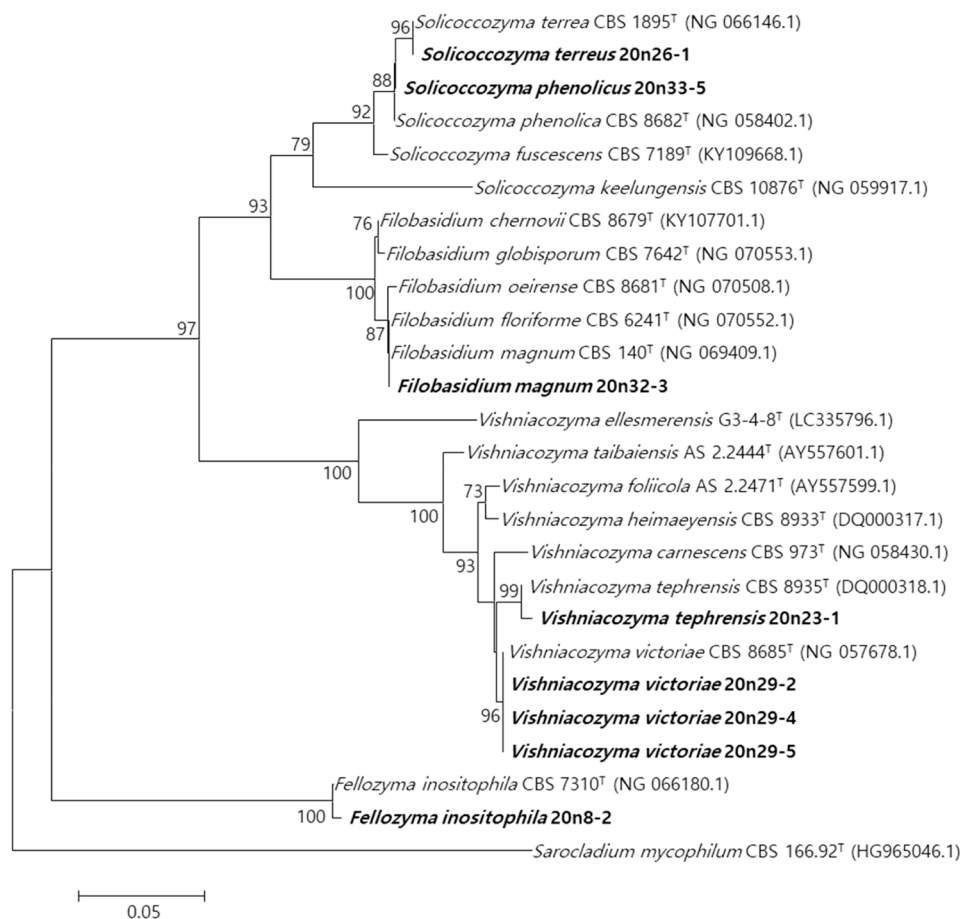


Fig. 3. Neighbor-joining phylogenetic tree based on 26S rRNA gene sequences shows the phylogenetic relationships between the isolated strains and their closest relatives. The sequence of *Sarocladium mycophilum* CBS 166.92^T was used as the outgroup. Bootstrap values (>70%) are shown at each branch. Bar, 0.05 substitutions per nucleotide position.

ted from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description *Fellozyma inositophila* 20n8-2

Colonies are white colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n8-2 is positive for glucose, 2-keto-D-gluconate, L-arabinose, D-xylose, inositol, 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; weak positive for adonitol, xylitol, D-galactose and D-sorbitol; but negative for glycerol.

Strain 20n8-2 (KACC 37096, OK560106) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description of *Filobasidium magnum* 20n32-3

Colonies are cream colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain

20n32-3 is positive for glucose, 2-keto-D-gluconate, L-arabinose, D-xylose, N-acetyl-D-glucosamine, D-cellobiose, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-trehalose, and D-melezitose; weak positive for xylitol, D-galactose, inositol, D-sorbitol, d-methyl-D-glucoside and D-raffinose; but negative for glycerol and adonitol.

Strain 20n32-3 (KACC 37089, OK560112) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description *Solicoccozyma phenolicus* 20n33-5

Colonies are pale-yellow colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n33-5 is positive for glucose, 2-keto-D-gluconate, L-arabinose, D-xylose, D-sorbitol, N-acetyl-D-glucosamine and D-cellobiose; weak positive for D-galactose, inositol and D-trehalose; but negative for glycerol, adonitol, xylitol, d-methyl-D-glucoside, D-lactose (bovine origin),

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

| Strain ID | 20n15-2 | 20n8-2 | 20n32-3 | 20n6-6 | 20n33-5 | 20n26-1 | 20n23-1 | 20n29-2 | 20n29-4 | 20n29-5 |
|--------------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Morphological characteristics | | | | | | | | | | |
| Shape | Oval | Oval | Oval | Oval | Oval | Oval | Oval | Oval | Oval | Oval |
| Vegetative reproduction | Budding | Budding | Budding | Budding | Budding | Budding | Budding | Budding | Budding | Budding |
| API 20C AUX | | | | | | | | | | |
| Glucose | + | + | + | + | + | + | + | w | - | - |
| Glycerol | w | - | - | + | - | - | - | - | - | - |
| 2-Keto-D-gluconate | - | + | + | w | + | + | - | + | w | + |
| L-Arabinose | - | + | + | + | + | + | + | - | - | - |
| D-Xylose | + | + | + | + | + | + | + | w | w | w |
| Adonitol | w | w | - | - | - | - | + | - | - | - |
| Xylitol | w | w | w | + | - | - | w | - | - | - |
| D-Galactose | - | w | w | + | w | - | w | - | - | - |
| Inositol | w | + | w | w | w | w | + | w | w | + |
| D-Sorbitol | + | w | w | + | + | + | + | w | w | w |
| d-Methyl-D-glucoside | - | + | w | - | - | - | w | - | - | - |
| N-Acetyl-D-glucosamine | - | + | + | + | + | + | + | - | w | + |
| D-Cellobiose | w | + | + | w | + | + | + | - | w | w |
| D-Lactose (bovine origin) | - | + | + | - | - | - | + | - | - | - |
| D-Maltose | + | + | + | + | - | - | + | w | + | + |
| D-Saccharose (Sucrose) | + | + | + | + | - | - | + | w | + | + |
| D-Trehalose | + | + | + | + | w | w | + | w | - | - |
| D-Melezitose | + | + | + | + | - | - | + | w | w | w |
| D-Raffinose | + | + | w | + | - | - | + | w | + | + |

Strains: 1, 20n15-2; 2, 20n8-2; 3, 20n32-3; 4, 20n6-6; 5, 20n33-5; 6, 20n26-1; 7, 20n23-1; 8, 20n29-2; 9, 20n29-4; 10, 20n29-5. All data were obtained in this study. +, positive; w, weakly positive; -, negative.

D-maltose, D-saccharose (sucrose), D-melezitose and D-raffinose.

Strain 20n33-5 (KACC 37097, OK560109) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description *Solicoccozyma terreus* 20n26-1

Colonies are pale-yellow colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n26-1 is positive for glucose, 2-keto-D-gluconate, L-arabinose, D-xylose, D-sorbitol, N-acetyl-D-glucosamine and D-cellobiose; weak positive for inositol and D-trehalose; but negative for glycerol, adonitol, xylitol, D-galactose, d-methyl-D-glucoside, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-melezitose and D-raffinose.

Strain 20n26-1 (KACC 37098, OK560050) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description *Vishniacozyma tephrensii* 20n23-1

Colonies are cream colored after 3 days of incubation

on YM agar at 10°C. In the API 20C AUX test, strain 20n23-1 is positive for glucose, L-arabinose, D-xylose, adonitol, inositol, D-sorbitol, N-acetyl-D-glucosamine, D-cellobiose, D-lactose (bovine origin), D-saccharose (sucrose), D-trehalose, D-melezitose and D-raffinose; weak positive for xylitol, D-galactose and d-methyl-D-glucoside; but negative for glycerol and 2-keto-D-gluconate.

Strain 20n23-1 (KACC 37099, OK560050) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description *Vishniacozyma victoriae* 20n29-2

Colonies are cream colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n29-2 is positive for 2-keto-D-gluconate; weak positive for glucose, D-xylose, inositol, D-sorbitol, D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose and D-raffinose; but negative for glycerol, L-arabinose, adonitol, xylitol, D-galactose, d-methyl-D-glucoside, N-acetyl-D-glucosamine, D-cellobiose and D-lactose (bovine origin).

Strain 20n29-2 (KACC 37102, OK559987) was isolated

ted from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description *Vishniacozyma victoriae* 20n29-4

Colonies are pale yellow colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n29-4 is positive for D-maltose, D-saccharose (sucrose) and D-raffinose; weak positive for 2-keto-D-gluconate, D-xylose, inositol, D-sorbitol, N-acetyl-D-glucosamine, D-cellobiose and D-melezitose; but negative for glucose, glycerol, L-arabinose, adonitol, xylitol, D-galactose, d-methyl-D-glucoside, D-lactose (bovine origin) and D-trehalose.

Strain 20n29-4 (KACC 37103, OK559987) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

Description *Vishniacozyma victoriae* 20n29-5

Colonies are pale yellow colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX test, strain 20n29-5 is positive for 2-keto-D-gluconate, inositol, N-acetyl-D-glucosamine, D-maltose, D-saccharose (sucrose) and D-raffinose; weak positive for D-xylose, D-sorbitol, D-cellobiose and D-melezitose; but negative for glucose, glycerol, L-arabinose, adonitol, xylitol, D-galactose, d-methyl-D-glucoside, D-lactose (bovine origin) and D-trehalose.

Strain 20n29-5 (KACC 37104, OK559742) was isolated from a soil sample, Namhansanseong, Gwangju city, Gyeonggi-do, Republic of Korea.

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