**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 *Torulaspora* (2 strains) of the phylum *Ascomycota*; the genera *Erythrobasidium* (1 strain), *Leucosporidium* (4 strains), *Usitentyloma* (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), *Dothioraėmonium* (1 strain), *Fellozyma inositophila* (1 strain), *Filobasidium magnum* (1 strain), *Solicoccozyma phenolicus* (1 strain), *Solicoccozyma terreus* (1 strain), *Vishniacozyma tephrensis* (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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DOI:10.12651/JSR.2021.10.4.336

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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 focuses on the isolation and description of unrecorded species in the phylum *Ascomycota* and *Basidiomycota*.

The phylum *Ascomycota* consists of three subphyla: *Pezizomycotina* (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 ballistococnidia and fermentation. The feature of *Piskurozymace-
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 Namhanseong, 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.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Strain ID</th>
<th>Most closely related species</th>
<th>26S rRNA similarity</th>
<th>Isolation source</th>
<th>Record in Korea</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascomycota</strong></td>
<td><strong>Dothideomycetes</strong></td>
<td><strong>Dothideales</strong></td>
<td><strong>Dothioraceae</strong></td>
<td>20n15-2</td>
<td><em>Dothiora cannabinae</em></td>
<td>590/592 (99%)</td>
<td>Soil</td>
<td>Unreported</td>
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<tr>
<td></td>
<td><strong>Hypocreales</strong></td>
<td></td>
<td><strong>Sarocladiaceae</strong></td>
<td>20n6-6</td>
<td><em>Sarocladium strictum</em></td>
<td>592/593 (99%)</td>
<td>Soil</td>
<td>Unreported</td>
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<tr>
<td><strong>Sordariomycetes</strong></td>
<td></td>
<td></td>
<td><strong>Saccharomycetales</strong></td>
<td>20n23-9</td>
<td><em>Tetrapispora iriomotensis</em></td>
<td>577/577 (100%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>20n36-2</td>
<td><em>Torulaspora delbrueckii</em></td>
<td>595/596 (99%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>20n36-4</td>
<td><em>Torulaspora delbrueckii</em></td>
<td>583/585 (99%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
<tr>
<td><strong>Cystobasidiomycetes</strong></td>
<td></td>
<td></td>
<td><strong>Erythrobasidiales</strong></td>
<td>20n6-5</td>
<td><em>Erythrobasidium hasegavianum</em></td>
<td>591/592 (99%)</td>
<td>Soil</td>
<td>Reported</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20n21-1</td>
<td><em>Leucosporidium golubesi</em></td>
<td>607/608 (99%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
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<td></td>
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<td></td>
<td>20n21-4</td>
<td><em>Leucosporidium golubesi</em></td>
<td>607/608 (99%)</td>
<td>Soil</td>
<td>Reported</td>
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<td></td>
<td>20n22-2</td>
<td><em>Leucosporidium golubesi</em></td>
<td>608/609 (99%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>20n21-3</td>
<td><em>Leucosporidium golubesi</em></td>
<td>608/609 (99%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
<tr>
<td><strong>Microbotryomycetes</strong></td>
<td></td>
<td></td>
<td><strong>Ustilentylomataceae</strong></td>
<td>20n6-1</td>
<td><em>Ustilentyloma graminis</em></td>
<td>604/604 (100%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
<tr>
<td></td>
<td><strong>Leucosporidiales</strong></td>
<td></td>
<td></td>
<td>20n6-2</td>
<td><em>Ustilentyloma graminis</em></td>
<td>628/628 (100%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
<tr>
<td><strong>Basidiomycota</strong></td>
<td></td>
<td></td>
<td><strong>Chrysozymaceae</strong></td>
<td>20n8-2</td>
<td><em>Fellozyma inositophila</em></td>
<td>599/601 (99%)</td>
<td>Soil</td>
<td>Unreported</td>
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<td></td>
<td></td>
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<td></td>
<td>20n2-4</td>
<td><em>Sampaiozyma ingeniensis</em></td>
<td>607/607 (100%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>20n2-9</td>
<td><em>Sampaiozyma ingeniensis</em></td>
<td>610/611 (99%)</td>
<td>Soil</td>
<td>Reported</td>
</tr>
<tr>
<td><strong>Filobasidiomycetes</strong></td>
<td></td>
<td></td>
<td><strong>Filobasidiales</strong></td>
<td>20n32-3</td>
<td><em>Filobasidium magnum</em></td>
<td>616/616 (100%)</td>
<td>Soil</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>20n33-5</td>
<td><em>Solicoccozyma phenolicus</em></td>
<td>622/622 (100%)</td>
<td>Soil</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td><strong>Piskurozymaceae</strong></td>
<td></td>
<td></td>
<td>20n26-1</td>
<td><em>Solicoccozyma terreus</em></td>
<td>638/639 (99%)</td>
<td>Soil</td>
<td>Unreported</td>
</tr>
<tr>
<td><strong>Tremellomycetes</strong></td>
<td></td>
<td></td>
<td><strong>Tremellales</strong></td>
<td>20n23-1</td>
<td><em>Wishniaczyma tephrensis</em></td>
<td>600/602 (99%)</td>
<td>Soil</td>
<td>Unreported</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>20n29-2</td>
<td><em>Wishniaczyma victoriae</em></td>
<td>616/616 (100%)</td>
<td>Soil</td>
<td>Unreported</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>20n29-4</td>
<td><em>Wishniaczyma victoriae</em></td>
<td>613/613 (100%)</td>
<td>Soil</td>
<td>Unreported</td>
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<td></td>
<td>20n29-5</td>
<td><em>Wishniaczyma victoriae</em></td>
<td>616/616 (100%)</td>
<td>Soil</td>
<td>Unreported</td>
</tr>
</tbody>
</table>
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), d-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, lactose, d-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 isola-
Strain 20n8-2 (KACC 37096, OK560106) was isolated 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, 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),
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-glucanate, l-arabinose, d-xylose, adonitol, inositol, d-sorbitol, N-acetyl-d-glucosamine, d-cellobiose, d-lactose (bovine origin), d-maltose, d-saccharose (sucrose), d-melezitose and d-raffinose; weak positive for xylitol, d-galactose and d-methyl-d-glucoside; but negative for glycerol and 2-keto-d-glucanate.

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

**Description Vishniacozyma tephrensis 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-maltose, 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-glucanate.

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

**Acknowledgements**

This work was 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).

**References**


