

Report of two unrecorded yeast species in the class *Tremellomycetes*

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The purpose of this study is to isolate and identify wild yeasts from the soil samples collected in Daegu and Daejeon City, Republic of Korea. Among 15 strains isolated in this study, 13 strains were previously reported and two strains had not been reported in Republic of Korea. To identify 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 tests were done using API 20C AUX kit. All strains were assigned to the phylum *Basidiomycota*. The two unrecorded yeast strains, PG2-2-10C and DJ2-14-10C, belong to the genus *Holtermanniella* (family *Holtermanniaceae*, order *Holtermanniales*, class *Tremellomycetes*) and *Goffeauzyma* (family *Filobasidiaceae*, order *Filobasidiales*, class *Tremellomycetes*), respectively. The two unrecorded yeast strains had oval shape and polar budding cells. This research describes the morphological and biochemical properties of the two unreported yeast species that had not officially reported in Korea.

Keywords: *Goffeauzyma*, *Holtermanniella*, unrecorded yeasts

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INTRODUCTION

In this study, wild yeasts were isolated from the soil samples collected in Daegu and Daejeon City, Republic of Korea. As a result of isolation and identification, many yeast strains were shown to be previously reported species and a few unrecorded species were found. The unreported species were identified to belong to the genera *Holtermanniella* and *Goffeauzyma*.

The genus *Holtermanniella* is a basidiomycetous yeast in the class *Tremellomycetes*, phylum *Basidiomycota*. *Holtermanniella* species were isolated from plants and fruits (Wuczkowski *et al.*, 2011). The genus *Holtermanniella* comprises five distinct species and has *Holtermanniella takashimae* as the type species (<https://www.mycobank.org>). Members of the genus *Holtermanniella* have been consistently isolated from cold regions, with two out of the five known species (*Holtermanniella wattica* and *Holtermanniella nyarrowii*) originally discovered in Antarctica. Consequently, the majority of *Holtermanniella* species exhibit limited growth above 25°C. Furthermore, three *Holtermanniella* species have demonstrated significant concentrations of polyunsaturated fatty acids (particularly linolenic acid) in comparison to *Holtermannia corniformis* and *Saccharomyces cerevisiae*,

both of which lack this lipid (Reiersøl and de Menna, 1958; Golubev *et al.*, 2004; Guffogg *et al.*, 2004). Such fatty acid compositions align with the characteristic profile of yeasts adapted to cold environments (McMurrugh and Rose, 1973; Libkind *et al.*, 2008; Rossi *et al.*, 2009).

The genus *Goffeauzyma* is a basidiomycetous yeast in the class *Tremellomycetes*, phylum *Basidiomycota*. *Goffeauzyma* species were isolated from soil (Boekhout *et al.*, 2011a; Fonseca *et al.*, 2011a; 2011b). The genus *Goffeauzyma* comprises nine distinct species and has *Goffeauzyma gastrica* as the type species (<https://www.mycobank.org>). The genus *Goffeauzyma* is classified within the family *Filobasidiaceae*. Typical characteristics associated with the family *Filobasidiaceae* include hyphal structures that may exhibit clamp connections and possess haustorial branches. The holobasidia are tubular or elongated, featuring terminally sessile basidiospores. Both pseudohyphae and true hyphae may sporadically emerge. Sexual reproduction manifests in select species, while budding cells are evident. Ballistoconidia are notably absent (Sampaio, 2011a; 2011b). Fermentation processes are not observed, and nitrate is utilized by several species. The predominant coenzyme Q system comprises either CoQ-9 or CoQ-10 (Liu *et al.*, 2015).

As a result of this study, two unrecorded yeast strains

were found in domestic ecosystems of Korea, and we investigated the phenotypic characteristics of the unrecorded species.

MATERIALS AND METHODS

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

The cell morphologies of strains were done by the LEICA (DM500), using yeast strains incubated in 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 kit (bioMérieux, France) was used according to the manufacturer’s instructions.

The genomic DNA was extracted after incubated on YPD agar for 3 days. The D1/D2 region of the 26S rRNA gene sequence was amplified by PCR using NL1 (5'-GCA TATCAATAAGCGGAGGA AAAG-3') and NL4 (5'-GGT CCGTGTTTCAAGACGG-3') primers (Kurtzman and Robnett, 1998; Fell *et al.*, 2000).

Pairwise sequence comparisons were made using the

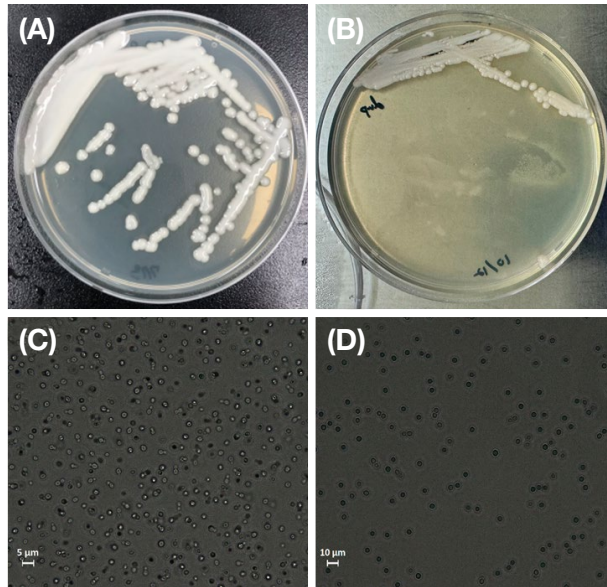


Fig. 1. Morphology of cells from the unrecorded strains incubated at 10°C. The colonies of *Holtermanniella wattica* PG2-2-10C (A), *Goffeauzyma gastrica* DJ2-14-10C (B). The budding of *Holtermanniella wattica* PG2-2-10C (C), *Goffeauzyma gastrica* DJ2-14-10C (D). All strains were grown after 3 days on YPD agar. Bars, 5 µm and 10 µm, respectively.

Table 1. A list of yeasts strains isolated from soil in Republic of Korea. All strains were cultured under 10°C for 3 days.

Phylum	Class	Order	Family	Strain	Most closely related species	26S rRNA similarity	Record in Korea
Basidiomycota	Tremellomycetes	Holtermannietales	Holtermanniaceae	PG2-2-10C	<i>Holtermanniella wattica</i>	524/526 (99%)	Unreported
				DJ2-14-10C	<i>Goffeauzyma gastrica</i>	475/475 (100%)	Unreported
		Filobasidiales	Piskurozymaceae	PG3-1-10C	<i>Solicozozyma aerea</i>	525/527 (99%)	Reported
				PG2-3-10C	<i>Solicozozyma terricola</i>	527/527 (100%)	Reported
				PG2-4-10C	<i>Solicozozyma terra</i>	515/515 (100%)	Reported
				PG2-6-10C	<i>Solicozozyma terra</i>	522/522 (100%)	Reported
				PG3-2-10C	<i>Solicozozyma terra</i>	531/531 (100%)	Reported
				DJ2-1-10C	<i>Solicozozyma terricola</i>	503/503 (100%)	Reported
				DJ2-3-10C	<i>Solicozozyma terricola</i>	512/512 (100%)	Reported
				DJ2-9-10C	<i>Solicozozyma terricola</i>	520/520 (100%)	Reported
				DJ2-10-10C	<i>Solicozozyma terricola</i>	531/531 (100%)	Reported
				DJ2-13-10C	<i>Solicozozyma terricola</i>	507/507 (100%)	Reported
				DJ1-3-10C	<i>Solicozozyma terricola</i>	516/516 (100%)	Reported
				DJ2-16-10C	<i>Solicozozyma phenolica</i>	527/527 (100%)	Reported
				DJ2-18-10C	<i>Solicozozyma phenolica</i>	533/533 (100%)	Reported

Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1997) and to align with the related sequences retrieved from GenBank. The MYCOBANK (<https://www.mycobank.org/>) database identified type strains for each species and gene sequence of close strains were obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>) for 26s rDNA.

The phylogenetic trees based on the D1/D2 domain of LSU rRNA gene sequence were reconstructed by 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) and bootstrap analysis was done (1,000 replicates) (Felsenstein, 1985).

RESULTS AND DISCUSSION

Fifteen wild yeasts strains were collected from soil samples in Daegu and Daejeon City in Korea. The yeast

strains were identified by analyzing D1/D2 domain of 26s rRNA gene sequence similarities that were calculated by the NCBI BLAST. Based on the result of the phylogenetic analyses, two yeast strains were identified as domestic unrecorded yeast species. The taxonomic composition and identification results are listed in Table 1. Strain PG2-2-10C was closely related to *Holtermanniella wattica* with 99.62% D1/D2 sequence similarity. Strain DJ2-14-10C was closely related to *Goffeauzyma gastrica* with 100.00% D1/D2 sequence similarity.

All strains were assigned to the families *Holtermanniaceae* (1 strain), *Filobasidiaceae* (1 strain), and *Piskurozymaceae* (13 strains) of the phylum *Basidiomycota*. The unrecorded yeast strains PG2-2-10C and DJ2-14-10C belong to the phylum *Basidiomycota* and the genera *Goffeauzyma* and *Holtermanniella*, respectively. The phylogenetic analysis shows that the strain PG2-2-10C is closely related to *Holtermanniella wattica* (Fig. 2). The phylogenetic analysis shows that the strain DJ2-14-10C is

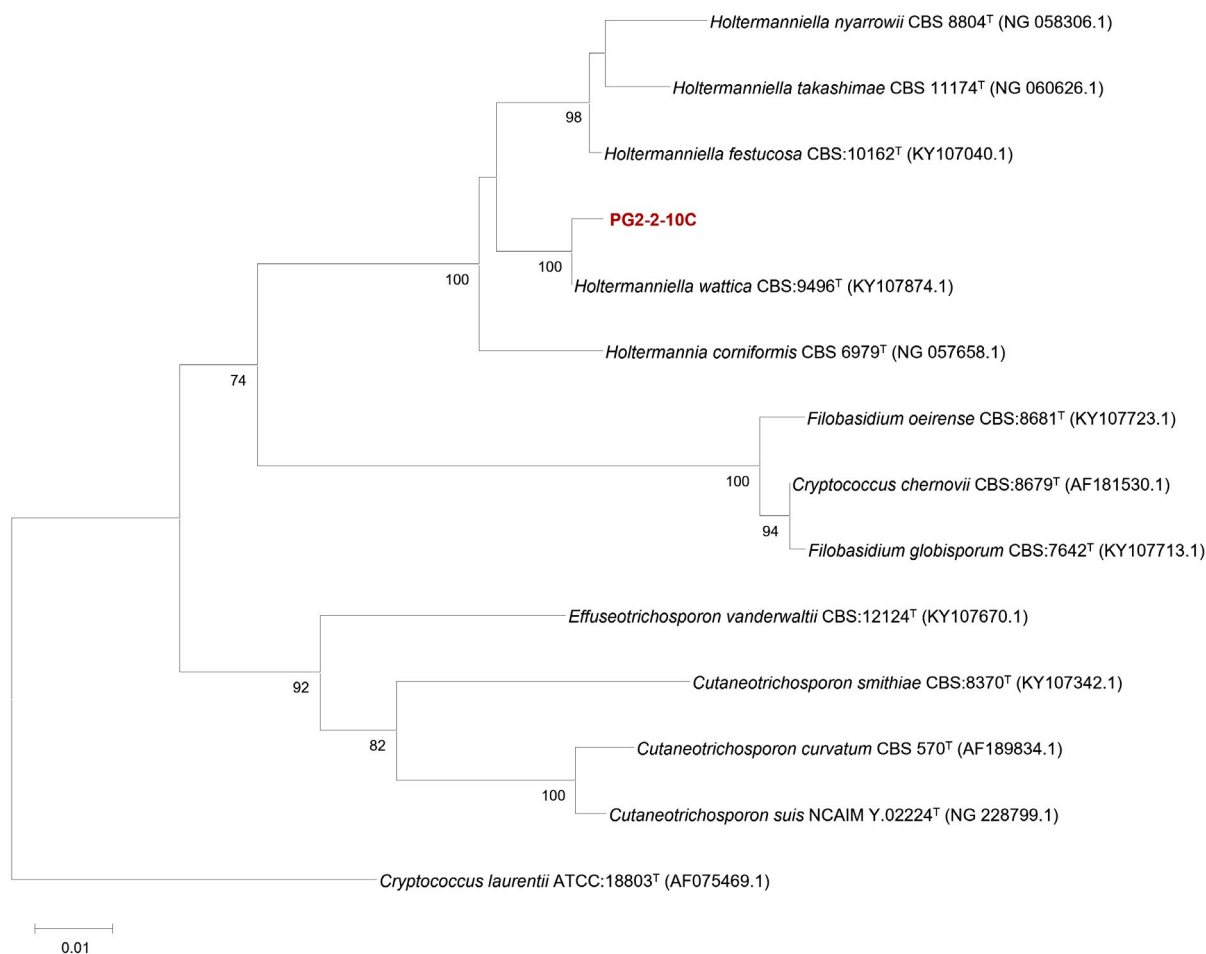


Fig. 2. A neighbor-joining phylogenetic tree reconstructed from comparative analysis of 26S rRNA gene sequences showing the relationships of strain PG2-2-10C with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position (Trees, 1987).

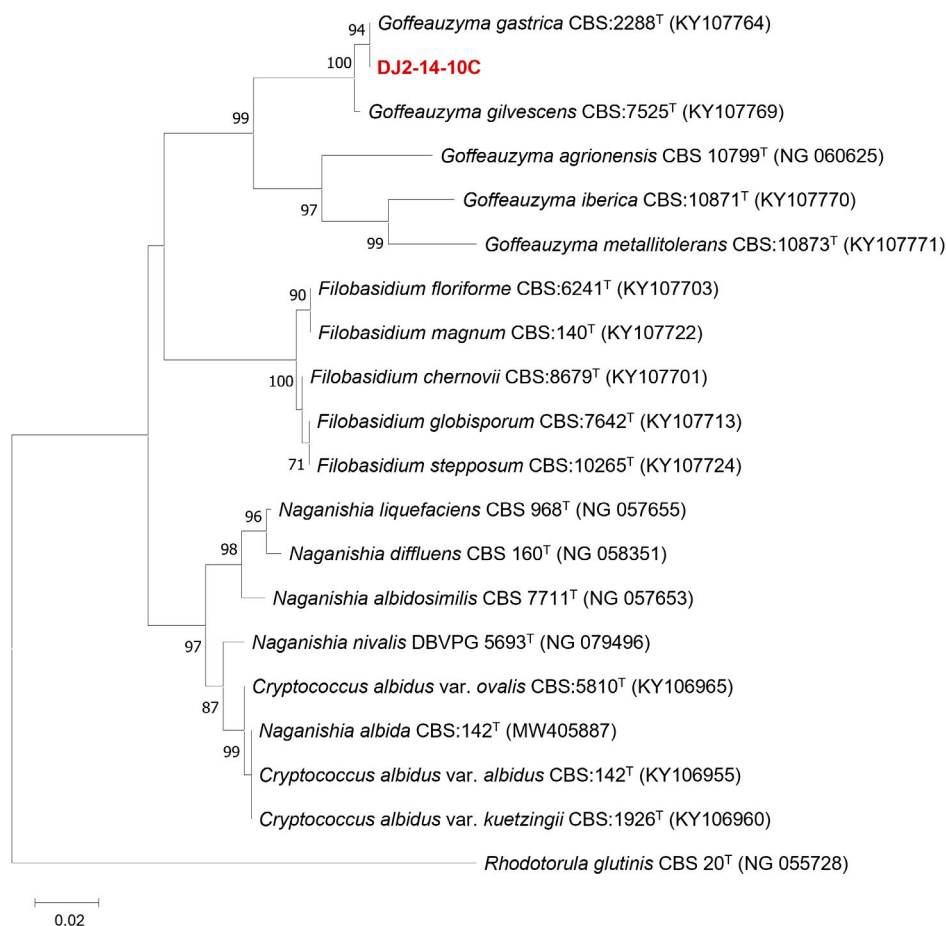


Fig. 3. A neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain DJ2-14-10C with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position (Trees, 1987).

closely related to *Goffeauzyma gastrica* (Fig. 3).

Result of carbon source assimilation test using API 20C AUX kit is described in species description. Characteristics of two unrecorded strains and related species are shown in Table 2.

Description of *Holtermanniella wattica* PG2-2-10C

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 10°C. In the API 20C AUX test, strain PG2-2-10C is positive for D-melezitose, inositol, glucose, D-sorbitol, D-saccharose (sucrose), calcium-2-keto-D-gluconate, D-xylose, N-acetyl-D-glucosamine, D-cellobiose, D-maltose, and D-trehalose; weak positive for methyl- α -D-glucopyranoside and D-raffinose; but negative for adonitol, D-galactose, D-lactose (bovine origin), xylitol glycerol, and L-arabinose.

Strain PG2-2-10C (KACC 410362) was isolated from soil collected in Daegu, Republic of Korea.

Description of *Goffeauzyma gastrica* DJ2-14-10C

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 DJ2-14-10C is positive for calcium 2-keto-D-gluconate, L-arabinose, glucose, D-trehalose, D-galactose, D-maltose, and D-melezitose; weak positive for D-xylose and D-cellobiose; but negative for methyl- α -D-glucopyranoside adonitol, xylitol, inositol, D-sorbitol, N-acetyl-D-glucosamine, glycerol, D-saccharose (sucrose), D-lactose (bovine origin), and D-raffinose.

Strain DJ2-14-10C was isolated from soil collected in Daejeon, Republic of Korea.

CONFLICTS OF INTEREST

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

Table 2. Microbiological characteristics of the unrecorded yeasts strains and closely related species

Strain ID	1	2	3	4	5	6
Morphological characteristics						
Shape	Oval	Oval	Oval	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding	Budding	Budding	Budding
API 20C AUX						
Glucose	+	+	+	+	+	-
Glycerol	-	-	-	w	ND	w
Calcium 2-keto-gluconate	+	+	ND	+	ND	ND
L-arabinose	-	+	-	+	+	+
D-xylose	+	w	+	+	ND	+
Adonitol	-	-	ND	ND	ND	ND
Xylitol	-	-	ND	w	+	+
D-galactose	-	+	+	w	+	+
Inositol	+	-	+	+	ND	+
D-sorbitol	+	-	-	-	ND	+
Methyl- α -D-glucopyranoside	w	-	ND	ND	+	ND
N-acetyl-D-glucosamine	+	-	ND	+	ND	-
D-cellobiose	+	w	+	+	ND	+
D-lactose (bovine origin)	-	-	+	w	-	+
D-maltose	+	+	-	+	+	+
D-saccharose (sucrose)	+	-	ND	ND	-	ND
D-trehalose	+	+	+	w	ND	+
D-melezitose	+	+	-	+	+	ND
D-raffinose	w	-	+	w	ND	+

Taxa: 1, *Holtermanniella wattica* PG2-2-10C; 2, *Goffeauzyma gastrica* DJ2-14-10C; 3, *Holtermanniella wattica* CBS:9496^T; 4, *Holtermanniella festucosus* CBS:10162^T; 5, *Goffeauzyma gastrica* CBS:2288^T; 6, *Cryptococcus agrionensis* CBS:10799^T (Russo *et al.*, 2010).
+, positive; w, weakly positive; -, negative; ND, no data.

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