

New record of *Vishniacozyma pseudocarnescens*, basidiomycetous yeast species isolated from flowers in Korea

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In the course of our survey of microbial diversity, a total of seven basidiomycetous yeast strains were isolated from flowers sampled across the four provinces of the Republic of Korea, of which two strains were identified as an unrecorded species. Comparative analysis was conducted for identification of strains using the D1/D2 region of the large subunit (LSU) rRNA gene sequences, observation of cell morphologies, and carbon assimilation tests. Seven strains were classified within the phylum *Basidiomycota*. Two unrecorded strains, JRP1-10 (KACC 410703) and KC-1 (KACC 410704), were identified as *Vishniacozyma pseudocarnescens*, belonging to the family *Bulleribasidiaceae*, order *Tremellales*, class *Tremellomycetes*. Two strains had ellipsoidal-shaped and monopolar budding cells and smooth and cream-colored colonies after cultivation at 25°C for 3 days. Here, we present the morphological and biochemical characteristics of the unrecorded yeast species, *Vishniacozyma pseudocarnescens*, in the Republic of Korea.

Keywords: unrecorded yeasts, *Vishniacozyma*

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INTRODUCTION

As part of our survey on microbial diversity, yeast strains were isolated from flowers in the four provinces of the Republic of Korea, including Chungcheongbuk-do, Gyeongsangnam-do, Jeollabuk-do, and Jeollanam-do. Following isolation, several yeast strains were identified, of which two strains represent a previously unrecorded species belonging to the genus *Vishniacozyma*, family *Bulleribasidiaceae*.

The family *Bulleribasidiaceae* was primarily proposed by Liu *et al.* (2015). At the time of writing, the family comprised six genera: *Bulleribasidium*, *Deroxomyces*, *Dioszegia*, *Hannaella*, *Niellozyma*, and *Vishniacozyma* (<https://www.mycobank.org>). The type genus is *Bulleribasidium* (Sampalo *et al.*, 2002). Members of the family are characterized by formation of hyphae, absence of basidiocarps, sexual reproduction in some species, occasional presence of ballistoconidia, and budding cells (Liu *et al.*, 2015). Except for one teleomorphic species, *Bulleribasidium oberjochense*, all members of the family have been documented only in an asexual state.

The genus *Vishniacozyma* was first described by Liu *et al.* (2015). Currently, the genus, represented by the type

species, *V. carnescens*, consists of 21 recognized species (<https://www.mycobank.org>). Members of the genus have been isolated from various environments, such as soil, plant leaves, grapes, and subglacial ice (Maeng *et al.*, 2022). They are characterized by thin-walled hyphae with clamp connections and the presence of H-shaped conidia and ellipsoidal ballistoconidia. Under culture conditions, poorly developed pseudohyphae and budding cells are present with occasional presence of ballistoconidia. Members of the genus are further characterized based on the presence of CoQ-9 or CoQ-10 as the main CoQ system (Liu *et al.*, 2015).

In the present study, we report the unrecorded yeast species, *Vishniacozyma pseudocarnescens*, in the Republic of Korea. This species was recently proposed as a new basidiomycete yeast species during a survey of yeast diversity along the intertidal zone of the Chinese coastline (Zhu *et al.*, 2023).

MATERIALS AND METHODS

Flower samples were collected from the four provinces of the Republic of Korea, including Chungcheongbuk-do,

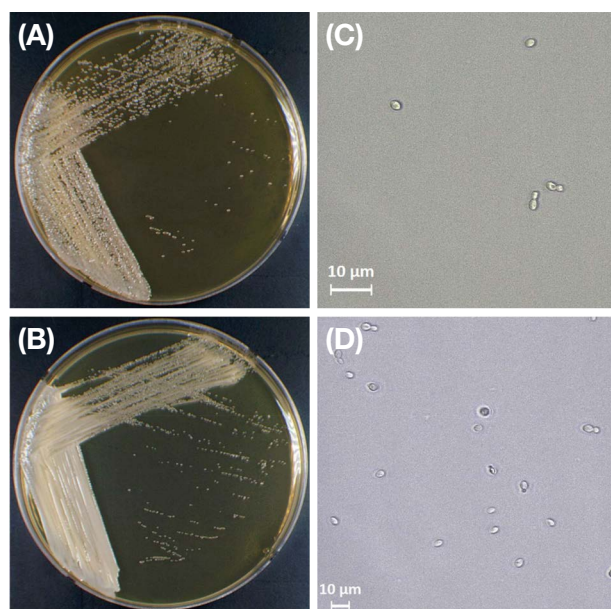


Fig. 1. Cell morphology of unrecorded strains. Colonies of *Vishniacozyma pseudocarnescens* JRP1-10 (A) and *Vishniacozyma pseudocarnescens* KC-1 (B). Budding patterns of *Vishniacozyma pseudocarnescens* JRP1-10 (C) and *Vishniacozyma pseudocarnescens* KC-1 (D). Strains were grown at 25°C on YMA for 3 days.

Gyeongsangnam-do, Jeollabuk-do, and Jeollanam-do between 2018 and 2019. Harvested flowers were diluted with sterilized 0.85% (w/v) NaCl solution supplemented with 17 mg/L chloramphenicol and 1.25 g/L propionic acid. The diluted samples were spread onto yeast malt extract agar (YMA) and incubated at 25°C for 3 days. Among the purified isolates, strains JRP1-10 and KC-1 isolated from flowers in Jeollabuk-do were examined for the present study. Two strains were deposited and preserved in a metabolically inactive state at the Korean Agricultural Culture Collection (KACC), Republic of Korea.

The cell morphology and budding pattern were observed by the LEICA (DM500) after cultivation at 25°C on YMA for 3 days. Images of phase contrast microscopy and the colonies of two strains are shown in Fig. 1. Biochemical characteristics were examined using API 20C AUX kit (bioMérieux) according to the manufacturer's instructions.

The genomic DNA was extracted after cultivation at 25°C on YMA for 3 days. The D1/D2 region of the large subunit (LSU) rRNA gene was amplified by PCR using NL1 (5'-GCATATCAATAAGCGGAGGA AAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') primers (Kurtzman and Robnett, 1998). The PCR products were examined by agarose gel electrophoresis and purified and sequenced by Macrogen (Daejeon, Republic of Korea). The obtained nucleotide sequences of the LSU rRNA gene were assembled using the SeqMan II software (DNASTAR).

Table 1. A list of yeast strains isolated from flowers in Korea and their taxonomic affiliations.

Phylum	Class	Order	Family	Strain ID	Most closely related species	26S rRNA similarity	Record in Korea
Basidiomycota	Tremellomycetes	Tremellales	<i>Bulleribasidiaceae</i>	JRP1-10 (PP237213)	<i>Vishniacozyma pseudocarnescens</i> (OR077057)	567/568 (99.82%)	Unreported
				KC-1 (PP237214)	<i>Vishniacozyma pseudocarnescens</i> (OR077057)	567/568 (99.82%)	Unreported
		Filobasidiales	<i>Bulleraceae</i>	WSW-35 (OK287394)	<i>Bullera alba</i> (KY106261)	611/611 (100%)	Reported
				FSTC4-2 (MW171255)	<i>Filobasidium magnum</i> (NG069409)	614/615 (99.84%)	Reported
Microbotryomycetes	Sportidiobolales	Sportidiobolales	<i>Sporidiobolaceae</i>	TAU-1 (OK287358)	<i>Rhodotorula graminis</i> (NG068963)	592/592 (100%)	Reported
				CNSJ-11 (OK287359)	<i>Sporobolomyces phaffii</i> (AY070011)	590/590 (100%)	Reported
				MGLH-11 (MW171260)	<i>Rhodotorula taiwanensis</i> (GU646863)	579/579 (100%)	Reported

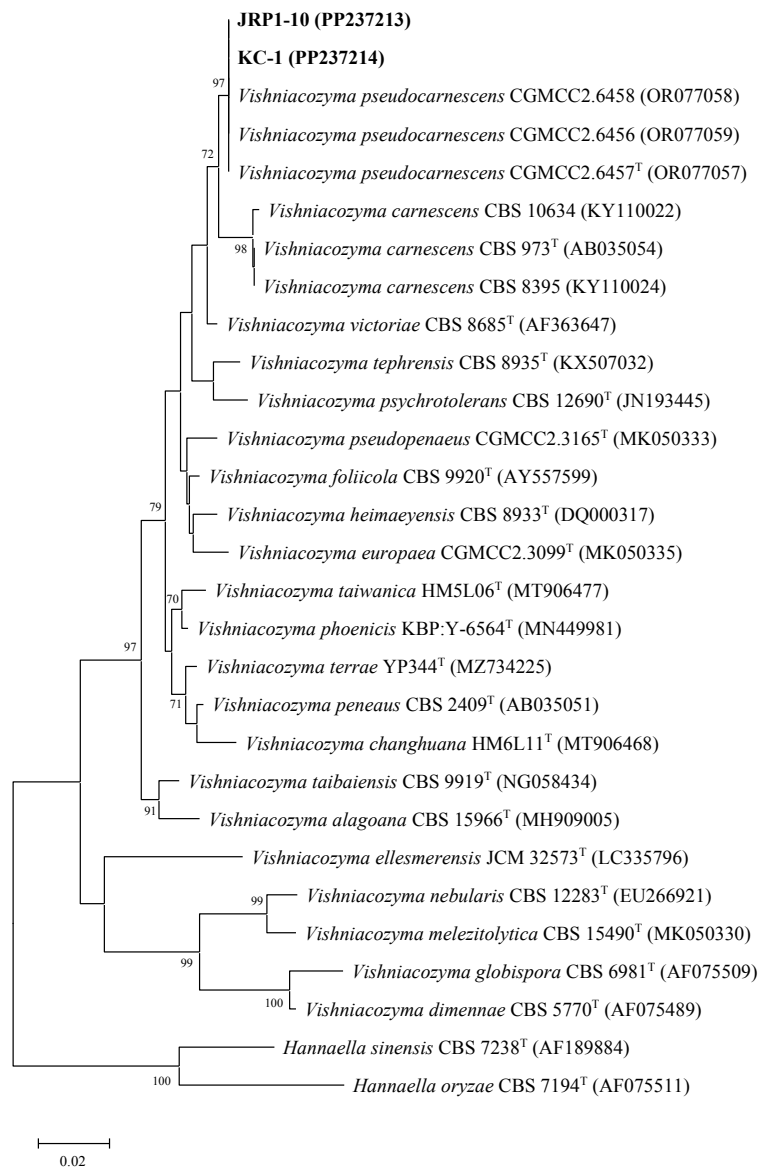


Fig. 2. Neighbor-joining phylogenetic tree based on the D1/D2 domain of LSU rRNA gene sequences of unrecorded strains and closely related strains. Bootstrap values (>70%) were obtained from 1,000 replicates and presented as percentages at branching points. Bar, 0.01 substitutions per nucleotide position.

The Basic Local Alignment Search Tool (BLAST) was employed for conducting pairwise sequence comparisons (Altschul *et al.*, 1997) and CLUSTAL W for aligning sequences with closely related strains obtained from NCBI (<https://www.ncbi.nlm.nih.gov>).

The phylogenetic tree based on the D1/D2 domain of LSU rRNA gene sequences was constructed by the neighbor-joining algorithm available in MEGA 11 program with 1,000 bootstrap replicates (Tamura *et al.*, 2021). Evolutionary distance was calculated according to the maximum composite likelihood method (Tamura *et al.*, 2004).

RESULTS AND DISCUSSION

A total of seven yeasts strains were isolated from the flowers sampled in the four provinces of the Republic of Korea. Comparative analysis using the D1/D2 domain of LSU rRNA gene sequences revealed that five strains were previously recorded, while the remaining two strains were identified as an unrecorded species previously in the Republic of Korea. Taxonomic identification of seven strains is summarized in Table 1.

Seven strains were assigned to four families, including *Bulleribasidiaceae* (2 strains), *Bulleraceae* (1 strain), *Filo-*

Table 2. Morphological and biochemical characteristics of unrecorded strains and closely related strains.

Strain ID	1	2	3	4	5
Morphological characteristics					
Shape	Ellipsoidal	Ellipsoidal	Ellipsoidal	Ellipsoidal	Encapsulated
Reproduction	Budding	Budding	Budding	Budding	Budding
Pseudomycelium	ND	ND	Not found	ND	Not found
Biochemical characteristics					
Glycerol	W	W	+	L	+
2-Keto-D-Gluconate	+	+	ND	+	+
L-Arabinose	+	+	+	ND	+
D-Xylose	+	+	W	+	+
Adonitol	W	W	ND	ND	ND
Xylitol	W	W	+	+	+
D-Galactose	W	+	+	+	+
Inositol	W	W	+	L	ND
D-Sorbitol	+	W	ND	ND	ND
α -Methyl-D-Glucoside	W	W	+	ND	ND
<i>N</i> -Acetyl-D-Glucosamine	+	+	+	W	ND
D-Cellobiose	W	+	ND	ND	ND
D-Lactose (bovine origin)	+	W	W	+	+
D-Maltose	+	+	W	+	+
D-Saccharose	W	+	ND	ND	ND
D-Trehalose	+	W	W	+	+
D-Melezitose	W	W	W	+	+
D-Raffinose	W	W	W	+	+

Strains: 1, *V. pseudocarnescens* JRP1-10; 2, *V. pseudocarnescens* KC-1; 3, *V. pseudocarnescens* CGMCC2.6457^T (Data from Zhu et al., 2023); 4, *V. carnescens* CBS 973^T (Data from Abu-Mejdad et al., 2019; Takashima et al., 2003); 5, *V. victoriae* G5 (Data from Montes et al., 1999). Symbols: +, positive; L, latent; W, weak; ND, no data.

basidiaceae (1 strain), *Sporidiobolaceae* (3 strains) of the phylum *Basidiomycota*. Two unrecorded strains JRP1-10 and KC-1 belong to the family *Bulleribasidiaceae*, order *Tremellales*, class *Tremellomycetes*. They exhibited a close relationship to *Vishniacozyma pseudocarnescens* CGMCC2.6457 with 99.82% similarity for the D1/D2 domain of LSU rRNA gene sequences. According to the phylogenetic analysis, strains JRP1-10 and KC-1 formed a clade with *Vishniacozyma pseudocarnescens* (Fig. 2).

Biochemical characteristics of the two unrecorded strains and closely related strains are summarized in Table 2. In general, two unrecorded strains JRP1-10 and KC-1 exhibited similar characteristics with the closely related strains. However, they were distinguished from the type strain of *V. pseudocarnescens* in its biochemical traits by showing the strong ability to assimilate D-xylose and D-maltose.

Description of *Vishniacozyma pseudocarnescens* JRP1-10

Cells are ellipsoidal shaped and budding is monopolar.

Colonies are smooth and cream-colored after 3-day incubation at 25°C on YMA. According to API 20C AUX test, strain JRP1-10 is positive for 2-keto-D-gluconate, L-arabinose, D-xylose, D-sorbitol, *N*-methyl-D-glucosamine, D-lactose (bovine origin), D-maltose, and D-trehalose; and weakly positive for glycerol, adonitol, xylitol, D-galactose, inositol, α -methyl-D-glucoside, D-cellobiose, D-saccharose, D-melezitose, and D-raffinose.

Strain JRP1-10 (KACC 410703) was isolated from the flower of *Kerria japonica* f. *pleniflora* (Witte) Rehder in Jeonju City, Jeollabuk-do, Republic of Korea.

CONFLICTS OF INTEREST

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

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