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An unrecorded ascomycetous yeast species, *Metschnikowia rancensis*, isolated from flower, *Pseudocydonia sinensis* in Korea

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During a survey of microbial diversity in the Republic of Korea, three unrecorded ascomycetous yeast strains were isolated from flower, *Pseudocydonia sinensis*. Three strains, designated as SINF-1Y (KACC 410756), SINF-2Y (KACC 410757), and SINF-3Y (KACC 410758), were subjected to sequence analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene, observation of colony morphology, and determination of carbon assimilation patterns. The phylogenetic analysis of sequences identified three strains as *Metschnikowia rancensis*, belonging to the family *Metschnikowiaceae*, order *Saccharomycetales*, class *Saccharomycetes* within the phylum *Ascomycota*. Morphological characterization revealed ellipsoidal-shaped and polar budding cells that formed smooth and cream-colored colonies with entire margins. Three strains were distinguishable from the closely related strains in there assimilation of carbon sources. Based on the phylogenetic, morphological, and biochemical traits, three strains were determined as a previously unrecorded yeast species, *Metschnikowia rancensis*, in the Republic of Korea.

Keywords: *Metschnikowia*, Unrecorded yeasts

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Introduction

The yeast family *Metschnikowiaceae* consists of five distinct genera, *Aciculoconidium*, *Clavispora*, *Kodamaea*, *Metschnikowia*, and *Nectaromyces* (Vicente *et al*., 2020). Among these, the genus *Metschnikowia*, first described over a century ago, includes over 70 species and numerous related lineages within the expansive clade of budding yeasts (https://www.mycobank.org/; accessed July 2024).

Metschnikowia species are characterized by formation of multilateral budding of vegetative cells and one or two needle-shaped ascospores within elongate asci (Mendonça-Hagler *et al*., 1993). They have been found in flowers, flower nectar, fruits, plants, and pollinating insects, such as flies, bees, and beetles, mostly from tropical regions (Lachance *et al*., 2016; Chai *et al*., 2019). There are several reports of *Metschnikowia* species isolated from wildflowers in the Republic of Korea (Hong *et al*., 2001; Hyun *et al*., 2014; Kim *et al*., 2020).

The genus *Metschnikowia*, known as one of non-Saccharomyces wine yeasts, is considered as a promising tool for improving wine quality (Vicente *et al*., 2020). This is due to its fermentation capacity, enzymatic activity related to aromatic and color precursors, and antimicrobial effects against spoilage yeasts and molds. In addition, it holds a significant biotechnological potential in the food and agricultural sectors, particularly for low-cost lipid production and strong antimicrobial activity as a biological control agent (Sipiczki, 2020).

In a survey of microbial diversity in the Republic of Korea, three strains isolated from flowers were identified as an unrecorded yeast species, *Metschnikowia rancensis*. The species initially reported in 1984 as *Candida rancensis* was later proposed to be transferred to the genus, *Metschnikowia* (Ramírez and González, 1984; Kurtzman *et al*., 2018). This study aimed to describe this unrecorded yeast species, *Metschnikowia rancensis*, based on the phylogenetic, morphological, and biochemical characterization.

Materials and Methods

Flower samples of *Pseudocydonia sinensis* were col-

lected from Goseong-gun, South Gyeongsang, Republic of Korea in April 2024. The samples were diluted in distilled water and spread onto yeast extract peptone dextrose agar (YPD; MBcell, Republic of Korea) and potato dextrose agar (PDA; BD, United States) both supplemented with 0.2 g/L sodium propionate and 0.2 g/L chloramphenicol followed by incubated at 10°C for 5−7 days. Subsequently, pure isolates were obtained by repeated streak-plating on YPD. Among the purified isolates, strains SINF-1Y, SINF-2Y, and SINF-3Y were examined for the present study. Three strains were deposited and preserved in an inactive state at the Korean Agricultural Culture Collection (KACC), Republic of Korea. Taxonomic information of three strains and other species of the genus *Metschnikowia* registered in KACC is summarized in Table 1.

The genomic DNA of the isolates was extracted after cultivation at 25°C for 3 days on yeast malt extract agar (YMA). For phylogenetic analyses, sequences of the D1/D2 region of the large subunit (LSU) rRNA gene were determined using NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) primers (Kurtzman and Robnett, 1998). The nucleotide sequences of the LSU rRNA gene were provided by Macrogen, Republic of Korea and edited and assembled using SeqMan II software (DNASTAR). The obtained sequences were compared against yeast sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997). A phylogenetic tree was constructed using the maximum-likelihood (ML) method in MEGA 11 software (Tamura *et al*., 2021). The Kimura 2 parameter model was used with a discrete Gamma distribution (+G) with 5 rate categories, assuming that a certain fraction of sites are evolutionarily invariable (+I).

The colony morphology and budding cells were observed using an optical microscope (Zeiss DE/Axio Imager A1 microscope equipped with an AxioCam Icc3, Carl Zeiss, Germany) after cultivation at 25°C for 3 days on YMA. Images of the colonies and budding cells are presented in Fig. 1. Biochemical characteristics were assessed using API 20C AUX kit (bioMérieux) following the manufacturer's instructions.

Results and Discussion

Comparative analyses of the D1/D2 domain of the LSU rRNA gene sequences revealed that three strains represent a previously unrecorded species, *Metschnikowia rancensis*, in the Republic of Korea. They belong to the family *Metschnikowiaceae*, order *Saccharomycetales*, class *Saccharomycetes*, within the phylum *Ascomycota*. Three strains exhibited a close relationship with the type

Table 1. A list of yeast strains of the genus *Metschnikowia* 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
Ascomycota	*Saccharomycetes*	*Saccharomycetales*	*Metschnikowiaceae*	SINF-1Y (PQ014150)	*Metschnikowia rancensis* (NG055309)	508/509 (99.80%)	Unreported
				SINF-2Y (PQ014151)	*Metschnikowia rancensis* (NG055309)	500/501 (99.80%)	Unreported
				SINF-3Y (PQ014152)	*Metschnikowia rancensis* (NG055309)	502/503 (99.80%)	Unreported
				AZLY-1 (MZ333635)	*Metschnikowia reukaufii* (NG055410)	512/513 (99.81%)	Reported
				TO-2 (MN128575)	*Metschnikowia koreensis* (NG058340)	536/537 (99.81%)	Reported

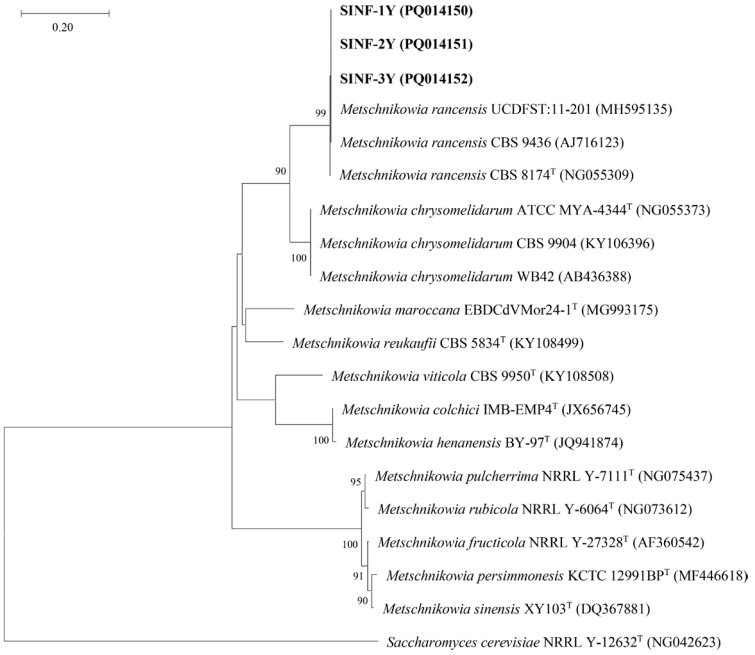

Fig. 1. Phylogeny of strains SINF-1Y, SINF-2Y, and SINF-3Y and the related species based on Maximum Likelihood analysis of the sequences of the D1/D2 domain of the large subunit rRNA gene. *Saccharomyces cerevisiae* NRRL Y-12632^{T} was used as an outgroup. Only bootstrap values above 70% from 1,000 replicates are shown. The scale bar indicates 0.2 nucleotide substitutions per position.

Table 2. Morphological and biochemical characteristics of strain SINF1-1Y and the closely related strains.

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

Strains: 1, *M. rancensis* SINF1-1Y; 2, *M. rancensis* CBS 8174^{T} (Data from Ramírez and González, 1984); 3, *M. chrysomelidarum* ATCC MYA-4344^{T} (Data from Nguyen *et al*., 2006); 4, *M. maroccana* EBDCdVMor24-1^{T} (Data from de Vega *et al*., 2018). Symbols: +, positive; −, negative; W, weak; V, variable; ND, no data.

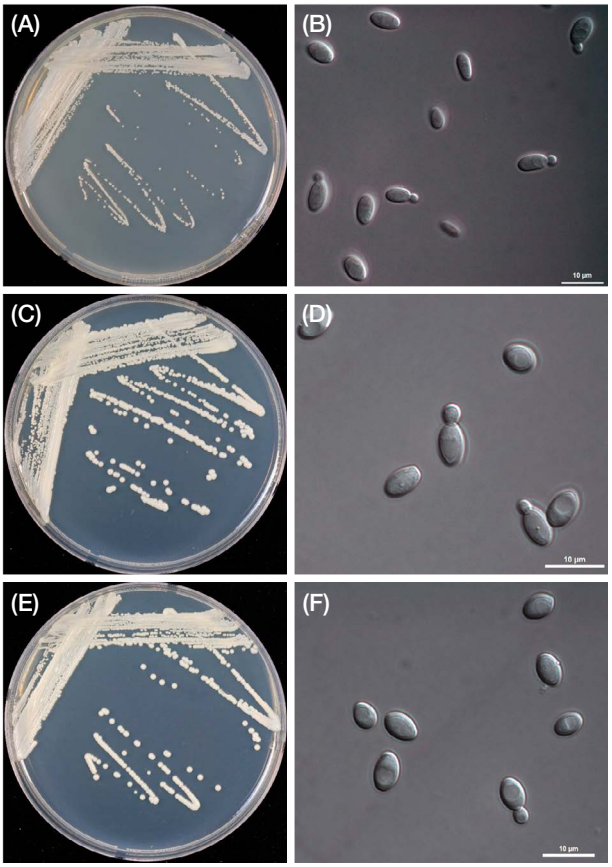

Fig. 2. Colony morphology and budding cells of strains SINF-1Y (A, B), SINF-2Y (C, D), and SINF-3Y (E, F). All strains were grown at 25ºC for 3 days on YMA. The scale bar indicates 10 μm. Images were captured at 1000× magnification.

strain *Metschnikowia rancensis* CBS 8174^T with 99.80% similarity for the D1/D2 domain of the LSU rRNA gene sequences. Phylogenetic analysis revealed that strains SINF-1Y, SINF-2Y, and SINF-3Y formed a stable cluster with *Metschnikowia rancensis* (Fig. 1).

Morphologically, strains SINF-1Y, SINF-2Y, and SINF-3Y had the ellipsoidal shape which is consistent with the type strain *Metschnikowia rancensis* CBS 8174^T and the closely related species (Fig. 2, Table 2). Biochemical characteristics of three strains and the closely related species are summarized in Table 2. The biochemical traits of strains SINF-2Y and SINF-3Y were same as those of strain SINF-1Y and thus not shown in Table 2. Strain SINF-1Y differed from the type strain *Metschnikowia rancensis* CBS 8174^T in its ability to utilize glycerol and from the closely related species *Metschnikowia chrysomelidarum* ATCC MYA-4344^T to utilize D-xylose and D-cellobiose. Furthermore, strain SINF-1Y showed different characteristics from *Metschnikowia maroccana* EBDCdVMor24-1^T for its capability to assimilate 2-keto-D-gluconate, D-xylose, xylitol, *N*-acetyl-D-glucosamine, and D-trehalose.

Description of *Metschnikowia rancensis* SINF-1Y

Cells are ellipsoidal with a polar budding pattern. Colonies are cream-colored and smooth with an entire margin after incubation at 25°C for 3 days on YMA. Based on the results of the API 20C AUX test, strain SINF-1Y is positive for Glycerol, 2-Keto-D-Gluconate, Adonitol, Xylitol, D-Sorbitol, *N*-Acetyl-D-Glucosamine, D-Cellobiose, D-Maltose, D-Saccharose, D-Trehalose, and D-Melezitose; weakly positive for D-Xylose; and negative for L-Arabinose, D-Galactose, Inositol, α-Methyl-D-Glucoside, D-Lactose, and D-Raffinose.

Strain SINF-1Y (KACC 410756) was isolated from flower of *Pseudocydonia sinensis* in Goseong-gun, South Gyeongsang, Republic of Korea.

Conflicts of Interest

These authors of this paper have no affiliation with any interests and is solely responsible for the paper.

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