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Characterization of two unrecorded yeast species, *Starmerella apicola* and *Symmetrospora symmetrica*, isolated from *Apis mellifera* and *Citrus sunki* in Republic of Korea

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The purpose of this study is to isolate and identify wild yeasts from *Apis mellifera* and *Citrus sunki* samples collected in Okcheon-gun and Jeju-si, Republic of Korea. Among ten strains, one strain had been previously reported, but nine strains were unreported in the Republic of Korea. To identify the wild yeast strains, pairwise sequence comparisons of the D1/D2 region of the 26S rRNA gene sequence were conducted using the Basic Local Alignment Search Tool (BLAST) (Fell *et al*., 2000). Cell morphologies were observed using a phase contrast microscope, and assimilation tests were performed using the API 20C AUX kit. The two unrecorded yeast strains, MBEE-1 and MMD-1, belong to the genus *Starmerella* (family *Saccharomycetales* incertae sedis, Order *Saccharomycetales*, Class *Saccharomycetes*) and the genus *Symmetrospora* (family *Symmetrosporaceae*, Order *Cystobasidiomycetes* incertae sedis, Class *Cystobasidiomycetes*), respectively. Both strains had oval-shaped and polar budding cells. This research elucidated the morphological and biochemical properties of these two previously unreported yeast species in Korea.

Keywords: 26s rRNA, *Starmerella*, *Symmetrospora*, unrecorded yeasts

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Introduction

In this study, wild yeasts were isolated from *Apis mellifera* and *Citrus sunki* samples collected in Okcheon-gun and Jeju-si, Republic of Korea. As a result of isolation and identification, many yeast strains were found to be previously reported species, while a few were identified as unrecorded species. The unreported species were classified within the genera *Starmerella* and *Symmetrospora*. The genus *Starmerella* is a member of the class *Saccharomycetes* within the phylum *Ascomycota*. This genus comprises 42 distinct species, with *Starmerella bombicola* designated as the type species (https://www.mycobank.org). The genus *Symmetrospora* belongs to the class *Cystobasidiomycetes* incertae sedis within the phylum *Basidiomycota*. This genus includes 16 distinct species, with *Symmetrospora gracilis* as the type species (https://www.mycobank.org).

The genus *Starmerella* was described to accommodate the sexual state of *Candida bombicola* (Rosa and Lachance, 1998). The cells range from spherical to ellipsoidal, and neither hyphae nor pseudohyphae are produced. During sexual reproduction, it is found that the conjugated asci usually form a single, roughened, asymmetrical ascospore (Teixeira *et al*., 2003; Santos *et al*., 2018; Shibayama *et al*., 2024). Coenzyme Q-9 is present, and the diazonium blue B reaction is negative. The type species is *Starmerella bombicola* (Kurtzman *et al*., 2011).

The genus *Symmetrospora*, which belongs to the family *Symmetrosporaceae*, is primarily defined by phylogenetic analysis of seven genes and an expanded LSU rRNA gene dataset. This analysis positions *Symmetrospora* as a sister clade to *Erythrobasidiales* within *Cystobasidiomycetes*. Characteristically, pseudohyphae and true hyphae are not observed in this genus, and ballistoconidia may be present or absent. When present, the ballistoconidia are typically symmetrical or nearly symmetrical, and ellipsoidal or ovoid in shape. The major coenzyme Q system is CoQ-10 (Wang *et al*., 2015).

As a result of this study, two previously unrecorded yeast strains were discovered in domestic ecosystems, and their phenotypic characteristics were thoroughly investigated.

Materials and Methods

Samples were collected from Okcheon-gun and Jeju-si, Republic of Korea. Each sample was thoroughly crushed, serially diluted in distilled water, and the resulting suspension was spread on Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA). The plates were then incubated at 25°C and 10°C for 3 days. The strains are preserved in a metabolically inactive state at the Korean Agricultural Culture Collection, Republic of Korea.

The cell morphologies of the strains were observed using a LEICA (DM500) microscope, with yeast strains incubated on YPD agar for 3 days. Phase contrast microscope images and colonies of strains MBEE-1 and MMD-1 are shown in Fig. 1. To characterize the biochemical features, the API 20C AUX (bioMérieux) test was performed according to the manufacturer's instructions.

The genomic DNA was extracted after incubation on YPD agar for 3 days. The D1/D2 region of the 26S rRNA gene sequence was amplified by PCR using NL1 (5′-GCATATCAATAAGCGGAGGAAA AG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) primers (Kurtzman and Robnett, 1998). Pairwise sequence comparisons were performed using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) to align with sequences of related species retrieved from GenBank. The MYCOBANK (https://www.mycobank.org/) database

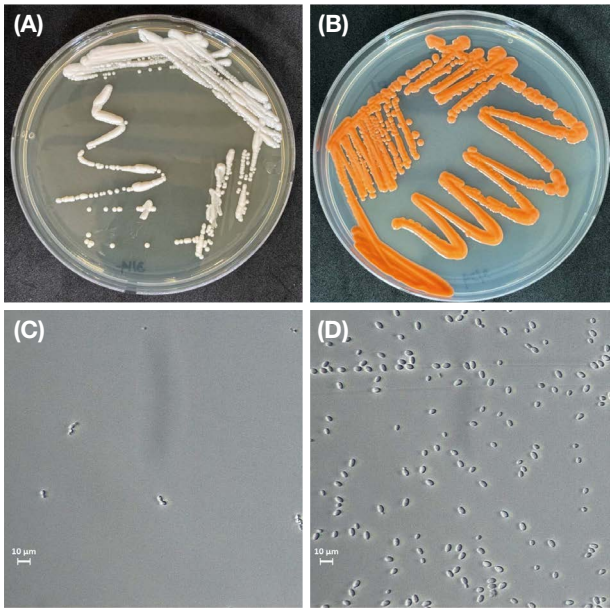

Fig. 1. Morphology of cells from the unrecorded strains MBEE-1 and MMD-1 incubated at 25°C. All strains were grown after 3 days on YPD agar. The colonies of *Starmerella apicola* MBEE-1 (A) and *Symmetrospora symmetrica* MMD-1 (B). The budding cells of *Starmerella apicola* MBEE-1 (C) and *Symmetrospora symmetrica* MMD-1 (D). Bars, 20 μm and 10 μm, respectively.

Table 1. Yeasts isolated strains from soil in Republic of Korea. All strains were cultured under 25°C and 10°C for 3 days.

Phylum	Class	Order	Family	Sample	Strain ID	Most closely related species	26S rRNA similarity	Record in Korea
Ascomycota	*Saccharomycetes*	*Saccharomycetales*	*Saccharomycetales* incertae sedis	*Apis mellifera*	MBEE-1	*Starmerella apicola*	479/480 (99%)	Unreported
					MBEE-4	*Starmerella apicola*	479/480 (99%)	Unreported
					MBEE-5	*Starmerella apicola*	479/480 (99%)	Unreported
					PBEE1-3	*Starmerella apicola*	479/480 (99%)	Unreported
					PBEE3-1	*Starmerella apicola*	479/480 (99%)	Unreported
					PBEE1-5	*Zygosaccharomyces rouxii*	581/581 (100%)	Reported
Basidiomycota	*Cystobasidiomycetes*	*Cystobasidiomycetes* incertae sedis	*Symmetrosporaceae*	*Citrus sunki*	MMD-1	*Symmetrospora symmetrica*	618/620 (99%)	Unreported
					PMD-2	*Symmetrospora symmetrica*	618/620 (99%)	Unreported
					PMD-3	*Symmetrospora symmetrica*	618/620 (99%)	Unreported
					PMD-4	*Symmetrospora symmetrica*	618/620 (99%)	Unreported

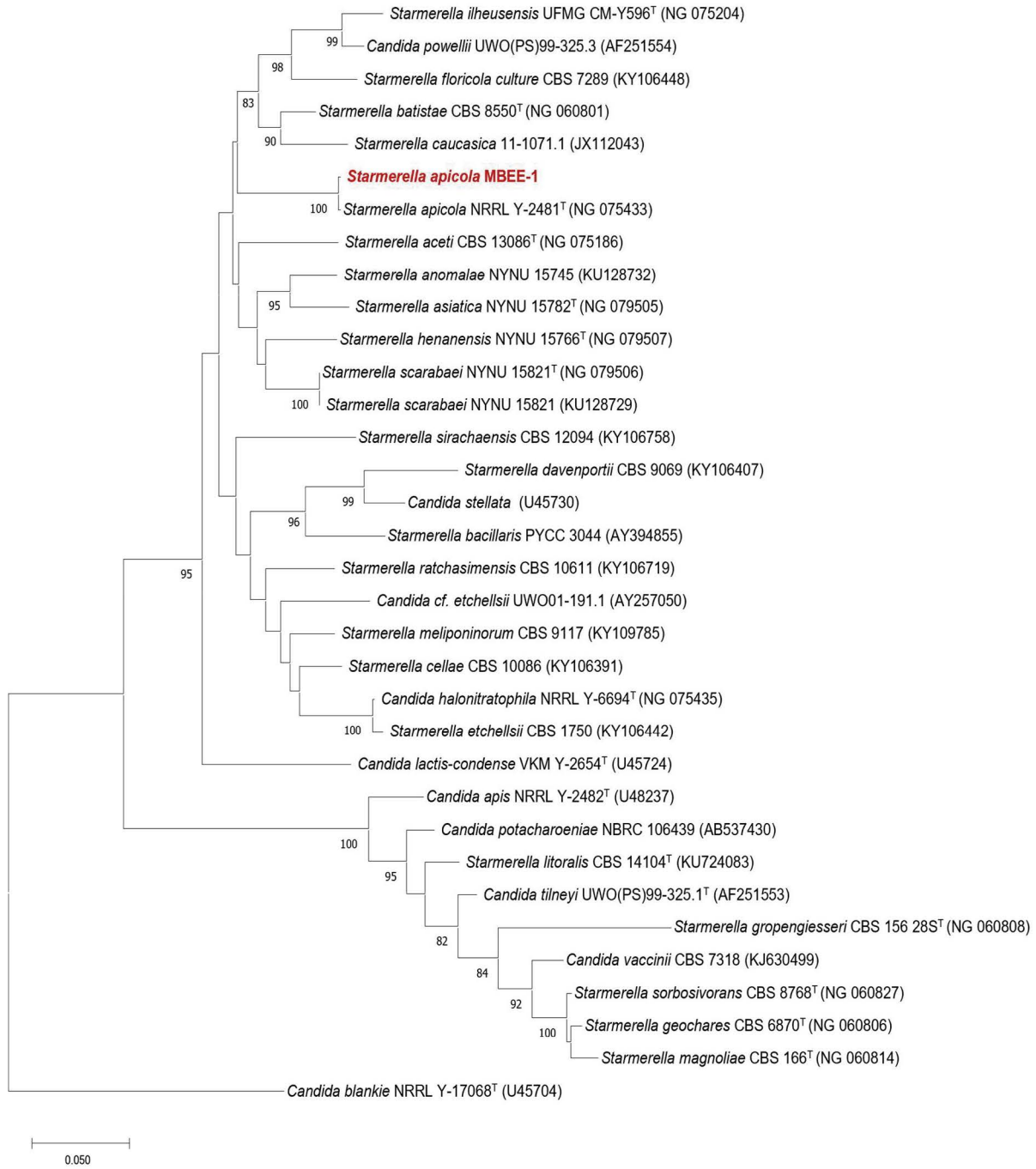

Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains MBEE-1 with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.05 substitutions per nucleotide position.

was used to identify strain types for each species, and closely related strains' gene sequences were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/) for the 26S rDNA in a row.

The phylogenetic trees based on the D1/D2 domain of the LSU rRNA gene sequence were reconstructed using the neighbor-joining algorithm (Saitou and Nei, 1987) of the MEGA 11 program (Tamura *et al*., 2021). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983) for neighborhood bond analysis, and the confidence levels of the clades were estimated through bootstrap analysis with 1,000 replicates (Felsenstein, 1985).

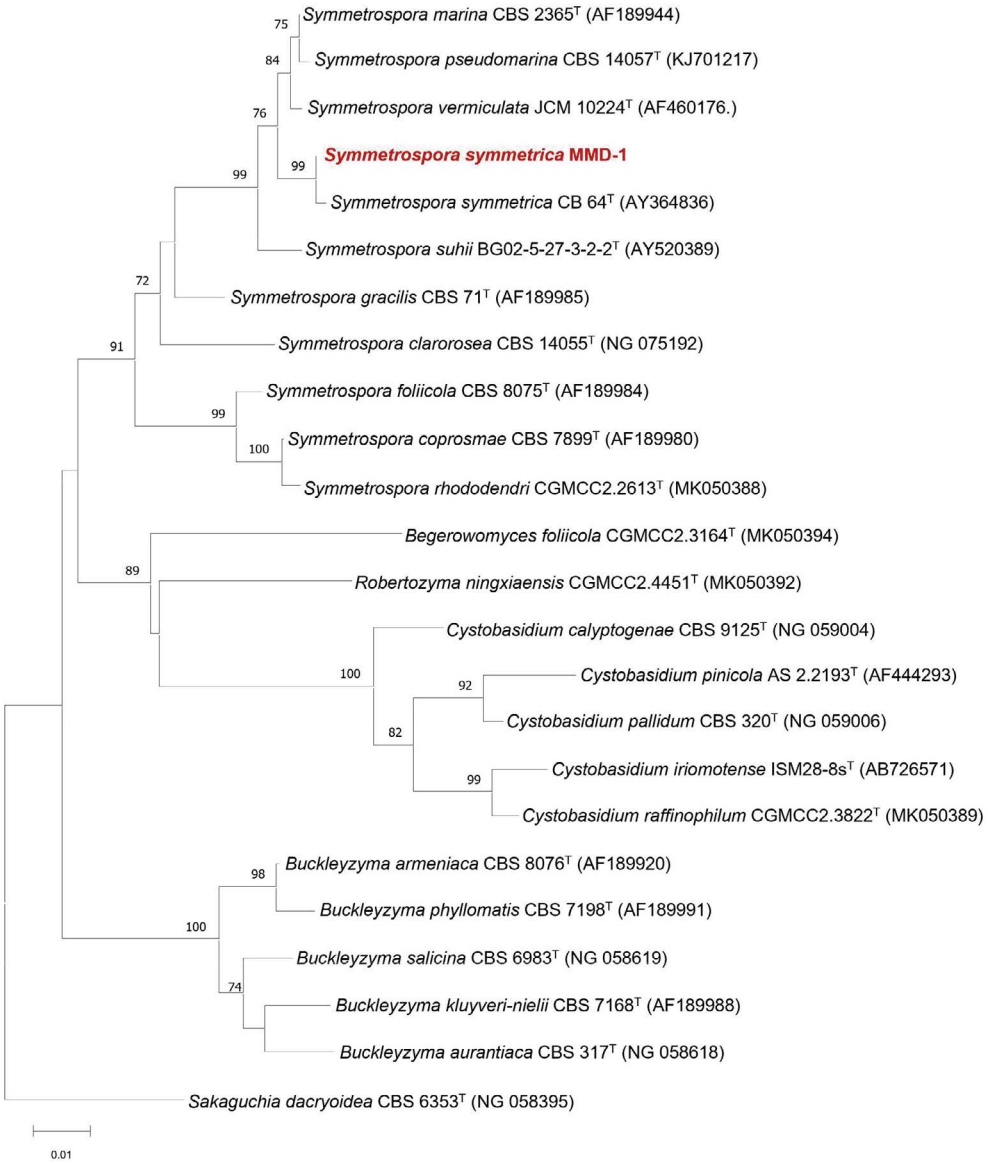

Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain MMD-1 with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

Results and Discussion

Among the ten wild yeast strains analyzed, six were isolated from *Apis mellifera* samples collected in Okcheon-gun, while the remaining four were derived from *Citrus sunki* samples obtained in Jeju-si. The yeast strains were identified by analyzing D1/D2 domain of the 26S rRNA gene sequence similarities, which were calculated using NCBI BLAST. Based on the D1/D2 domain of the 26S rRNA gene sequence, four yeast strains were identified as previously unrecorded species in Korea. The taxonomic composition and identification results are listed in Table 1.

Of the ten strains, the six strains isolated from *Apis mellifera* all belonged to the *Saccharomycetales incertae* sedis family of the phylum *Ascomycota*, while the four strains isolated from *Citrus sunki* all belonged to the *Symmetrosporaceae* family of the phylum *Basidiomycota*. The unrecorded yeast strain MBEE-1 belongs to the phylum *Ascomycota* and genus *Starmerella*, while MMD-1 belongs to the phylum *Basidiomycota* and genus *Symmetrospora*. The phylogenetic tree shows that the isolated strains are closely related to the *Starmerella* species and *Symmetrospora* species with the highest 26S rRNA gene

Table 2. Phenotypic characteristics of strain MBEE-1 and related species.

	1	2	3	4
Growth on				
Temp (°C)	10–25	19–37	25	4–40
Assimilation of:				
Glucose	+	+	+	+
Glycerol	+	+	−	+
L-Arabinose	W	−	−	−
D-Xylose	+	−	−	−
Xylitol	−	−	−	−
D-Galactose	−	−	−	−
Inositol	−	−	−	−
D-Sorbitol	+	W	+	W
Methyl-α-D-Glucopyranoside	−	−	ND	−
N-Acetyl-Glucosamine	−	−	−	−
D-Cellobiose	−	−	−	−
D-Lactose	−	−	−	−
D-Maltose	−	−	−	−
D-Saccharose	+	−	ND	−
D-Trehalose	−	−	−	W
D-Melezitose	−	−	−	−
D-Raffinose	+	+	+	+

Taxa: 1, *Starmerella apicola* MBEE-1; 2, *Starmerella apicola* CBS 2868^T; 3, *Starmerella vitae* CBS 15147^T; 4, Starmerella bombi CBS 5836^T.
+, positive; w, weakly positive; d, delay; −, negative; ND, no data.

Table 3. Phenotypic characteristics of strain MMD-1 and related species.

	1	2	3	4
Growth on				
Temp (°C)	10–25	19–30	19–30	12–40
Assimilation of:				
Glucose	+	+	+	+
Glycerol	+	+	+	−
Calcium 2-Keto-Gluconate	−	ND	−	ND
L-Arabinose	−	−	d	−
D-Xylose	+	−	d	−
Xylitol	W	ND	+	−
D-Galactose	+	−	−	+
Inositol	−	−	−	−
D-Sorbitol	−	−	−	−
Methyl-α-D-Glucopyranoside	−	−	d	−
N-Acetyl-Glucosamine	+	ND	ND	−
D-Cellobiose	−	+	+	−
D-Lactose	−	−	+	−
D-Maltose	−	−	+	−
D-Saccharose	−	ND	−	−
D-Trehalose	W	+	−	W
D-Melezitose	W	+	+	W
D-Raffinose	−	−	d	+

Taxa: 1, *Symmetrospora symmetrica* MMD-1; 2, *Symmetrospora symmetrica* CB 64^T; 3, *Symmetrospora marina* CBS 2365^T; 4, *Symmetrospora vermiculata* JCM 10224^T.
+, positive; w, weakly positive; d, delay; -, negative; ND, no data.

sequence similarity (Figs. 2 and 3), thereby supporting close relationships.

Description of *Starmerella apicola* MBEE-1

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 25°C. In the API 20C AUX test, strain MBEE-1 is positive for glucose, glycerol, D-xylose, D-sorbitol, D-saccharose, and D-raffinose; weakly positive for calcium 2-keto-gluconate, L-arabinose, and adonitol; and negative for xylitol, D-galactose, inositol, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, D-cellobiose, D-lactose, D-maltose, D-trehalose, and D-melezitose (Table 2).

Strain MBEE-1 (KCTC 37300) was isolated from *Apis mellifera* collected in Okcheon-gun, North Chungcheong Province, Republic of Korea.

Description of *Symmetrospora symmetrica* MMD-1

Cells are circular-shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, and orange cream-colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain MMD-1 is positive for glucose, glycerol, D-xylose, adonitol, D-galactose, and *N*-acetyl-glucosamine; weakly positive for xylitol, D-trehalose, and D-melezitose; but negative for calcium 2-keto-gluconate, L-arabinose, inositol, D-sorbitol, methyl-α-D-glucopyranoside, D-cellobiose, D-lactose, D-maltose, D-saccharose, and D-raffinose (Table 3).

Strain MMD-1 (KCTC 37301) was isolated from *Citrus sunki* collected in Jeju-si, 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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