Journal of Species Research 10(4):344-349, 2021

Isolation and characterization of unrecorded yeast species from Korea in the families *Debaryomycetaceae* and *Piskurozymaceae*

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The purpose of this study was to isolate and identify wild yeasts from soil of Gyeongju city, and *Haemadipsa rjukjuana* of Gageodo Island, characterizing unrecorded yeast strains from Korea. The molecular analysis of the D1/D2 domain of 26S rDNA of yeast was performed using the Basic Local Alignment Search Tool (BLAST). No official report exists describing these three species: one species in the genus *Candida*, one species in the genus *Debaryomyces*, and one species in the genus *Solicoccozyma*. *Candida saitoana* YL9, *Debaryomyces fabryi* YL1, and *Solicoccozyma terrea* 20g9-1 are recorded for the first time from Korea. All three strains were oval shaped and polar binding, while positive for glucose, D-xylose, and D-cellobiose. Morphological, physiological, and biochemical properties are described in the species descriptions.

Keywords: *Candida*, *Debaryomyces*, *Haemadipsa rjukjuana*, *Solicoccozyma*, unrecorded yeasts

DOI:10.12651/JSR.2021.10.4.344

Introduction

In 2020, 23 species of yeast were isolated from soil of Gyeongju city and Namhansanseong, Gwangju city, and from *Haemadipsa rjukjuana* collected from Gageodo Island, Gwangju city, Korea. We identified the characteristics of each species by separating the unrecorded species of the genus *Candida*, *Debaryomyces*, and *Solicoccozyma* for the purpose of yeast classification.

Haemadipsa rjukjuana is a segmented worm belonging to the phylum *Annelida*. Insects and yeast gain their own interests through interaction. The insect intestine differs among species but are hostile environments for many microorganisms (Stefanini, 2018). In particular, the leech is known to inhabit in tropical regions with high humidity (Won *et al*., 2014).

The family *Debaryomycetaceae* contains more than 100 yeast species, and members are reported to be isolated from a wide variety of substrates, such as soil, water, food, plant substrates, and animal-associated samples (Kurtzman *et al*., 2011; Urbina *et al*., 2013). Many of the yeast species belonging to the family are known to be associated with insects. Most of the classification groups included in the family *Debaryomycetaceae* form pseudohyphae and all teleomorphic species do not form septate hyphae except the genus *Spathaspora* (Hui *et al*., 2014).

The genus *Candida* represents *Ascomycetous* yeast in the family *Debaryomycetaceae*, order *Saccharomycetales*, subphylum *Saccharomycetes*, and phylum *Ascomycota*. The *Candida* clade consists of 163 species with the type species *Candida vulgaris*. *Candida saitoana* is similar to *Candida famata*. Species in the clade *Candida* have been isolated from various source such as soils (Martini and Martini, 1992), rotten wood, plants, insect (Suzuki *et al*., 1999), sea water (Van Uden and Zobell, 1962), clinical specimens, insects, rotting wood, dung, and spoiled food (Khunnamwong *et al*., 2015).

The genus *Debaryomyces* is represents *Ascomycetous* yeast in the family *Debaryomycetaceae*, order *Saccharomycetales*, subphylum *Saccharomycetes*v, and the phylum *Ascomycota*. The genus *Debaryomyces* consists of 80 species with the type species *Debaryomyces hansenii* (Zopf) Lodder&Kreger-van Rij. The strain *Debaryomyces fabryi* is physiologically similar to *Debaryomyces hansenii*, but genetically distinct. Half of known isolates in the clade *Debaryomyces* are from sake and rice vinegar fermentation, but *Debaryomyces fabryi* is isolated from skin infections (Suzuki, 2011).

The family *Piskurozymaceae* encompassed 2−4.5% of the total *Basidiomycota* sequences (Rosenfeld *et al*., 2019). Species in this family present budding cells and usually utilize nitrate (Liu *et al*., 2015; Kurtzman *et al*.,

Table 1. Yeasts strains isolated from soil and *Haemadipsa rjukjuana* collected in South Korea.

Phylum	Class	Order	Family	Strain ID	Most closely related species	26S rRNA similarity	Record in Korea
Ascomycota	*Saccharomycetes*	*Saccharomycetales*	*Debaryomycetaceae*	YL6	*Candida friedrichii*	549/554 (99)	Reported
				YL9	*Candida saitoana*	572/572 (100)	Unreported
				YL14		578/578 (100)	Unreported
				YL1	*Debaryomyces fabryi*	578/578 (100)	Unreported
				YL12	*Debaryomyces hansenii*	579/582 (99)	Reported
				YL5	*Meyerozyma caribbica*	567/569 (99)	Reported
				YL10		576/577 (99)	Reported
				YL13		550/555 (99)	Reported
				YL2	*Meyerozyma guilliermondii*	580/580 (100)	Reported
				YL3		583/584 (99)	Reported
				YL7		583/583 (100)	Reported
				YL11		583/583 (100)	Reported
				YL15		586/586 (100)	Reported
				YL16		581/581 (100)	Reported
				YL17		577/577 (100)	Reported
				20g24-1	*Yamadazyma scolyti*	546/549 (99)	Reported
Basidiomycota	*Tremellomycetes*	*Filobasidiales*	*Piskurozymaceae*	20g9-1	*Solicoccozyma terrea*	615/615 (100)	Unreported
		Tremellales	*Bulleribasidiaceae*	20n2-8	*Dioszegia zsoltii*	585/588 (99)	Reported
			Rhynchogastremataceae	20n9-3	*Papiliotrema aurea*	605/611 (99)	Reported
				20g25-1		605/610 (99)	Reported
				20g12-1	*Papiliotrema laurentii*	613/615 (99)	Reported
			Trimorphomycetaceae	20g3-1	*Saitozyma podzolica*	611/613 (99)	Reported
	Microbotryomycetes		*Chrysozymaceae*	20n28-7	*Sampaiozyma ingeniosa*	608/608 (100)	Reported

All strains were cultured at 10°C for 3 days.

Table 2. Microbiological characteristics of the unrecorded yeasts collected from South Korea.

Strain ID	1	2	3
Morphological characteristics			
Shape	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding
API 20C AUX			
Glycerol	−	w	−
2-Keto-D-gluconate	w	−	+
L-Arabinose	−	w	w
Adonitol	−	+	−
Xylitol	−	+	−
D-Galactose	+	+	−
Inositol	+	−	w
D-Sorbitol	−	+	w
N-Acetyl-D-glucosamine	w	+	−
D-Lactose (bovine origin)	w	−	−
D-Maltose	+	+	−
D-Saccharose (sucrose)	+	+	−
D-Trehalose	−	w	−
D-Melezitose	+	w	−
D-Raffinose	w	−	−

Taxa: 1, *Candida saitoana* YL9; 2, *Debaryomyces fabryi* YL1; 3, *Saitozyma terrea* 20g9-1.
All data were obtained in this study. +, positive; w, weakly positive; −, negative.

2017).

The genus *Solicoccozyma* represents *Basidiomycetous* yeast in the family *Piskurozymaceae*, order *Filobasidiales*, subphylum *Saccharomycetes*, and phylum *Basidiomycota*. Species in the clade *Solicoccozyma* have been isolated from soils (Yurkov, 2018). The *Solicoccozyma* clade consists of 11 recognized species with the type species *Solicoccozyma aeria*.

This study focuses on the description of three yeast species belonging to genera *Candida*, *Debaryomyces*, and *Solicoccozyma* that have not officially been reported in Korea.

Materials and Methods

Samples were collected from Gyeongju city, Gyeongsangbuk Province and Gageodo Island, Jeollanam Province in Korea. One strain is from the soil of Gyeongju city, and the other two from water used to wash *Haemadipsa rjukjuana* from Gageodo Island. The samples were diluted in distilled water step by step. The dilution was spread on YM and YNB agar mediums and incubated it at 10°C for 3−4 days. Table 1 summarizes strain IDs, sources of isolation, taxonomic composition, and identification results.

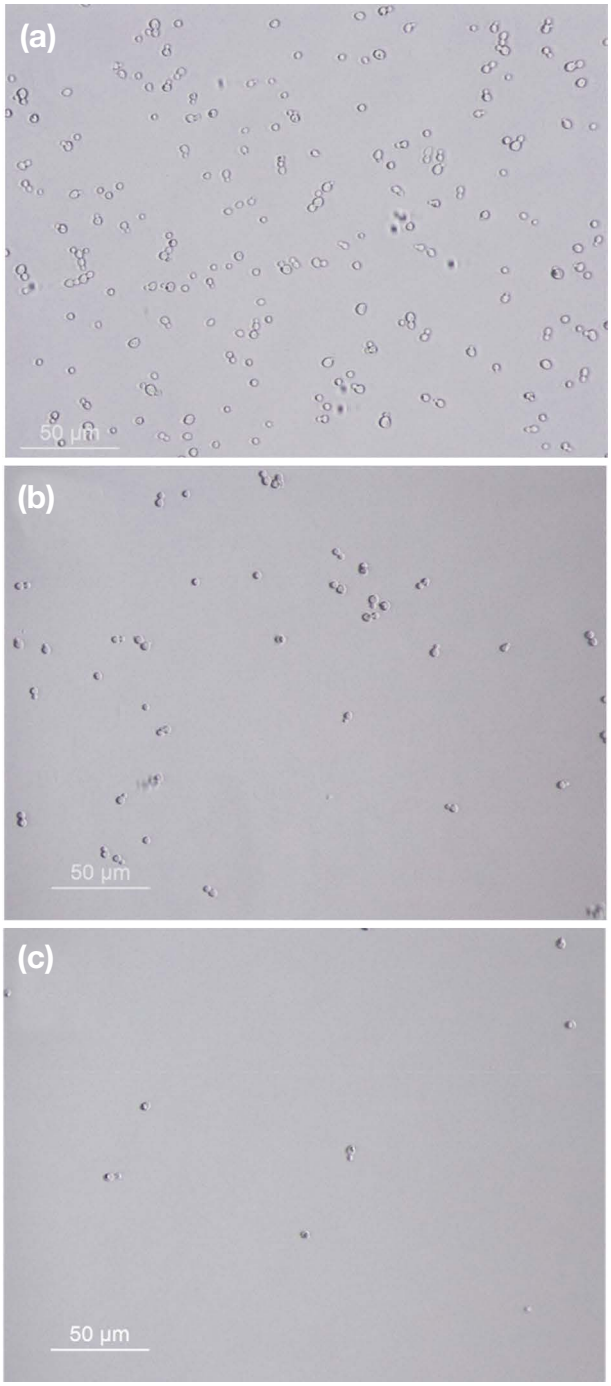

Fig. 1. Photomicrographs showing budding cells of strains YL1 (a), YL9 (b) and 20g9-1 (c). All strains were grown for 3 days on YM and YNB agar.

Cell morphologies of colonies were examined using a dissecting microscope (Leica, DM500). Photomicrographs of the strains YL9, YL1 and 20g9-1 are shown in Fig. 1. Biochemical characteristics were established using API 20C AUX (bioMérieux) according to the manufacturer's

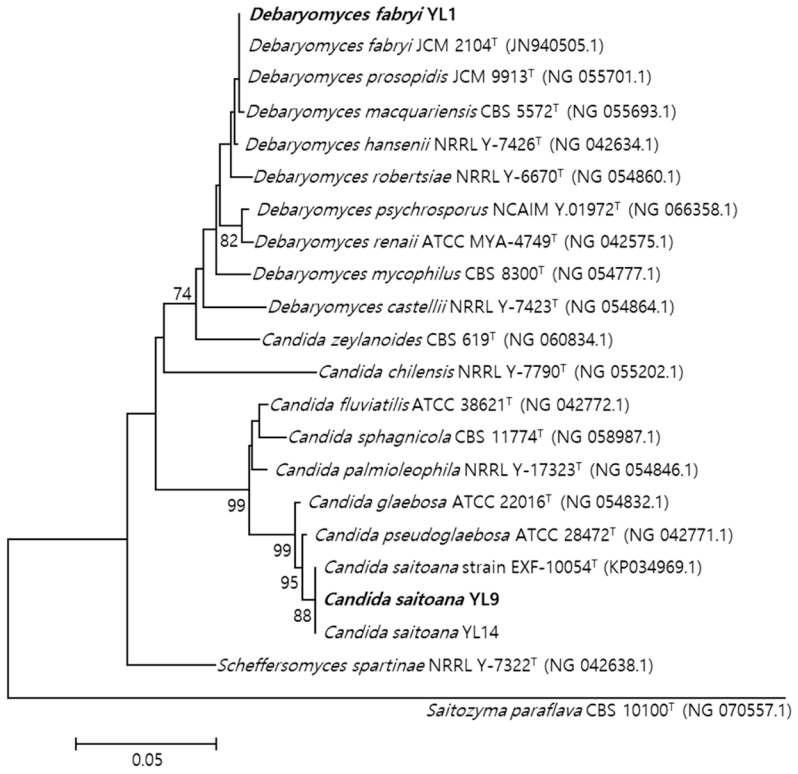

Fig. 2. Phylogenetic tree derived from neighbor-joining analysis based on the 26S rDNA gene sequences, showing the placement of strain YL1 in the *Debaryomyces* and YL9 in the *Candida saitoana*. *Saitozyma paraflava* CBS 10100^T was used as outgroup. Bootstrap values of above 70% are given at nodes based on 1,000 replicates. Bar, 0.05 substitutions per site.

instructions.

Genomic DNA was extracted from cultures. The D1/D2 domain of the LSU rRNA (26S) gene was amplified using PCR with NL1 and NL4 primers (Kurtzman and Robnett, 1998). We used Basic Local Alignment Search Tool (BLAST) (Altschul, 1997) for pairwise sequence comparisons and to align the sequences with related species retrieved from GenBank. The MycoBank (https://www.mycobank.org/) database identified strain types for each species and close strains gene sequence were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/) for 26S. The phylogenetic tree was reconstructed using the neighboring joining method of MEGA 7.0 (Kumar *et al*., 2016). The evolutionary distance was calculated using the two-parameter model of Kimura (Kimura, 1980) the confidence level of the class was estimated through 1000 bootstrap replicates (Felsenstein, 1985).

Results and Discussion

Based on 26S gene sequencing, two phyla were represented *Ascomycota* and *Basidiomycota*. Strains YL1 and YL9 belong to the phylum *Ascomycota*, while strain 20g9-1 belongs to the phylum *Basidiomycota*. Photomicrographs were used to observe the budding of the three strains (Fig. 1). The detailed physiological, morphological, and characteristics of the strain are included in the strain descriptions.

The strain YL9 was most closely related to *Candida saitoana* (CBS 940^T, 100% sequence similarity), YL1 was most closely related to *Debaryomyces fabryi* (CBS 789^T, 100%), and 20g9-1 was most closely related to *Solicoccozyma terrea* (CBS 1895^T, 100%). The three strains formed robust phylogenetic clades with their most closely related species (Fig. 2 and Fig. 3).

Based on phylogenetic analysis, we concluded that strain YL1 is a member of the genus *Debaryomyces* in the family *Debaryomycetaceae*, strain YL9 is a member of the genus *Candida* in the family *Debaryomycetaceae*, and strain 20g9-1 is a member of the genus *Solicoccozyma* in the family *Piskurozymaceae*. Therefore, we describe three previously unreported strains found in Korea.

Description of *Candida saitoana* YL9

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, and white colored after 3 days of incubation on YNB agar at 10°C. In the API 20C AUX, strain YL9 is positive for glucose, 2-keto-D-gluco-

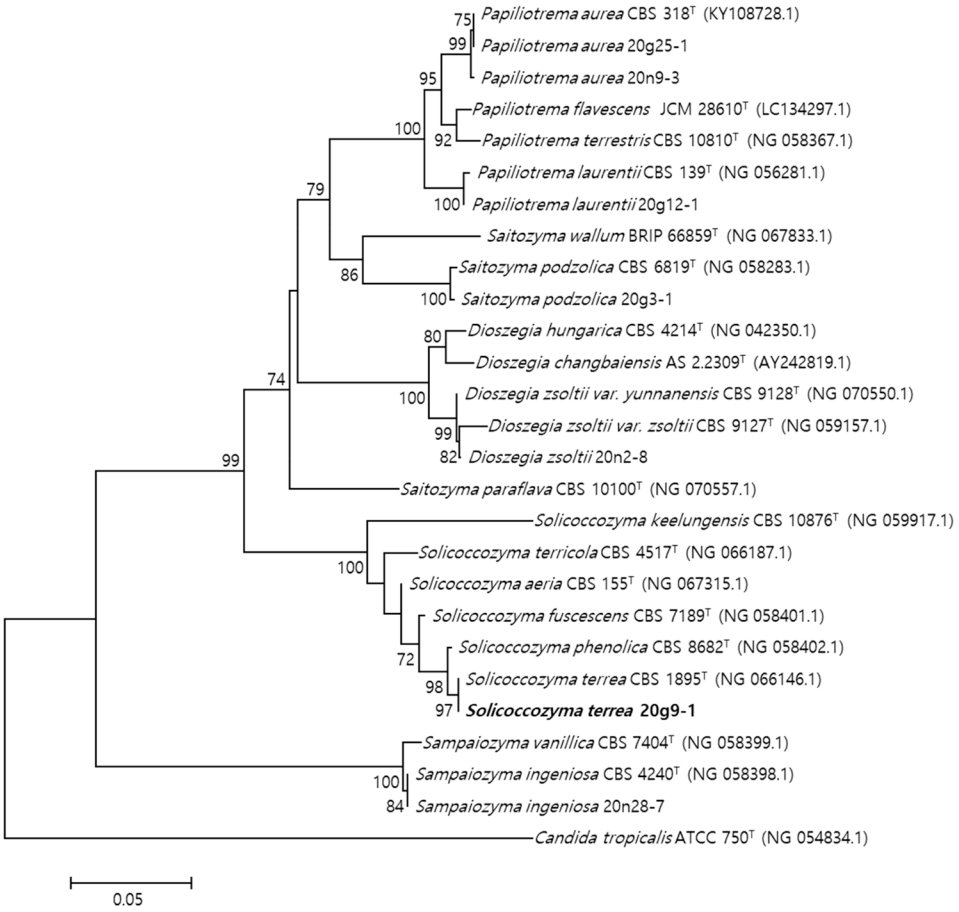

Fig. 3. Phylogenetic tree derived from neighbor-joining analysis based on the 26S rDNA gene sequences, showing the placement of strains 20g9-1 in the genus *Solicoccozyma*. *Candida tropicalis* ATCC 750^T was used as outgroup. Bootstrap values of above 70% are given at nodes based on 1,000 replicates. Bar, 0.05 substitutions per site.

nate (w), D-xylose, D-galactose, inositol, *N*-acetyl-D-glucosamine (w), D-cellobiose, D-lactose (bovine origin) (w), D-maltose, D-saccharose (sucrose), D-melezitose, and D-raffinose; but negative for glycerol, L-arabinose, adonitol, xylitol, D-sorbitol, *N*-methyl-D-glucoside, and D-trehalose. Strain YL9 (KACC 49811) was isolated from the soil collected in Gyeongju city, South Korea.

Description of *Debaryomyces fabryi* YL1

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, and beige colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX, strain YL1 is positive for glucose, 2-keto-D-gluconate, L-arabinose (w), D-xylose, adonitol, Xylitol (w), D-galactose (w), inositol (w), D-sorbitol (w), *N*-acetyl-D-glucosamine (w), D-cellobiose, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), and D-melezitose; but negative for glycerol, *N*-methyl-D-glucoside, D-trehalose, and D-raffinose. Strain YL1 (KACC 49810) was isolated from *Haemadipsa rjukjuana* collected in Gageodo Island, South Korea.

Description of *Solicoccozyma terrea* 20g9-1

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, and yellow-colored after 3 days of incubation on YM agar at 10°C. In the API 20C AUX, strain 20g9-1 is positive for glucose, 2-keto-D-gluconate, L-arabinose (w), D-xylose, inositol (w), D-sorbitol (w), and D-cellobiose; but negative for glycerol, adonitol, xylitol, D-galactose, *N*-methyl-D-glucoside, *N*-acetyl-D-glucosamine, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose, and D-raffinose. Strain 20g9-1 (KACC 48928) was isolated from *Haemadipsa rjukjuana* collected in Gageodo Island, South 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).

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Submitted: May 27, 2021
Revised: August 31, 2021
Accepted: August 31, 2021