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Research Note

Microbiological Characteristics and Physiological Functionality of New Records of Yeasts from Wild Flowers in Yokjido, Korea

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Abstract Two new yeast records, *Cryptococcus adeliensis* YJ19-2 and *Cryptococcus uzbekistanensis* YJ10-4 were screened from 60 yeasts strains that were isolated and identified from wild flowers in Yokjido, Gyeongsangnam-do, Korea. The morphological and cultural characteristics of the newly recorded yeasts and the physiological functionalities of the supernatants and cell-free extracts obtained from their cultures were investigated. The two newly recorded yeasts did not form ascospores and pseudomycelia. They also grew well in yeast extract-peptone-dextrose broth. *C. uzbekistanensis* YJ10-4 grew in a vitamin-free medium and was also tolerant to sugar and salt. Antihypertensive angiotensin I-converting enzyme inhibitory activity of the supernatant from *C. adeliensis* YJ19-2 was high (71.8%) and its cell-free extract also showed very high (81.2%) antidiabetic á-glucosidase inhibitory activity.

Keywords Morphological characteristics, New yeast records, Wild flowers, Yokjido

The GRAS strains of yeasts are traditionally used in the preparation of various Korean fermented foods including traditional rice wines and soy sauces [1-3]. Recently, some bioactive agents, such as antihypertensive angiotensin I-converting enzyme (ACE) inhibitors [4], ribonucleotides [5, 6] anti-angiogenic compounds [7], and antidementia β-secretase inhibitors [8], have been produced using *Saccharomyces cerevisiae*. However, almost all of the fungal strains were isolated only from soy sauce, traditional rice wine and their by-productts (*meju* or *nuruk*). Only a few researchers have isolated useful yeast strains from natural sources such as wild flowers, fruits, or cereals. It is necessary to isolate and characterize new yeast strains from natural sources and to screen them for their potential industrial use, as well as to further establish a yeast mycoflora map. We have previously isolated various new yeast strains from wild flowers in Daejeon city, Gejoksan [9, 10], Oseosan, Baekamsan [11, 12], coastal and inland areas [13], Gyeonggi-do and the Jeju island [14] in Korea. Furthermore, we have reported the production of the anti-gout xanthine oxidase inhibitor from one of these strains [14].

We have also isolated several yeast strains from wild flowers in Ulleungdo and Yokjido, Korea [15]. In this study, we describe screening of new records of yeasts from Yokjido, Gyeongsangnam-do, Korea, and evaluated their morphological characteristics and the physiological functions of the supernatants and cell-free extracts obtained from their cultures for the production of bioactive agents.

The morphological and cultural characteristics of the new records of yeasts were investigated according to theprotocols described in a previous paper [16]. Ascospore and pseudomycelium formation test were performed as follows: the new yeast strains were cultured in yeast extract-peptone-dextrose (YPD) medium at 30°C for 24 hr and, then cultured for 5 days in an ascospore medium containing potassium acetate 1%, yeast extract 0.1% and dextrose 0.05%. The ascospores were then observed by microscopy. Furthermore, the new yeast strains were cultured at 30°C for 7 days in YPD medium, yeast extract-malt extract medium, potato dextrose medium, and glucose-peptone-yeast extract agar containing dextrose 4%, peptone 0.5% and yeast extract 0.5%. Pseudomycelium formation was determined by observation of the shape of each cell in these cultures. The physiological functionality of supernatants

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and cell-free extracts from the two new yeast strains were investigated as follows: the newly recorded yeasts were cultured in the YPD medium at 30°C for 2 days. After centrifugation at 10,000 ×g for 15 min, the supernatants and cells were obtained. The cells were disrupted by vortexing with sonication, centrifuged at 12,000 ×g for 20 min. The mixture was filtered to obtain the cell-free extract and supernatants. The physiological functionalities of these cell-free extracts and supernatants were determined as described in previous papers [8, 17-19].

Screening for unrecorded yeasts. New records of yeasts from Yokjido, Korea were screened from 60 yeast strains from Yokjido by searching KERIS, PubMed, and other fungal taxonomy databases [9, 11, 20]. *Cryptococcus uzbekistanensis* YJ10-4 and *Cryptococcus adeliensis* YJ19-2, which were isolated from *Chrysanthemum coronarium* in Yokjido, Korea, were finally screened as two new records of yeasts.

Phylogenetic analyses on these two newly recorded yeasts were performed using MEGA 5.1. The phylogenetic tree was constructed based on the large-subunit rDNA D1/D2 domain sequence. *C. adeliensis* YJ19-2 was closely grouped to *C. adeliensis* JN400747.1, KC433766.1 and JX188117.1 (Fig. 1). *C. uzbekistanensis* YJ10-4 was closely grouped to *C. uzbekistanensis* KC006556.1 (Fig. 1). Finally, we reconfirmed the two newly recorded yeasts as *C. adeliensis* YJ19-2 and *C. uzbekistanensis* YJ10-4 and submitted their sequences to the GenBank database with accession nos. KJ410348 (*C. adeliensis* YJ19-2) and KJ410347 (*C. uzbekistanensis* YJ10-4).

C. uzbekistanensis was first isolated by Powel *et al.* [21] from an immunocompromised patient with lymphoma, and Yalcyn *et al.* [22] reported the molecular characterization and lipase profiling of *C. uzbekistanensis* isolated from environments contaminated with petroleum. Sipiczki [23] condueted a molecular taxonomic analysis of *C. adeliensis* isolated from *Verbascum* flowers, and Velázquez *et al.* [24] and Scorzetti *et al.* [25] reported xylanase production from *C. adeliensis*.

Characteristics of the newly recorded yeasts. The morphological and cultural characteristics of the two newly recorded yeasts are summarized in Table 1 and Fig. 2. *C. uzbekistanensis* YJ10-4 was round in shape, while *C. adeliensis* YJ19-2 was oval-shaped. Neither of the strains, formed ascospores or pseudomycelia.

The two newly recorded yeasts grew well in YPD medium, yeast extract-malt extract medium, and potato-dextrose broth. *C. uzbekistanensis* YJ10-4 grew in vitamin-free medium, was tolerant to sugar and salt and also grew in 50% glucose and 5% NaCl-YPD broth. The cell extracts dbtained from *C. adeliensis* YJ19-2 also showed urease activity.

We investigated the assimilation of various carbon sources by the two newly recorded yeasts using the API 20C AUX yeast identification kit (BioMériux, Marcy-I'Etoile, France) (Table 2). Both the yeasts utilized D-glucose, 2-keto-D-gluconate, L-arabinose, D-cellobiose, D-maltose, D-saccharose and D-raffinose. However, D-xylose, D-sorbitol,

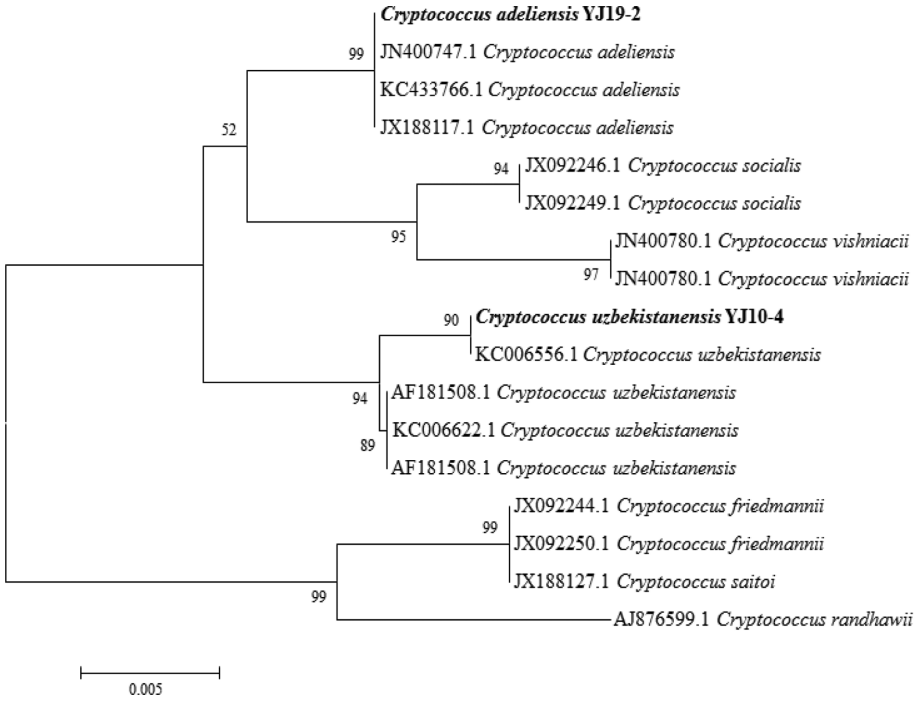

Fig. 1. Phylogenetic tree of *Cryptococcus adeliensis* YJ19-2 and *Cryptococcus uzbekistanensis* YJ10-4 based on nucleotide sequences of large subunit rDNA. The tree was generated by the neighbor-joining method using MEGA 5.1.

Table 1. Morphological and cultural characteristics of the newly reported yeast strains isolated from wild flowers in Yokjido, Gyeongsangnam-do, Korea

	Cryptococcus adeliensis YJ19-2	*Cryptococcus uzbekistanensis* YJ10-4
Morphological characteristics		
Shape	O	R
Vegetalle reproduction	B	B
Size (μm)	4.5 × 3.4	4.5 × 4.0
Ascospore	-	-
Pseudomycelium	-	-
Cultural and physiological characteristics		
Growth on YM	+	+++
Growth on YPD	+++	+++
Growth on PD	+	+
Color on YPD	PB	I
Growth on vitamine-free medium	-	+++
Growth in 50% Glucose-YPD	-	+
Growth in 5% NaCl-YPD	-	+
Growth in 20% NaCl-YPD	-	-
Groth in temperature/pH range	20~25°C/4	25~30°C/4~5
Urease activity	+	-

O, oval; R, round; B, budding; YM, yeast malt; YPD, yeast extract-peptone-dextrose; PD, peptone-dextrose; PB, pink beige; I, ivory.

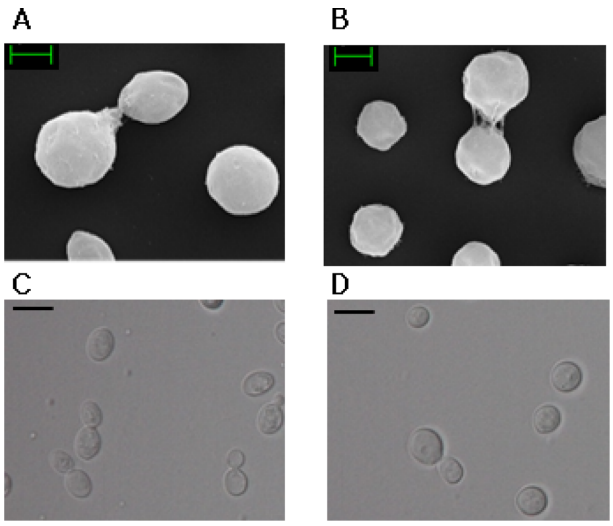

Fig. 2. Morphological characterization of *Cryptococcus uzbekistanensis* YJ10-4 (A, C) and *Cryptococcus adeliensis* YJ19-2 (B, D). A, B, Scanning electron microscopy; C, D, Optical microscopy (scale bars: A, B = 5 μm, C, D = 2 μm).

methyl-α-D-glucopyranoside and D-melezitose were only utilized by *C. uzbekistanensis* YJ10-4. In particular, *C. adeliensis* YJ19-2 utilized only seven kinds of carbon sources that were utilized by *C. uzbekistanensis* YJ10-4. Furthermore, the two newly recorded yeasts did not ferment all of the carbon sources tested, such as D-glucose, D-galactose, D-fructose, sucrose, maltose, lactose, mannose, raffinose, starch, cellobiose, and sorbitol (data not shown).

We compared the morphological and cultural characteristics of these new yeast strains with those of the known strains of the respective species. *C. adeliensis* from Antarctica [25], which was obtained from the CBS-KNAW Fungal Biodiversity Center was very similar to *C. adeliensis* YJ19-2 in that both the strains appeared globose to subglobose, measured (3~9) × (2~7) μm in 5% malt extract medium, and looked cream colored after being cultured for 30 days. *C. adeliensis* also did not form ascospores and pseudomycelium or true mycelium. Its optimal growth

Table 2. Carbon source assimilation by *Cryptococcus uzbekistanensis* YJ10-4 and *Cryptococcus adeliensis* YJ19-2

	Cryptococcus uzbekistanensis YJ10-4	*Cryptococcus adeliensis* YJ19-2
D-Glucose	+	+
D-Galactose	-	-
L-Arabinose	+	+
D-Xylose	+	-
D-Cellobiose	+	+
D-Lactose	-	-
D-Saccharose	+	+
D-Trehalose	-	-
D-Maltose	+	+
N-Acetyl-glucosamine	-	-
D-Melezitose	+	-
D-Raffinose	+	+
Methyl-α-D-glucopyranoside	+	-
2-Keto-D-gluconate	+	+
Glycerol	-	-
Adonitol	-	-
Inositol	-	-
D-Sorbitol	+	-
Xylitol	-	-

+, growth (assimilation); –, no growth (no assimilation).

Table 3. Physiological functionalities of the supernatants and cell-free extracts of the cultures of the newly reported yeast strains obtained from Yokjido, Korea

	Cryptococcus uzbekistanensis YJ10-4		*Cryptococcus adeliensis* YJ19-2	
	Supernatant	Cell-free extract	Supernatant	Cell-free extract
ACE inhibitory activity (%)	61.4 ± 0.7	29.5 ± 0.2	71.5 ± 0.4	37.2 ± 0.5
XOD inhibitory activity (%)	n.d	11.9 ± 0.1	n.d	n.d
SOD-like activity (%)	n.d	n.d	n.d	n.d
Antioxidant activity (%)	11.1 ± 0.6	3.0 ± 0.2	9.4 ± 0.1	9.3 ± 0.1
α-Glucosidase inhibitory activity (%)	35.7 ± 0.7	69.1 ± 0.5	36.4 ± 0.5	81.2 ± 0.1
Tyrosinase inhibitory activity (%)	39.2 ± 0.8	20.6 ± 0.5	22.1 ± 0.4	14.8 ± 0.7

ACE, angiotensin I-converting enzyme; n.d, not detected; XOD, xanthine oxidase; SOD, superoxide dismutase.

temperature ranged from 25~30°C and it did not grow in 50% glucose-YPD and 20% NaCl-YPD medium.

The first isolated *C. uzbekistanensis* strain was from s patient with lymphoma [21]; it was obtained from the CBS-KNAW Fungal Biodiversity Center, and the yeast extrac was very similar to the extract of the new yeast strain (*C. uzbekistanensis* YJ10-4) in terms of urease activity and growth of the yeast in 50% glucose-YPD. *C. uzbekistanensis* was round, approximately 5 μm in diameter, and asexually reproduced by budding. It did not form ascospores and pseudomycelia.

Physiological functionalities of the newly recorded yeasts. The physiological functionalities of the supernatants and cell-free extracts obtained from the newly recorded yeasts were investigated (Table 3). The antihypertensive ACE-inhibitory activities of supernatants from *C. adeliensis* YJ19-2 and *C. uzbekistanensis* YJ10-4 were 71.8% and 61.4%, respectively, and the inhibitory activities of the supernatants were also higher than those of the cell-free extracts (37.2% and 29.5%, respectively). These results were higher than those of the biomass from *S. cerevisiae* KCTC 7904 (42.1%), *Pichia anomala* (31.0%) [17], and its mutant (16.0%) [6]. Moreover, these results were similar to those for *Pichia anomala* KCCM 11473 (72.0%) and *Kluyveromyces fragilis* KCTC 7260 (68.9%) but lower than that for edible mushrooms, *Pleurotus cornucopiae* (78.0%) [16].

The antidiabetic α-glucosidase-inhibitory activity of the cell-free extract from *C. adeliensis* YJ19-2 was very high (81.2%). This inhibitory activity was higher than that of *Aspergillus oryzae* N157-1 (48.3%) [26], but was lower than that of *Pichia burtonii* Y257-7 (90.9%) isolated from traditional Korean fermented foods [18]. Tyrosinase-inhibitory activity in the *C. uzbekistanensis* YJ10-4 supernatant was 39.2%, and the other physiological functionalities were either not detected or weak (15%).

From the results described above, we conclude that antihypertensive ACE-inhibitory activity (71.8%) and antidiabetic α-glucosidase-inhibitory activity (81.2%) of a new yeast strain, *C. adeliensis* YJ19-2, were higher than those of other known yeasts and the other new yeast strain, *C. uzbekistanensis* YJ10-4. Therefore, *C. adeliensis* YJ19-2 would be very useful for food or medicinal applications as a potent bioactive compound-producing yeast

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