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Research Article

Isolation and Identification of Yeasts from Wild Flowers Collected around Jangseong Lake in Jeollanam-do, Republic of Korea, and Characterization of the Unrecorded Yeast *Bullera coprosmaensis*

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Abstract Several types of yeasts were isolated from wild flowers around Jangseong Lake in Jeollanam-do, Republic of Korea and identified by comparing the nucleotide sequences of the PCR amplicons for the D1/D2 variable domain of the 26S ribosomal DNA using Basic Local Alignment Search Tool (BLAST) analysis. In total, 60 strains from 18 species were isolated, and *Pseudozyma* spp. (27 strains), which included *Pseudozyma rugulosa* (7 strains) and *Pseudozyma aphidis* (6 strains), was dominant species. Among the 60 strains, *Bullera coprosmaensis* JS00600 represented a newly recorded yeast strain in Korea, and its microbiological characteristics were investigated. The yeast cell has an oval-shaped morphology measuring 1.4 × 1.7 μm in size. *Bullera coprosmaensis* JS00600 is an asporous yeast that exhibits no pseudomycelium formation. It grew well in vitamin-free medium as well as in yeast extract-malt extract broth and yeast extract-peptone-dextrose (YPD) broth, and it is halotolerant growing in 10% NaCl-containing YPD broth.

Keywords *Bullera coprosmaensis*, Characteristics, Jangseong Lake, Unrecorded yeast, Wild flowers

Generally, yeasts are heterotrophic with relatively simple nutritional needs and live as facultative anaerobe. Therefore, they should be wildly distributed in natural habitats such as in flowers and fruits, cereals, and plant debris in the surface area of soils. However, yeasts are exclusively isolated from various fermentation foods or their raw materials including meju, a traditional Korean fermented soybean [1]. It is necessary to isolate wild yeasts from natural sources and screen for the yeast mycoflora.

We have previously isolated wild yeasts from mountains [2] and fields [3], city gardens [4] and farm villages [5], islands [6], and coastal areas [7] and identified them with molecular biological tools. Furthermore, we have studied the production of bioactive compounds from these wild yeasts. Recently, we screened for new yeast records from among yeasts found in Ulleungdo [8], Yokjido [9], and in orchards and an arboretum [10] and their mycological characteristics were investigated.

In this study, we isolated and identified wild yeast strains from flowers around Jangseong Lake in Jeollanam-do, Republic of Korea. We also found an unrecorded yeast, and their mycological characteristics were investigated.

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MATERIALS AND METHODS

Isolation, identification of wild yeasts and screening of unrecorded yeast. Forty-eight wild flowers found lakeside around Jangseong Lake in Jeollanam-do, Republic of Korea were collected in August 2014, and several wild yeasts were isolated and identified as previously described [11].

Unrecorded yeasts in Korea were screened by searching

Table 1. Yeast species isolated from wild flowers of Jangseong lakeside in Jeollanam-do, Korea

No.	Putative species	Isolated No.	GenBank No.	Identity, No. (%)
1	*Bullera coprosmaensis*	JS00599	FN428908.1	620/626 (99)
		JS00600	AF444705.1	620/625 (99)
2	*Candida* sp.	JS00602	JN936885.1	446/447 (99)
3	*Cryptococcus flavescens*	JS00612	FJ743610.1	613/618 (99)
		JS00631	JQ964205.1	606/611 (99)
		JS00636	JQ964205.1	597/611 (98)
4	*Cryptococcus flavus*	JS00589	FN428891.1	596/601 (99)
		JS00629	EU177572.1	636/639 (99)
		JS00598	EU177572.1	636/640 (99)
5	*Cryptococcus laurentii*	JS00587	AM931019.1	613/628 (98)
6	*Hannaella oryzae*	JS00621	JQ754134.1	635/641 (99)
7	*Hanseniaspora opuntiae*	JS00632	KC111446.1	611/615 (99)
8	*Metschnikowia koreensis*	JS00584	KJ507292.1	507/513 (99)
		JS00585	KJ507292.1	548/553 (99)
		JS00588	KJ507292.1	541/552 (98)
		JS00590	KJ507292.1	511/517 (99)
		JS00610	KJ507292.1	545/553 (99)
		JS00611	KJ507292.1	545/552 (99)
		JS00614	KJ507292.1	545/553 (99)
		JS00595	KJ507292.1	539/552 (98)
9	*Metschnikowia* sp.	JS00623	JX257178.1	546/553 (99)
		JS00625	JX257178.1	544/553 (98)
10	*Pseudozyma antarctica*	JS00630	AB566343.1	645/648 (99)
11	*Pseudozyma aphidis*	JS00592	JN940520.1	643/649 (99)
		JS00601	HQ641278.1	478/485 (99)
		JS00628	KJ917976.1	581/592 (98)
		JS00641	HQ647300.1	523/534 (98)
		JS00642	JN940520.1	639/655 (98)
		JS00622	JX049426.1	401/409 (98)
12	*Pseudozyma rugulosa*	JS00593	JN940523.1	644/650 (99)
		JS00608	JN940523.1	643/648 (99)
		JS00616	JN940523.1	605/607 (99)
		JS00617	JN940523.1	645/648 (99)
		JS00618	JN940523.1	644/649 (99)
		JS00619	JN940523.1	607/608 (99)
		JS00624	JN940523.1	646/648 (99)
		JS00637	JN940523.1	605/607 (99)
		JS00596	JN940523.1	641/649 (99)
		JS00604	JN940523.1	646/648 (99)
		JS00613	JN940523.1	639/647 (99)
		JS00615	JN940523.1	644/648 (99)
		JS00620	JN940523.1	643/648 (99)
		JS00626	JN940523.1	643/648 (99)
		JS00627	JN940523.1	642/648 (99)
		JS00633	JN940523.1	642/648 (99)
		JS00634	JN940523.1	643/648 (99)
13	*Pseudozyma tsukubaensis*	JS00639	JQ219313.1	586/592 (99)
		JS00640	AB089373.1	586/594 (99)
		JS00643	JQ219313.1	615/623 (99)
14	*Rhodosporidium fluviale*	JS00591	KJ507302.1	579/581 (99)
		JS00638	KJ507302.1	567/572 (99)
		JS00594	KJ507302.1	497/502 (99)
		JS00597	KJ507302.1	610/614 (99)
15	*Rhodotorula graminis*	JS00603	EU563930.1	611/617 (99)
		JS00605	EU563930.1	608/617 (99)
		JS00607	EU563930.1	615/617 (99)
		JS00609	EU563930.1	613/617 (99)
16	*Rhodotorula minuta*	JS00606	AB026000.2	636/639 (99)
17	*Sirobasidium magnum*	JS00586	AF075475.1	564/577 (98)
18	*Yamadazyma mexicanum*	JS00635	JX188248.1	515/518 (99)

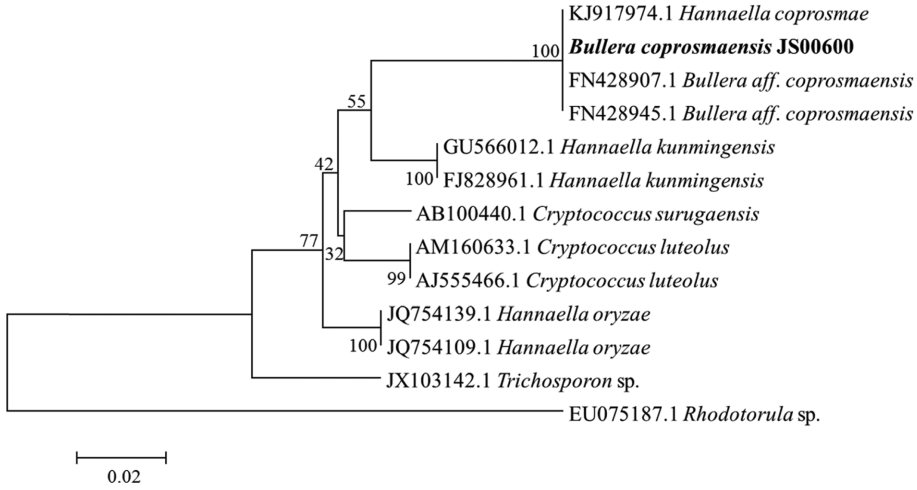

Fig. 1. Phylogenetic tree of the unrecorded yeast *Bullera coprosmaensis* JS00600 (NIBR No. KOSPFGC000129189) from this study based on the nucleotide sequences of large subunit 26S ribosomal DNA. The tree was generated by the neighbor-joining method, using MEGA v5.1.

KERIS, PubMed, and other fungal taxonomy databases.

Microbiological characteristics of the unrecorded yeast. The morphological and cultural characteristics of the unrecorded yeast were investigated as previously described [10, 12]. To determine whether the strain forms an ascospore, the unrecorded yeast was cultured in yeast extract-peptone-dextrose (YPD) medium at 30°C for 24 hr and then, cultured for 5 days in an ascospore-forming medium containing potassium acetate 1%, yeast extract 0.1%, and dextrose 0.05%. The strain was then observed with a microscope for ascospore formation. The unrecorded yeast was successively 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 glucose 4%, peptone 0.5%, and yeast extract 0.5%. Pseudomycelium formation was determined by observing the shape of cell in the cultures.

For scanning electron microscopy (SEM), JS00600 strain was cultured in YPD medium and then kept in 20% glycerol stock. The stock was diluted using a 0.05 M carcodylate buffer. The diluted solution was centrifuged at 1,300 rpm for 1 min to obtain yeast cell pellet. The pellet was used for fixation. The strain was also cultured in potato-dextrose-broth medium at shaking speed of 150 rpm in darkness at 30°C for 48 hr. The sample were fixed in 2.5% paraformaldehyde-glutaraldehyde buffer with 0.05 M phosphate (pH 7.2) for 2 hr, washed using the carcodylate buffer, post-fixed in 1% osmium tetroxide in the same buffer for 1 hr, and washed again using the same buffer, dehydrated in graded ethanol followed by isoamyl acetate, and then dried under a fume hood. Finally, the samples were covered in gold in a sputter coater and observed with the Hitachi S4700 field emission scanning electron microscope (Hitachi, Tokyo, Japan).

The physiological functionalities of the supernatants and cell-free extracts from the unrecorded yeast were determined as follows: the unrecorded yeast was cultured in YPD medium at 30°C for 2 days. After centrifugation at 10,000 ×g for 15 min, the supernatants and cells were separated. The cells were disrupted by vortexing with sonication and centrifuged at 12,000 ×g for 20 min. The mixture was filtered to obtain cell-free extracts and supernatants. The physiological functionalities of the cell-free extracts and supernatants

Table 2. Morphological and cultural characteristics of the unrecorded yeast *Bullera coprosmaensis* JS00600

Characteristic		*Bullera coprosmaensis* JS00600
Morphological characteristics		
Shape		O
Vegetative reproduction		B
Size (μm)		1.4 × 1.7
Ascospore		–
Pseudomycelium		–
Cultural and physiological characteristics		
Growth on YM		+++
YPD		+++
PD		+++
Vitamin-free medium		+++
Color on YPD		C
Osmotolerance		
Glucose (%)	5	++
	10	++
	20	+
	50	–
NaCl (%)	1	++
	5	+
	10	+
	20	–

O, oval-shaped; B, budding; +++, very good growth; ++ or +, good growth; –, no growth; C, cream color; YM, yeast extract-malt extract medium; YPD, yeast extract-peptone-dextrose broth; PD, potato-dextrose medium.

were determined as previously described [6, 11, 12].

Statistical analysis. Each experiment was performed at least three times, and all quantitative data are expressed as the mean ± SD values.

RESULTS AND DISCUSSION

Isolation and identification of yeasts from wild flowers around Jangseong Lake in Jeollanam-do, Republic of Korea. Sixty yeast strains belonging to 18 species were isolated from 48 kinds of blossoms found lakeside around Jangseong Lake during early August in 2014 (Table 1). Among them, the *Pseudozyma* spp. including 17 strains of *Pseudozyma rugulosa*, 6 strains of *Pseudozyma aphidis*, and 3 strains of *Pseudozyma tsukubaensis* were dominant species.

Screening of the unrecorded yeast, *Bullera coprosmaensis* JS00600. The unrecorded yeast *Bullera coprosmaensis* JS00600 (NIBR No. KOSPFGC000129189) was found among the sixty yeast strains isolated in this study. *B. coprosmaensis* JS00600 was isolated from *Plantago asiatica* which was in full bloom on August 4, 2014.

A phylogenetic tree for the unrecorded yeast *B. coprosmaensis* JS00600 was constructed based on the large-subunit rDNA D1/D2 domain sequence using the MEGA 5.1 program. *B. coprosmaensis* JS00600 was closely grouped to *B.* aff. *coprosmaensis* FN428907.1 (Fig. 1). Therefore, we reconfirmed the newly recorded yeast as *B. coprosmaensis* JS00600 and submitted its sequence to the GenBank database with the accession number KT277522.

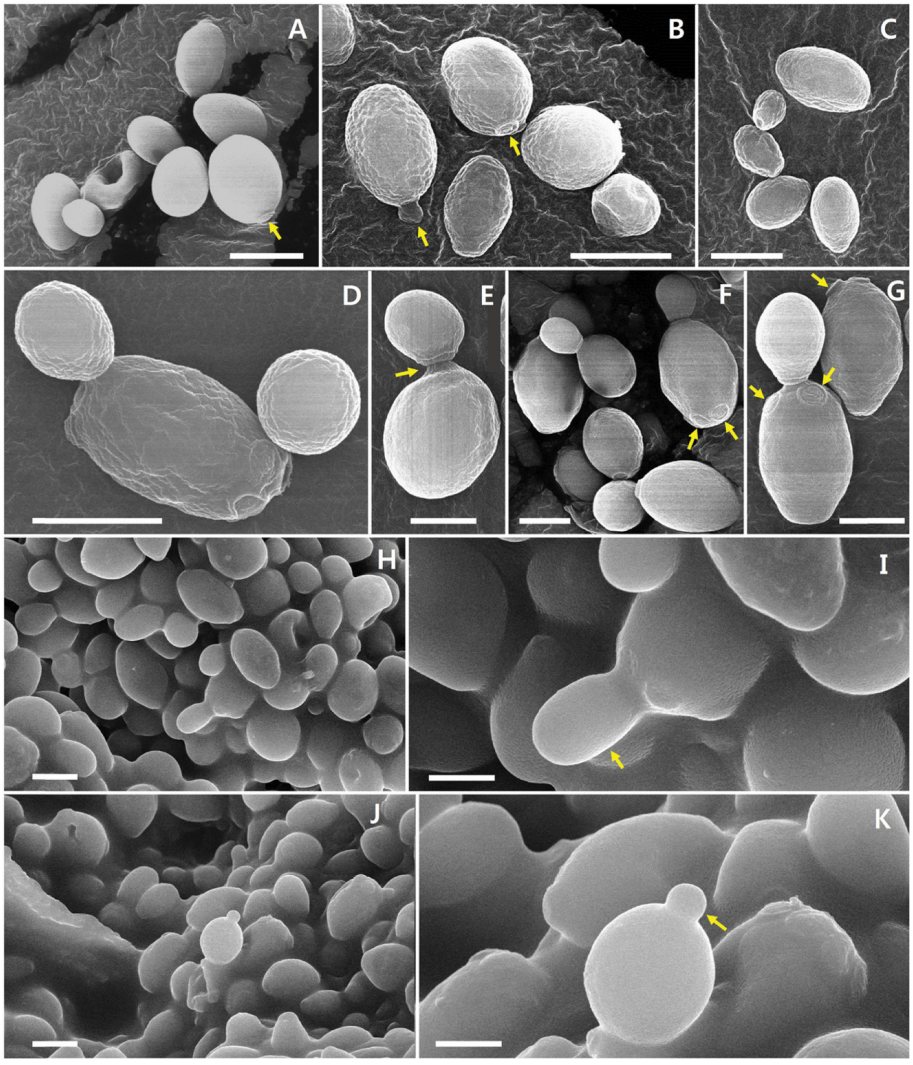

Fig. 2. Scanning electron microscopy (SEM) of vegetative cells of *Bullera coprosmaensis* JS00600 strain in different media and conditions (A~G and H~K, photos from two samples cultured in potato-dextrose-broth and yeast extract-peptone-dextrose [diluted from glycerol stock stored at −80°C], respectively). A~C, Typical cell shapes of JS00600; D, E, Cell shapes and budding forms with age barrier (arrow); A, B, F, G, Budding form and cells budding off daughter cells, and scars (arrows); H, J, Cell shapes and budding forms; I, K, Their magnified forms and cells budding off daughter cell (arrows) (scale bars = 2 μm).

B. coprosmaensis was the first reported from the surface of plants materials collected in New Zealand [13]. Few studies on *B. coprosmaensis* have been done.

Characteristics of the unrecorded yeast *Bullera coprosmaensis* JS00600. The morphological and cultural characteristics of *B. coprosmaensis* JS00600 are presented in Table 2 and Fig. 2. *B. coprosmaensis* JS00600 has an oval shaped morphology and a budding system for vegetative reproduction. Fig. 1 shows the SEM of JS00600 strain in different media and conditions. The typical shapes of vegetative cells of JS00600 were ellipsoidal to oval, showing commonly single cell forms and budding system. The strain did not form an ascospore and pseudomycelium. *B. coprosmaensis* JS00600 grew well in YPD medium, yeast extract-malt extract medium, and vitamin-free medium.

The osmotolerance of *B. coprosmaensis* JS00600 against glucose and NaCl were investigated. *B. coprosmaensis* JS00600 grew well in YPD medium containing 20% glucose and 10% NaCl, respectively. Few studies on halophilic yeasts have been done except for *Zygosaccharomyces rouxii* from soybeans [14] and halotolerant protease-producing *Saccharomyces lipolytica* [15] and *Hansenula polymorpha* S-9 from traditional Meju in a previous study [14, 16]. It is generally known that halophilic microorganisms produce halotolerant enzymes with some advantages such as preventing microbial contamination in the enzyme industry and enhancing the flavor of salted foods during aging. Therefore, *B. coprosmaensis* JS00600 in this study should be very useful in preparing halotolerant enzymes and bioactive compounds for the food and medical industry.

Table 3 shows the resistance of the unrecorded yeast *B. coprosmaensis* JS00600 to heavy metals and cycloheximide.

Table 3. Resistance to heavy metals and chemicals of the unrecorded yeast, *Bullera coprosmaensis* JS00600

Metals and chemicals		Growth
$HgCl_2$, $CuSO_4$, $AgNO_3$, $Na_2S_2O_5$	400 ppm	–
$Pb(NO)_2$	400 ppm	++
	800 ppm	+
$C_2H_3O_2Li$	400 ppm	++
	800 ppm	+
Sodium alginate	400 ppm	+
Cycloheximide	50 ppm	–
Ethanol	5%	–

++, very good growth; +, good growth; and –, no growth on yeast extract-peptone-dextrose medium.

B. coprosmaensis JS00600 exhibited tolerance to 800 ppm Pb^{2+} and Li^{+}, whereas it showed growth inhibition for 400 ppm Hg^{2+}, Cu^{2+}, Ag^{+}, $Na_2S_2O_5$, and 50 ppm cycloheximide. Lee *et al.* [17] reported some *Saccharomyces* sp. and *Zygosaccharomyces* sp. from traditional *Meju* were resistant to 800 ppm Pb^{2+}.

Assimilation and fermentation on carbon sources. Assimilation and fermentation of *B. coprosmaensis* JS00600 were investigated with several kinds of sugars and sugar alcohol (Table 4). *B. coprosmaensis* JS00600 assimilated glucose, maltose, sucrose, raffinose and cellobiose in hexose. Additionally, arabinose, xylose in pentose and sorbitol, xylitol N-acetyl glucosamine and calcium 2-keto-gluconate were also assimilated. However, only glucose and galactose in sugars used in this study were fermented by *B. coprosmaensis* JS00600.

Physiological functionalities. To obtain data for application of *B. coprosmaensis* JS00600 in functional foods or the medicinal industry, we prepared supernatants and cell-free extracts of *B. coprosmaensis* JS00600, and then,

Table 4. Carbon source assimilation (fermentation) by unrecorded yeast, *Bullera coprosmaensis* JS00600

Carbon source assimilation (fermentation)	*Bullera coprosmaensis* JS00600
D-Glucose	+(+)
Glycerol	–
Calcium 2-keto-gluconate	+
L-Arabinose	+(–)
Xylose	+(–)
Adonitol	–
Xylitol	+(–)
D-Galactose	+(+)
Inositol	–
D-Sorbitol	+(–)
Methyl-αD-glucopyranoside	–
N-Acetyl-glucosamine	+
D-Cellobiose	+(–)
D-Lactose	–
D-Maltose	+(–)
D-Saccharose (sucrose)	+(–)
D-Trehalose	–
D-Melezitose	–
D-Raffinose	+(–)

+, good growth; and –, no growth on yeast extract-peptone-dextrose medium.

Table 5. Physiological functionalities of unrecorded yeast *Bullera coprosmaensis* JS00600

	ACE inhibitory activity (%)	α-Glucosidase inhibitory activity (%)	Antioxidant activity (%)	SOD-like activity (%)	XOD inhibitory activity (%)	Tyrosinase inhibitory activity (%)
Cell-free extract	75.0 ± 0	94.7 ± 0.2	n.d	n.d	8.9 ± 0.7	12.5 ± 0.4
Supernatant	n.d	46.0 ± 0.4	5.2 ± 0.4	n.d	n.d	18.6 ± 0

ACE, angiotensin I-converting enzyme; SOD, superoxide dismutase; XOD, xanthine oxidase; n.d, not detected or < 5%.

some of their physiological functionalities were determined (Table 5). The cell-free extracts exhibited a very high hypoglycemic α-glucosidase inhibitory activity of 94.7% and an antihypertensive angiotensin I-converting enzyme inhibitory activity of 75.0%. This α-glucosidase inhibitory activity was similar to that of *Pichia burtonii* (90.9%) in our previous paper [18]. *B. coprosmaensis* JS00600 newly recorded in this study should be useful in the production of bioactive anti-diabetic and anti-hypertensive agents.

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