

Characteristics of Two Unrecorded Yeasts from Wild Flowers in Ulleungdo, Korea

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ABSTRACT : Two unrecorded yeasts, *Meyerozyma caribbica* UL5-1 and *Pichia silvicola* UL6-1 were screened from 58 yeasts which were isolated from wild flowers in Ulleungdo in Gyeongsangbuk-do, Korea. The morphological and cultural characteristics of these unrecorded yeasts were investigated. Both yeasts were oval in shape and formed pseudomycelia. *P. silvicola* UL6-1 formed ascospore, but *M. UL5-1* did not. *P. silvicola* UL6-1 and *M. caribbica* UL5-1 also grew in vitamin-free medium and 5% NaCl-containing yeast extract-peptone-dextrose medium. The two unrecorded yeasts assimilated glucose, galactose, xylose, cellobiose, trehalose, glycerol and sorbitol, and also fermented glucose, fructose and mannose. The supernatant of both *M. caribbica* UL5-1 and *P. silvicola* UL6-1 showed high antihypertensive angiotensin I-converting enzyme inhibitory activity of 84.2% and 82.6%, respectively. Cell-free extract of *P. silvicola* UL6-1 also showed very high anti-diabetic α -glucosidase inhibitory activity (85.8%).

KEYWORDS : Characteristics, Ulleungdo, Unrecorded yeasts, Wild flowers

Yeast produce various bioactive agents [1-4] including an antihypertensive angiotensin I-converting enzyme inhibitor [5]. Most of the yeasts that produce these compounds have been isolated from fermented foods or their raw materials [3,4,6]. Recently, we isolated and identified various yeasts, including unrecorded yeasts, from wild flowers growing in cities [7,8], mountains [9], inland and coastal areas [10,11], and, islands, such as Jeju Island, Korea [12,13].

In a previous paper [13], we reported the diversity of yeasts present on wild flowers in Ulleungdo and Yokjido, Korea; we also reported other characteristics of the unrecorded yeasts isolated from Yokjido [14]. Here, we describe the mycological characteristics of the unrecorded yeasts isolated from Ulleungdo, Korea.

We investigated the morphological characteristics of the unrecorded yeasts using previously described methods [13]. The physiological functions of the yeasts were determined as follows. The selected unrecorded yeasts were cultured in yeast extract-peptone-dextrose (YPD) medium at 30°C for 2 d. After centrifugation at 10,000 × g for 15 min, supernatants and cells were obtained. The cells were disrupted by vortexing with sonication and then centrifuged at 12,000 × g for 20 min. Cell-free extract was obtained and the supernatant was used to determine the physiological functionalities of the yeasts.

Antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activity was assayed using the method published by Cushman and Cheung [15]. Antioxidant activity was assayed using the method of Lee *et al.* [4] using antioxidant activity (DPPH) as a substrate. Tyrosinase inhibitory activity was assayed by the method of Kim *et al.* [16]. Xanthine oxidase inhibitory activity and superoxide dismutase (SOD)-like activity were determined using a modification of a previously described method [9]. α -Glucosidase inhibitory activity was assayed using the method described Kang *et al.* [17] using *p*-nitrophenyl- β -D-glucopyranoside (PNPG) as a substrate.

The unrecorded yeasts were screened from 58 yeasts isolated in Ulleungdo by searching Keris, PubMed and other fungal taxonomy databases. The previously unrecorded yeasts were identified as *Meyerozyma caribbica* UL

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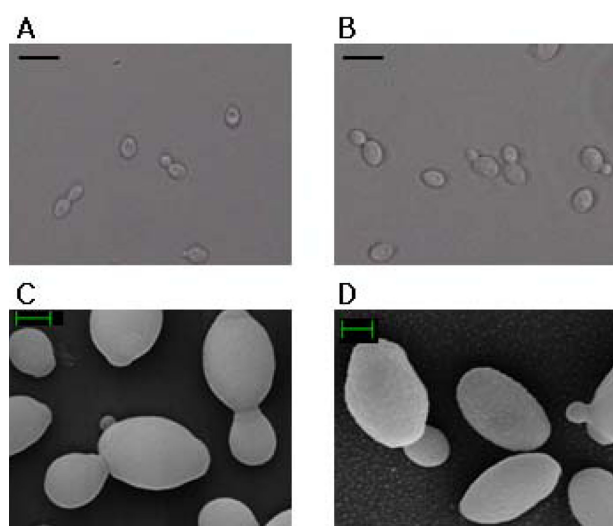
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Table 1. Microbiological and cultural characteristics of the newly reporting yeasts from wild flowers of Ulleungdo, Korea

	<i>Meyerozyma caribbica</i> UL5-1	<i>Pichia silvicola</i> UL6-1
Morphological characteristics		
Shape	O ¹⁾	O
Vegetative reproduction	B ²⁾	B
Size (μm)	2.6×2	3.4×2.6
Ascospore	-	+
Pseudomycelium	+	+
Cultural and physiological characteristics		
Growth on YM	++	+++
Growth on YPD	+++	+++
Growth on PD	++	+++
Color on YPD	W ³⁾	W
Growth on Vitamin-free medium	++	++
Growth in 50% Glucose-YPD	+	-
Growth in 5% NaCl-YPD	++	+
Growth in 20% NaCl-YPD	+	-
Growth in temp. / pH range	25-37°C / 4-5	20-30°C / 4-8
Urease activity	-	-

¹⁾O, Oval ²⁾B, Budding ³⁾W, White**Fig. 1.** Morphological characterization of *Meyerozyma caribbica* UL5-1 (A,C) and *Pichia silvicola* UL6-1 (B,D). A and B, Optical microscope (scale bar=1 μm); C and D, Scanning electron microscope (scale bars=5 μm).

5-1 and *Pichia silvicola* UL6-1; they were isolated from *Duchesnea chrysantha* and *Rubia akane* in Ulleungdo.

Table 2. Comparison of carbon sources assimilation between *Meyerozyma caribbica* UL5-1 and *Pichia silvicola* UL6-1

	<i>Meyerozyma caribbica</i> UL5-1	<i>Pichia silvicola</i> UL6-1
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. Comparison of carbon sources fermentation between *Meyerozyma caribbica* UL5-1 and *Pichia silvicola* UL6-1

Carbon sources	<i>Meyerozyma caribbica</i> UL5-1	<i>Pichia silvicola</i> UL6-1
D-glucose	+	+
Fructose	+	+
Mannose	+	+
Maltose	-	-
D-galactose	+	-
Sucrose	+	-
Lactose	-	-
Raffinose	+	-
Starch	-	-
Sorbitol	-	-
Cellobiose	-	+

*+, Fermentable, -; not fermentable

Mycological characteristics of the unrecorded yeasts

The morphological and cultural characteristics of the two unrecorded yeasts are summarized in Table 1 and Fig. 1. The two unrecorded yeasts were both oval in shape

Table 4. Physiological functionalities of unrecorded yeasts from Ulleungdo, Korea

	<i>Meyerozyma caribbica</i> UL5-1		<i>Pichia silvicola</i> UL6-1	
	Supernatant	Cell-free ext.	Supernatant	Cell-free ext.
ACE* inhibitory activity (%)	84.2(±0.5)	29.4(±0.8)	82.6(±0.7)	14.9(±0.7)
XOD inhibitory activity (%)	n.d**	7.6(±0.5)	n.d	14.6(±0.5)
SOD-like activity (%)	n.d	n.d	n.d	n.d
Antioxidant activity (%)	14.9(±0.6)	2.1(±0.1)	12.0(±0.1)	n.d
α-Glucosidase inhibitory activity (%)	n.d	66.4(±0.2)	n.d	85.8(±0.7)
Tyrosinase inhibitory activity (%)	19.5(±0.2)	19.9(±0.2)	28.4(±0.5)	7.4(±0.1)

*ACE, Angiotensin I-converting enzyme; XOD, Xanthine oxidase; SOD, Superoxide dismutase.

**n.d; not detected.

did not form ascospores and pseudomycelia. Both grew well in YPD, yeast extract-malt extract (YM) and potato-dextrose (PD) broth and also grew in vitamin-free medium. *M. caribbica* UL5-1 was found to be halophilic and thermotolerant, and grew well in 20% NaCl-YPD broth at 37°C. Both yeasts were negative for urease activity.

We previously reported about a thermotolerant yeast isolated from wild flowers [14], traditional *meju* [6], and halotolerant yeasts from Korean fermented soy sauce, *Doenjang* and *Gochujang* [18].

We investigated the assimilation and fermentation of the two unrecorded yeasts on various carbon sources, using previously described methods [18] (Table 2 and 3). *M. caribbica* UL5-1 utilized several types of hexose, pentose and sugar alcohol such as D-glucose, D-galactose, D-maltose, D-saccharose, D-trehalose, D-melezitose, D-raffinose, D-cellobiose, D-xylose, L-arabinose, glycerol, 2-keto-D-glucuronate, D-sorbitol, methyl-α-D-glucopyranoside and N-acetyl-glucosamine. However, it was not able to utilize D-lactose, xylitol, or inositol. *Pichia silvicola* UL6-1 utilized only D-glucose, D-galactose, D-trehalose, D-cellobiose, glycerol and D-sorbitol. Furthermore, *M. caribbica* UL5-1 fermented D-glucose, fructose, mannose, D-galactose, sucrose and raffinose, whereas *P. silvicola* UL6-1 fermented only D-glucose, fructose, mannose and cellobiose.

Physiological functionalities of the unrecorded yeasts

The physiological functionalities of the supernatants and cell-free extracts from the unrecorded yeasts were investigated (Table 4). The antihypertensive ACE inhibitory activities of supernatants from *M. caribbica* UL5-1 and *Pichia silvicola* UL6-1 were 84.2% and 82.6%, respectively, approximately 50% higher than those of their cell-free extracts.

These results were higher than those of *Saccharomyces*

cerevisiae KCTC 7904 (42.1%)[13], *Pichia anomala* (31.0%), *Pichia anomala* KCCM 11473 (72.0%)[19], or *Pleurotus cornucopiae* (78.0%)[20].

The anti-obesity α-glucosidase inhibitory activity of cell-free extract from *Pichia silvicola* UL6-1 was also very high 85.8%. These results were higher than those of *Aspergillus oryzae* N157-1 (48.3%)[17] or *Pichia burtonii* (90.9%)[3] isolated from Korean traditional fermented foods. Tyrosinase inhibitory activity and the other functionalities were either not detected or were very weak (15%).

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