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

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

|                  | Meyerozyma caribbica UL5-1 | Pichia silvicola UL6-1 |
|------------------|----------------------------|------------------------|
| **Morphological characteristics** |                          |                        |
| Shape            | O<sup>1</sup>              | O                      |
| Vegetative reproduction | B<sup>2</sup>    | B                      |
| Size (µm)        | 2.6×2                      | 3.4×2.6                |
| Ascospore        | -                          | +                      |
| Pseudomycelium   | +                          | +                      |
| **Cultural and physiological characteristics** |                          |                        |
| Growth on YM     | ++                         | +++                    |
| Growth on YPD    | +++                        | +++                    |
| 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  | -                          | -                      |

<sup>1</sup>O, Oval; <sup>2</sup>B, Budding; <sup>3</sup>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).

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

| Carbon sources     | Meyerozyma caribbica UL5-1 | Pichia silvicola UL6-1 |
|--------------------|-----------------------------|------------------------|
| D-glucose          | +*                         | +                      |
| D-galactose        | +                          | +                      |
| L-arabinose        | +                          | -                      |
| D-xylene           | +                          | +                      |
| 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            | -                          | -                      |

<sup>*</sup>+; 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     | Meyerozyma caribbica UL5-1 | Pichia silvicola UL6-1 |
|--------------------|-----------------------------|------------------------|
| D-glucose          | +*                         | +                      |
| Fructose           | +                          | +                      |
| Mannose            | +                          | +                      |
| Maltose            | -                          | -                      |
| D-galactose        | +                          | -                      |
| Sucrose            | +                          | -                      |
| Lactose            | -                          | -                      |
| Raffinose          | +                          | -                      |
| Starch             | -                          | -                      |
| Sorbitol           | -                          | -                      |
| Cellobiose         | -                          | +                      |

<sup>*</sup>+; 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...
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-xyllose, L-arabinose, glycerol, 2-keto-D-gluconate, 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, D-sorbitol, glyceral 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%), *P. 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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