Mycobiology 2014 June, 42(2): 198-202
http://dx.doi.org/10.5941/MYCO.2014.42.2.198
piSSN 1229-8093 • eISSN 2092-9123
© The Korean Society of Mycology

*Corresponding author
E-mail: biotech8@pcu.ac.kr

Received March 10, 2014
Revised April 7, 2014
Accepted June 10, 2014

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

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

Se-Hee Hyun and Jong-Soo Lee*
Department of Biomedicinal Science and Biotechnology, Paichai University, Daejeon 302-735, Korea

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 pseudomyecelia. 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 α-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-products (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 the protocols described in a previous paper [16]. Ascospore and pseudomyecelium 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%. Pseudomyecelium formation was determined by observation of the shape of each cell in these cultures. The physiological functionality of supernatants...
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] conducted 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 obtained 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 (BioMérieux, Marcy-l’Etoile, France) (Table 2). Both the yeasts utilized D-glucose, 2-keto-D-gluconate, L-arabinose, D-cellobiose, D-maltose, D-sorbitol, D-saccharose and D-raffinose. However, D-xylose, D-sorbitol,
In particular, \textit{C. adeliensis} YJ19-2 utilized only seven kinds of carbon sources that were utilized by \textit{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. \textit{C. adeliensis} from Antarctica [25], which was obtained from the CBS-KNAW Fungal Biodiversity Center was very similar to \textit{C. adeliensis} YJ19-2 in that both the strains appeared globose to sub-globose, measured (3–9) × (2–7) μm in 5% malt extract medium, and looked cream colored after being cultured for 30 days. \textit{C. adeliensis} also did not form ascospores and pseudomycelium or true mycelium. Its optimal growth

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

|                      | \textit{Cryptococcus adeliensis} YJ19-2 | \textit{Cryptococcus uzbekistanensis} YJ10-4 |
|----------------------|----------------------------------------|--------------------------------------------|
| **Morphological characteristics** |                                        |                                            |
| Shape                | O                                      | R                                          |
| Vegetative reproduction | B                                      | B                                          |
| Size (μm)            | 4.5 × 3                                 | 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 vitamin-free medium | -                                  | +++                                        |
| Growth in 50% Glucose-YPD | -                                    | +                                          |
| Growth in 5% NaCl-YPD | -                                      | +                                          |
| Growth in 20% NaCl-YPD | -                                      | -                                          |
| Growth 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.

### Table 2. Carbon source assimilation by \textit{Cryptococcus uzbekistanensis} YJ10-4 and \textit{Cryptococcus adeliensis} YJ19-2

|                     | \textit{Cryptococcus uzbekistanensis} YJ10-4 | \textit{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).
temperature ranged from 25–30°C and it did not grow in 50% glucose-YPD and 20% NaCl-YPD medium.

The first isolated \textit{C. uzbekistanensis} strain was from a patient with lymphoma [21]; it was obtained from the CBS-KNAW Fungal Biodiversity Center, and the yeast extract was very similar to the extract of the new yeast strain (\textit{C. uzbekistanensis} Y10-4) in terms of urease activity and growth of the yeast in 50% glucose-YPC. \textit{C. uzbekistanensis} was round, approximately 5 μm in diameter, and asexually reproduced by budding. It did not form ascospores and pseudomycelia.

\textbf{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 \textit{C. adeliensis} YJ19-2 and \textit{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 \textit{C. adeliensis} 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). Moreover, these results were similar to those for \textit{Pichia anomala} KCCM 11473 (72.0%) and \textit{Kluyveromyces fragilis} KCTC 7260 (68.9%) but lower than that for edible mushrooms, \textit{Pleurotus cornucopiae} (78.0%) [16].

The antidiabetic \(\alpha\)-glucosidase-inhibitory activity of the cell-free extract from \textit{C. adeliensis} YJ19-2 was very high (81.2%). This inhibitory activity was higher than that of \textit{Aspergillus oryzae} N157-1 (48.3%) [26], but was lower than that of \textit{Pichia burtonii} Y257-7 (90.9%) isolated from traditional Korean fermented foods [18]. Tyrosinase-inhibitory activity in the \textit{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 \(\alpha\)-glucosidase-inhibitory activity (81.2%) of a new yeast strain, \textit{C. adeliensis} YJ19-2, were higher than those of other known yeasts and the other new yeast strain, \textit{C. uzbekistanensis} YJ10-4. Therefore, \textit{C. adeliensis} YJ19-2 would be very useful for food or medicinal applications as a potent bioactive compound-producing yeast.

\textbf{ACKNOWLEDGEMENTS}

This study was funded by the project on survey and excavation of Korean indigenous species of NIBR under the Ministry of Environment, Republic of Korea.

\textbf{REFERENCES}

1. Jang IT, Kim YH, Yi SH, Lim SI, Lee JS. Screening of a new fibrinolytic substances-producing yeast. Kor J Mycol 2011; 39:227-8.
2. Kim JH, Kim NM, Lee JS. Physiological characteristics and ethanol fermentation of thermotolerant yeast \textit{Saccharomyces cerevisiae} OE-16 from traditional meju. Korean J Food Nutr 1999;12:490-5.
3. Lee JS, Choi YJ, Kwon SJ, Yoo JY, Chung DH. Screening and characterization of osmotolerant and gas-producing yeasts from traditional Doenjang and Kochujang. Food Sci Biotechnol 1996;5:54-8.
4. Kim JH, Lee DH, Jeong SC, Chung KS, Lee JS. Characterization of antihypertensive angiotensin 1-converting enzyme inhibitor from \textit{Saccharomyces cerevisiae}. J Microbiol Biotechnol 2004;14:1318-23.
5. Kim JH, Lee BH, Lee JS. Production of ribonucleotides by autolysis of \textit{Hansenula anomala} grown on Korean ginseng steaming effluent. J Biosci Bioeng 2002;93:318-21.
6. Lee JS, Hyun KW, Jeong SC, Kim JH, Choi YJ, Miguez CB. Production of ribonucleotides by autolysis of \textit{Pichia anomala} mutant and some physiological activities. Can J Microbiol 2004;50:489-92.
7. Jeong SC, Lee DH, Lee JS. Production and characterization of an anti-angiogenic agent from \textit{Saccharomyces cerevisiae} K-7. J Microbiol Biotechnol 2000;16:1904-11.
8. Lee DH, Lee DH, Lee JS. Characterization of a new antidementia \(\beta\)-secretase inhibitory peptide from \textit{Saccharomyces cerevisiae}. Enzyme Microb Technol 2007;42:83-8.
9. Kang MG, Hyun SH, Ryu JI, Min JH, Kim HK, Lee JS. Note on newly isolated yeasts from wild flowers in Daejeon city, Korea. Kor J Mycol 2012;40:174-6.
10. Min JH, Hyun SH, Kang MG, Lee HB, Kim CM, Kim HK, Lee JS. Isolation and identification of yeasts from wild flowers of Daejeon city and Chungcheongnam-do in Korea. Kor J Mycol 2012;40:141-4.

11. Hyun SH, Lee HB, Kim CM, Lee JS. New records of yeasts from wild flowers in coast near areas and inland areas, Korea. Kor J Mycol 2013;41:74-80.

12. Min JH, Ryu JJ, Kim HK, Lee JS. Isolation and identification of yeasts from wild flowers in Gyejoksan, Oseosan and Beakamsan of Korea. Kor J Mycol 2013;41:47-51.

13. Kim JH, Lee BH, Lee JS. Production of ribonucleotides by autolysis of Hansenula anomala grown on Korean ginseng steaming effluent. J Biosci Bioeng 2002;93:318-21.

14. Min JH, Lee HB, Lee JS, Kim HK. Identification of yeasts isolated from wild flowers collected in coast areas of Korea based on the 26S rDNA sequences. Kor J Mycol 2013;41:185-91.

15. Hyun SH, Mun HY, Lee HB, Kim HK, Lee JS. Isolation of yeasts from wild flowers in Gyonggi-do Province and Jeju Island in Korea and production of anti-gout xanthine oxidase inhibitor. Korean J Microbiol Biotechnol 2013;41:383-90.

16. Jang JH, Jeong SC, Kim JH, Lee YH, Ju YC, Lee JS. Characterisation of a new antihypertensive angiotensin 1-converting enzyme inhibitory peptide from Pleurotus cornucopiae. Food Chem 2011;127:412-8.

17. Kim NM, So SH, Lee SG, Song JE, Seo DS, Lee JS. Physiological functionality and enzyme activity of biomass from Pichia anomala grown on ginseng-steaming effluent. Mycobiology 2008;36:148-51.

18. Kim YH. Production and in-vivo anti-diabetic activity of α-glucosidase inhibitor from Pichia burtonii Y257-7 [dissertation]. Daejeon: University of Paichai; 2014.

19. Noro T, Oda Y, Miyase T, Ueno A, Fukushima S. Inhibitors of xanthine oxidase from the flowers and buds of Daphne genkwa. Chem Pharm Bull (Tokyo) 1983;31:3984-7.

20. Hyun SH, Lee HB, Lee JS. Characteristics of unrecorded yeasts, Rhodosporidium flaviale, Rhodosporidium paludigenum, Candida sp. 80-J-3 and Kluyveromyces thermotolerans isolated from wild flowers in Korea. Kor J Mycol 2013;41:181-4.

21. Powel MS, Alizadeh AA, Budvytiene I, Schaanen JM, Banaei N. First isolation of Cryptococcus uzbekistanensis from an immunocompromised patient with lymphoma. J Clin Microbiol 2012;50:1125-7.

22. Yalçýn HT, Corbacý C, Uçar FB. Molecular characterization and lipase profiling of the yeasts isolated from environments contaminated with petroleum. J Basic Microbiol 2013 May 26 [Epub]. http://dx.doi.org/10.1002/jobm.201300029.

23. Sipiczki M. Detection of yeast species also occurring in substrates associated with animals and identification of a novel dimorphic species in Verbascum flowers from Georgia. Antonie Van Leeuwenhoek 2013;103:567-76.

24. Velázquez E, del Villar M, Grondona I, Monte E, González-Villa T. Ultrastructural and chemotaxonomic analysis of a xylanolytic strain of Cryptococcus adeliensis isolated from sheep droppings in Spain. Arch Microbiol 2006;186:195-202.

25. Scorzetti G, Petrescu I, Yarrow D, Fell JW. Cryptococcus adeliensis sp. nov., a xylanase producing basidiomycetous yeast from Antarctica. Antonie Van Leeuwenhoek 2000;77:153-7.

26. Kang MG, Yi SH, Lee JS. Production and characterization of a new α-glucosidase inhibitory peptide from Aspergillus oryzae N159-1. Mycobiology 2013;41:149-54.