Endophytic Yeasts Colonize Roots of *Ulmus parvifolia* Jacq. and *Quercus salicina* Blume

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**Abstract**

**BACKGROUND:** Identification and characterization of endophytic yeasts inhabiting the roots of *Ulmus parvifolia* Jacq. and *Quercus salicina* Blume require biotechnological and culture-based techniques.

**METHODS AND RESULTS:** Homogenized *U. parvifolia* and *Q. salicina* root samples were spread onto four types of agar medium containing antibiotics, L-sorbose, and Triton X-100. In total, 25 yeast strains were isolated and subjected to phylogenetic analysis based on their internal transcribed spacer region sequences. The results revealed that the yeast genera *Cyberlindnera* (12 isolates) and *Cryptococcus* (1 isolate) were associated with roots of *U. parvifolia*; and the genera *Rhodotorula* (8 isolates), *Trichosporon* (3 isolates), and *Kluyveromyces* (1 isolate) were associated with roots of *Q. salicina*. Additionally, a *Kluyveromyces* isolate produced a detectable level of bioethanol. The yeast strains reported herein may be used in industrial production of biosurfactants and bioethanol.

**CONCLUSION:** Our findings revealed that the endophytic yeast genera *Cyberlindnera* and *Cryptococcus* predominated in roots of *U. parvifolia*. Additionally, *Kluyveromyces* isolates produced a detectable level of bioethanol.

**Key words:** Endophytic yeast, *U. parvifolia*, *Q. salicina*, roots, ITS gene

**Introduction**

Yeasts are important in various industries and in traditional food fermentation (Botha, 2011; Deak, 2009; Fonseca and Inacio, 2006; Raspor and Zupan, 2006; Tamang and Fleet, 2009). In this study, we investigated the endophytic yeast taxa associated with roots of *Ulmus parvifolia* and *Quercus salicina*, and evaluated their bioethanol production. To our knowledge, only one previous study has conducted phylogenetic analysis of endophytic yeast isolates (Rosen and Kunjappu, 2013).

*U. parvifolia* and *Q. salicina* evergreen trees are mainly distributed in southern Korea and surrounding islands (Lee, 2006). *U. parvifolia* (*U. parvifolia* Jacq.) is of the Ulmaceae family and is found in deciduous broad-leaf forests in the middle-southern region of Korea and on Jeju Island. *U. parvifolia* is distributed from 100m to approximately 500m above sea level (asl) on Jeju Island and hill slopes in Gotjawal and Orum, where it forms evergreen broad-leaf forests (Lee, 1996; Lee, 2003). Stem bark of *U. parvifolia* has diuretic and expectorant...
properties, and leaves harvested during summer and autumn are used to treat edema and kidney/bladder stones (Lee & Park, 2011). *Q. salicina* Blume of the Fragaeeae family is an evergreen tree species distributed on Jeju and Ulleung Islands, as well as various southern islands (Lee, 2003). It is distributed from 100 m to approximately 700m asl on hill slopes in the Gotjawal region, where it forms evergreen broad-leaf forests. It is used as a diuretic, anti-inflammatory, and to treat kidney stones (Kim et al., 2008; Lee et al., 2014).

Ethanol production by the non-conventional yeast genus *Kluyveromyces* has been reported (Choi et al., 2008; Goshima et al., 2013; Kang et al., 2010). Few studies have investigated the endophytic yeast communities of plant roots, which provide various habitats for microbial communities.

In this study, we evaluated the endophytic yeast taxa associated with roots of *U. parvifolia* and *Q. salicina*.

**Materials and Methods**

**Yeast isolation from roots of *U. parvifolia* and *Q. salicina***

*U. parvifolia* and *Q. salicina* on Dongbaekdongsan (latitude, 33 30 26.5'; longitude, 126 43 31.1'; 179m asl), Seonheul, Jocheon, and Jeju Islands were identified and characterized by Lee (1996). In this study, root samples were aseptically collected from Dongbaekdongsan, Jeju, and Gotjawal using clean forceps and placed in clean plastic bags. Roots were processed according to Kim et al. (2016a, 2017). Plant samples with soil were transported to the laboratory in Uljin, and processed within 1 week. The washed root samples were placed in Falcon tubes (Falconf, Los Angeles, CA) containing 10 mL of potassium phosphate buffer (10mM), and homogenized using a hand homogenizer (T10 basic; IKA, Germany). Homogenized and diluted samples (1 mL) were plated on sterile agar media using a glass spreader and incubated at 25C for 25 days. The agar media used were as follows: DG18 agar (MB Cell, Seoul), DOB with CSM agar (MP Bio, CA, USA), SCG agar (MB Cell, Seoul), and GPY agar. These media were supplemented with antibiotics (100 mg/L chloramphenicol and streptomycin), 0.1% Triton X-100, and 0.4% sorbose (Kim et al., 2016a).

**Culture of yeast isolates**

Yeasts were cultured on agar media in square plates (245×245×25 mm, Nunc Bio-Assay Dish; Thermo Scientific, Roskilde, Denmark). All colonies from one or two plates were picked and cultured separately. In total, 25 isolates were transferred to fresh plates three times and then processed for sequencing of their internal transcribed spacer (ITS) regions (Kim et al., 2016a; 2017).

**ITS sequencing**

Phylogenetic identification was performed as reported previously (Kim et al., 2016a; Kim et al., 2017; White et al., 1990). The ITS regions of the yeast isolates were sequenced using a PRISM BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and an ABI Prism 3730XL DNA Analyzer (Applied Biosystems) by Macrogen Inc. (Seoul, Korea). The nucleotide sequences obtained were deposited in DDBJ/EMBL/GenBank under the following accession numbers: LC229700LC229712 (13 *U. parvifolia* isolates) and LC229713LC229724 (12 *Q. salicina* isolates).
Phylogenetic analysis of yeast isolates

Multiple alignments of ITS sequences were performed using the Clustal Omega software on the EMBL website (http://www.ebi.ac.uk/tools/msa/clustalo/). A BLAST sequence similarity search was performed to identify the GenBank sequence of the yeast strain most closely related to each isolate. Phylogenetic trees were constructed by the neighbor-joining method using MEGA5 software for Windows (Tamura et al., 2011), including bootstrap analyses based on 1,000 samples, and evolutionary distances were calculated by the Kimura two-parameter method (Saitou and Nei, 1987).

Bioethanol and biosurfactant production

Bioethanol production was measured after anaerobic culture of three ethanol-producing isolates for 72 h. Bioethanol was measured by NICEM (National Instrumentation Center for Environmental Management, Seoul, Korea) by high-performance liquid chromatography (Hcolumn, Dionex Ultimate3000, USA), using a refractive index detector (ERC, RefractoMAX520, Japan) and ultraviolet light at 210nm. Surface tension of yeast cultures was measured using a Du Nouy ring tensiometer (Sigma Model 700, KSV Instruments Ltd., Helsinki, Finland).

Results and Discussion

Endophytic yeasts were isolated from homogenized roots of *U. parvifolia* and *Q. salicina*, and subjected to phylogenetic analysis using their ITS sequences. In total, 25 yeast isolates were obtained. The genera *Cyberlindnera* (12 isolates) and *Rhodotorula* (8 isolates) predominated (Fig. 2a). This is to our knowledge the first study to identify yeasts associated with *U. parvifolia* and *Q. salicina* roots.

In a previous study, the dominant yeast genus from *Mankyua chejuense* roots was *Cyberlindnera* (140 isolates), followed by *Candida* (11 isolates) and *Kluyveromyces* (isolate). *Dendropaxax morbifera* roots harbored *Vanderwaltozyma* (49.3%, 40 isolates), *Cryptococcus* (49.3%, 40 isolates), and *Kluyveromyces* (1.2%, 1 isolate). Moreover, the *Kluyveromyces* isolate exhibited a high level of bioethanol production (Kim et al., 2017).
C. saturnus is distributed worldwide in freshwater, soil, and leaf litter (Kurtzman 2011). Rhodotorula mucilaginosa is a ubiquitous basidiomycetous yeast species found in plants, soil, and aquatic environments, as well as extreme environments such as uranium leachate (Sampaio 2011). Trichosporon porosum is present in soil, trees, and bat guano, but its utility in biotechnology, food, agriculture, and medicine is unknown (Sugita 2011).

The Kluyveromyces isolates in this study were similar to K. hubeiensis, colonies of which on GPY medium are wheat-colored, have a rough texture, a round undulated margin, and umbonate elevation. K. hubeiensis ferments ethanol by the simultaneous saccharification and fermentation process (Choi et al., 2008).

U. parvifolia and Q. salicina are found in subtropical regions, the vegetation of which comprises mainly broad-leaved evergreen trees. Such trees predominate in the Seonheul-ri forest area of Korea. We investigated bioethanol and biosurfactant production by the Cyberlindnera and Rhodotorula (Fig. 2) isolates. As shown in Table 1, Kluyveromyces showed a high level of bioethanol production. However, none of the isolates produced detectable levels of biosurfactants. In contrast, Aureobasidium pullulans strains from wild flowers produce several types of biosurfactant (Kim et al., 2016b).

In conclusion, we isolated yeasts from roots of U. parvifolia and Q. salicina growing at Gotjawal, in the east of Jeju Island. The genera Cyberlindnera and Rhodotorula predominated, and Kluyveromyces produced a detectable level of bioethanol. These yeast isolates may have biotechnological applications as biosurfactant and bioethanol producers.

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References

Botha, A. (2011). The importance and ecology of yeasts in soil. Soil Biology and Biochemistry, 43(1), 1-8.

Choi, G. W., Han M. H., & Kim, Y. (2008). Development of glucoamylase & simultaneous saccharification and fermentation process for high-yield bioethanol. KSBB Journal, 23(6), 499-503.

Deak, T. (2009). Ecology and biodiversity of yeasts with potential value in biotechnology. Yeast biotechnology: diversity and applications (eds. Satyanarayana, T., and Kunze, G.), pp. 151-168, Springer Science + Business Media B.V., Dordrecht.

Fonseca, A., and Inacio, J. (2006). Phylloplane yeasts. Biodiversity and ecophysiology of yeasts (ed. Rosa, C. A., and Peter, G.), pp. 263-301, Springer, Berlin.

Goshima, T., Tsuji M., Inoue, H., Yano, S., Hoshino, T., & Matsushika, A. (2013). Bioethanol production from lignocellulosi biomass by a novel Kluyveromyces marxianus strain. Biosciencem, Biotechnology, and Biochemistry, 77(7), 1505-1510.

Kang, H. W., Kim, Y., Park J. Y., Min J. H., & Choi, G. W. (2010). Development of thermostable fusant, CHY1612 for lignocellosic simultaneous saccharification and fermentation. KSBB Journal, 25(6), 565-571.

Kim, J. I., Kim, H. H., Kim, S. U., Lee, K. T., Ham, I. H., & Whang, W. K. (2008). Antioxidative compounds from Q. salicina Blume Stem. Archives of Pharmacal Research, 31(3), 274.

Kim J. S. Kim D. S. Jeon S. M., & Ko S. H. (2016). Yeasts associated with roots of the endemic plant Markyua chojuense. Korean Journal of Environmental Agriculture, 35(2), 137-142.

Kim, J. S. Lee, I. K. Kim, D. W., & Yun, B. S. (2016). Aureosurfactin and 3-deoxyaureosurfactin, novel biosurfactants produced by Aureobasidium pullulans L3-GPY. The Journal of Antibiotics, 69, 759-761.

Kim, J. S., Kim, D. S., & Ko, S. H. (2017). Yeasts in internal roots of the rare plant Dendropana morbifera. Journal of Korean Oil Chemists’ Society, 34(1), 33-40.

Kurtzman, C. P. (2011). The Yeasts, a Taxonomic Study. Lindnera Kurtzman, Robnett & Basehoar-Powers (2008). The ds., vol. 3, (eds. C. P. Kurtzman, J. W. Fell & T. Bookhout). pp. 521-543, Amsterdam, Elsevier.

Lee, H. J., & Park, S. N. (2011). Antioxidative effect and active component analysis of Q. salicina Blume extracts. Journal of the Society of Cosmetic Scientists of Korea, 37(2), 143-152.

Lee, J. H., Lee, D. H., Choi, H. J., Suyama, Y., Kondo, T., Isagi, Y., & Choi, B. H. (2014). The distribution and population status of Quercus myrsinifolia (Fagaceae) on the Korean Peninsula. Korean Journal of Plant Taxonomy, 44(3), 165-170.

Lee, T. B. (2003). Coloured flora of Korea, pp. 1-914, Hyangmunsa, Seoul, Korea.

Lee, Y. N. (2006). New Flora of Korea, Vol. II. Kyo-Hak
Lee, W. T. (1996). Coloured standard illustrations of Korean plants, p. 152, Academy Publishing Co., Seoul, Korea.

Rasporg, P., & Zupan, J. (2006). Yeast in extreme environments. Biodiversity and ecophysiology of yeasts (eds. Rosa, C. A. & Peter, G.), pp. 370-417, Springer, Berlin.

Rosen M. J., & Kunjappu J. T. (2012). Surfactants in biology. Surfactants and Interfacial Phenomena, pp. 471-501, John Wiley & Sons, Hoboken, NJ, USA.

Saitou N., & Nei M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4(4), 406-425.

Sampaio, J. P. (2011). The Yeasts, a Taxonomic Study. Rhodotorula Harrison (1928). 5th eds, Vol. 3, (eds. C. P. Kurtzman, J. W. Fell & T. Boekhout), pp. 1873-1927, New York, Elsevier.

Sugita, T. (2011). The Yeasts: a Taxonomic Study. Trichosporon Behrend (1890). 5th eds, Vol. 3, (eds. C. P. Kurtzman, J. W. Fell & T. Boekhout), pp. 2015-2061, San Diego, CA, Elsevier.

Tamang, J. P., & Fleet, G. H. (2009). Yeasts diversity in fermented foods and beverages. Yeast biotechnology: diversity and applications (eds. Satyanarayana, T. and Kunze, G.), pp. 169-198, Springer Science+Business Media B. V., Dordrecht.

Tamura, K. Peterson, D. Peterson, N. Stecher, G, Nei, M, & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28(10), 2731-2739.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplication and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications (eds. Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J.), pp. 315-322, Academic press, San Diego, USA.