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Short communication

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

Jong-Shik Kim[1*] and Dae-Shin Kim[2]

[1]Gyeongbuk Institute for Marine Bio-Industry, Department of Research and Development, Uljin 36315, Korea
[2]World Heritage Office, Jeju Special Self-Governing Provincial Government, Jeju 63341, Korea

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ORCID
Jong-Shik Kim
http://orcid.org/0000-002-8181-1707

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 ancgtibiotics, 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*; and the genera *Rhodotorula* (8 isolates), *Trichosporon* (3 isolates), and *Kluyveromyces* (1 isolate) predominated in roots of *Q. salicina*. Additionally, *Kluyveromyces* isolates produced a detectable level of bioethanol.

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

*Corresponding author: Jong-Shik Kim
Phone: +82-54-720-3451; Fax: +82-54-780-3469;
E-mail: jskim@gimb.or.kr

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 Fragaceae 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; Leeetal.,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 Dongbackdongsan (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 (Falcon, 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%lsorbose (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).

(a)

(b)

Fig. 1a. Photographs of *U. parvifolia* habitatin Seonhel Gotjawal (A), flower (B), fruits (C), and roots (D).
Fig. 1b. Photographs of *Q. salicina* habitat in Seonhel Gotjawal (A), flower (B), fruits (C), and roots (D).

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).

Table 1. Bioethanol productivity of yeasts associated with roots of *U. parvifolia* and *Q. salicina*

Yeast	Lacticacid	Aceticacid	Ethanol
	(ppm)		
Kluyveromyces sp. EY12114-3-47	499.33	147.66	82221.40
Cyberlindnera sp. EY01123-1-2	304.09	415.27	13854.97
Cyberlindnera sp. EY01124-3-53	301.25	453.96	15274.35

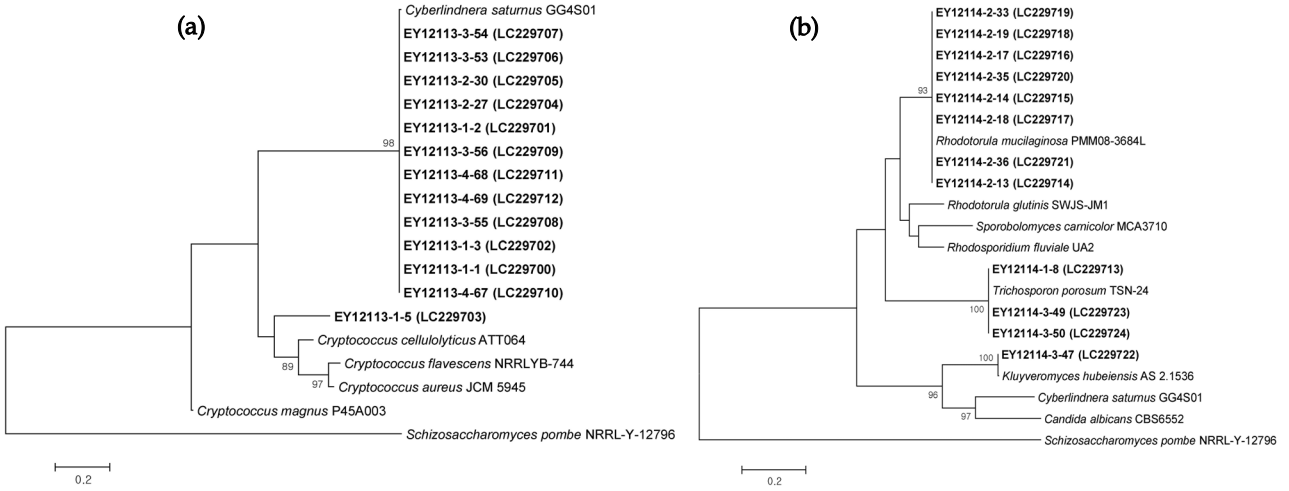

Fig. 2. Phylogenetic tree based on the ITS region sequences of yeast isolates from *U. parvifolia* (A) and *Q. salicina* (B) roots. Strains isolated in this study are indicated in bold. Numbers are confidence levels determined from 1,000 replicate bootstrap samplings.

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 1000 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 Noy 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* (1isolate). *Dendropanax 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 (Sugita2011).

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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