RESEARCH ARTICLE

Taxonomic Study of Indigenous *Lachancea* Species (Saccharomycetaceae, Saccharomycetales) and Description of Three Unrecorded Yeast Species in Korea

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ABSTRACT

The genus *Lachancea* holds significant potential for industrial applications in fermentation due to its high sugar tolerance. Globally, 11 species of *Lachancea* have been identified, with only one species previously reported in Korea. However, research on the genus *Lachancea* in Korea remains limited. In this study, a phylogenetic analysis was performed on 58 *Lachancea* strains, including those isolated during yeast diversity studies in Korea and strains preserved at the National Institute of Biological Resources (NIBR). This analysis revealed four yeast species, three of which had not been previously reported in Korea. Physiological and morphological characterization of these three newly identified species confirmed their identity as being consistent with their respective type strains, establishing them as indigenous to Korea. This paper provides a taxonomic description of these newly reported *Lachancea* species—*L. fermentati, L. kluyveri*, and *L. waltii*—isolated from natural environments in Korea.

Keywords: *Lachancea*, Unrecorded species, Yeast diversity

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INTRODUCTION

The genus *Lachancea* was proposed by Kurtzman (2003), following a reclassification of the "*Saccharomyces* complex" based on multigene sequence analyses [1]. The five species assigned to this novel genus are *Kluyveromyces thermotolerans*, *K. waltii*, *Saccharomyces kluyveri*, *Zygosaccharomyces cidri*, and *Z. fermentati*. Following the proposal of the *Lachancea* genus, several species have been added to it, including *L. meyersii* [2], *L. dasiensis* [3], *L. nothofagi* [4], *L. mirantina* [5], *L. lanzarotensis* [6], and *L. quebecensis* [7]. The 11 species currently comprising the genus *Lachancea* are characterized by the absence of true hyphae, 1–4 spherical ascospores, ability to ferment glucose and at least one other sugar, and an inability to assimilate nitrate [1,4]. The species of this genus are cosmopolitan and inhabit various niche, including soil, seawater, plants, animals, food, and even humans [8].

In recent years, the interest in application of non-*Saccharomyces* species in wine-making has increased, to generate higher quantities and greater diversities of metabolites [9]. In this regard, the most studied non-*Saccharomyces* species are *Hanseniaspora uvarum*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Schizosaccharomyces pombe*, and *Pichia kluyveri* [10]. *Lachancea thermotolerans* is useful in oenological applications [11], and its various commercial strains, such as strain Kt 421 (Viniflora® CONCERTO™; Chr. Hansen, Hoersholm, Denmark), strain Laktia (LAKTIA™; Lallemand, Montreal, Canada), are currently available. The application of *L. thermotolerans* in wine fermentation is being investigated to address the problem of increasing sugar content and decreasing acidity of grapes due to global warming, which affects the acidity of wine [12]. Research has been conducted on the oenological characteristics of *L. fermentati* and *L. lanzarotensis* as well [13].

In Korea, Hyun et al. [14] isolated *L. thermotolerans* (synonym *Kluyveromyces thermotolerans*) from wild flowers, which was an unrecorded yeast species. Since then, *L. thermotolerans* has been reported in several regions and from various sources, including plants [15], soil, and water [16] in Korea. In addition to *L. thermotolerans*, other species such as *L. cidri*, *L. fermentati*, and *L. kluyveri* have also been isolated in Korea. However, information on their taxonomic characteristics or preserved strains remains scarce.

During our investigation into the diversity of indigenous yeasts in Korea, we found that the genus *Lachancea* accounted for the highest number of isolates in environments with high sugar concentrations (10%) and the presence of alcohol (5%), highlighting the physiological traits of this genus.

In this study, we investigated yeast strains isolated from natural environments and those preserved at the National Institute of Biological Resources (NIBR). Our objective was to identify the species of the genus *Lachancea* indigenous to Korea and to describe the taxonomic characteristics of the three unrecorded species: *L. fermentati*, *L. kluyveri*, and *L. waltii*.

MATERIALS AND METHODS

Strain selection

A phylogenetic analysis was conducted on a total of 70 strains. These included 57 strains from the *Lachancea* genus isolated in this study, 1 strain preserved at the NIBR, and 12 strains selected from the data in the literature (Table 1). Considering the diversity of the isolated sources, five strains of three *Lachancea* species were selected as representative strains for morphological, physiological, and phylogenetic analyses.

Table 1. Strains and isolation sources of yeast species used to analyze the diversity within the *Lachancea* genus

Species	Strain	Source	Origin	NCBI Acc. No.	
				ITS	LSU
Lachancea cidri	CBS 5666	Plant (*Araucaria* sp.)	Chile	KY103940	KY108207
	NRRL Y-12634^T	Beverage (cider)	France	NR165978	NG055075
L. fermentati	NIBRFGC000500447	Air	Gongju, CN	OQ326709	OQ318459
	NIBRFGC000500449	Air	Jeonju, JB	OQ326710	OQ318460
	NIBRFGC000500450	Air	Jeonju, JB	PP588996	PP589417
	NIBRFGC000500487	Plant (*Rosa multiflora*)	Jeonju, JB	OQ326712	OQ318462
	NIBRFGC000509731	Air & insect	Incheon	OQ326721	OQ318471
	NIBRFGC000509732	Air & insect	Incheon	OQ326722	OQ318472
	NIBRFGC000509733	Air & insect	Incheon	OQ326723	OQ318473
	NIBRFGC000509734	Plant (rotten wood)	Gwangju	OQ326724	OQ318474
	NIBRFGC000509735	Plant (*Crataegus pinnatifida*)	Yangpyeong, GG	OQ326725	OQ318475
	NIBRFGC000509736	Plant (*Lindera obtusiloba*)	Yangpyeong, GG	OQ326726	OQ318476
	NIBRFGC000511204	Plant (*Euscaphis japonica*)	Jeju	PP589007	PP589504
	NIBRFGC000511205	Plant (*Aralia elata*)	Jeju	PP589009	PP589608
	NIBRFGC000511216	Plant (*Huperzia serrata*)	Jeju	PP589006	PP589503
	NIBRFGC000512338	Plant (*Liriope platyphylla*)	Gochang, JB	PP589003	PP589420
	NIBRFGC000512339	Plant (*Commelina communis*)	Gochang, JB	PP589010	PP589419
	NIBRFGC000512340	Plant (*Callicarpa japonica*)	Gochang, JB	PP589002	PP589418
	NIBRFGC000512341	Plant (*Persicaria filiformis*)	Gochang, JB	PP588997	PP589421
	NIBRFGC000512342	Plant (*Vicia unijuga*)	Gochang, JB	PP589005	PP589423
	NIBRFGC000512343	Plant (*Vicia venosa*var. *cuspidata*)	Gochang, JB	PP588994	PP589359
	NIBRFGC000512344	Plant (*Vicia venosa*var. *cuspidata*)	Gochang, JB	PP588995	PP589578
	NIBRFGC000512345	Plant (*Agrimonia pilosa*)	Gochang, JB	PP589000	PP589373
	NIBRFGC000512346	Plant (*Scutellaria indica*)	Gochang, JB	PP588998	PP589502
	NIBRFGC000512347	Plant (*Platycarya strobilacea*)	Gochang, JB	PP589004	PP589422
	NIBRFGC000512348	Plant (*Ligustrum obtusifolium*)	Gochang, JB	PP589001	PP589536
	NIBRFGC000512372	Air	Gwangyang, JN	PP588999	PP589607
	NIBRFGC000512373	Air	Gwangyang, JN	PP589008	PP589546
	NRRL Y-1559^T	Beverage (sediment of peppermint)	Unknown	NR130666	NG055076
L. kluyveri	NIBRFGC000511155	Plant (*Pinus koraiensis*)	Taebaek, GW	PP589027	PP589424
	NRRL Y-12651^T	Insect (*Drosophila pinicola*)	Unknown	NR138159	NG055066
L.meyersii	CBS 8951^T	Seawater	USA	AY645657	AY645656
	CBS 9924	Seawater	Bahamas	AY645659	AY645658
L. nothofagi	CBS 11399	Plant (*Nothofagus antarctica*)	Unknown	KY103991	KY108256
	CBS 11611^T	Plant (*Nothofagus* spp.)	Argentina	NR155331	NG058329
L. thermotolerans	NIBRFGC000500451	Air	Jeonju, JB	OQ326711	OQ318461
	NIBRFGC000500466	Plant (*Dendranthema boreale*)	Gongju, CN	MZ575158	MZ575124
	NIBRFGC000500467	Plant (*Smilax china*)	Gongju, CN	MZ575159	MZ575125
	NIBRFGC000500468	Plant (*Dendranthema zawadskii* var. *latilobum*)	Gongju, CN	MZ575157	MZ575123
	NIBRFGC000500488	Plant (*Rosa multiflora*)	Jeonju, JB	OQ326713	OQ318463
	NIBRFGC000506493	Mushroom (*Geastrum* sp.)	Sungju, GB	OQ326719	OQ318469
	NIBRFGC000509938	Plant (*Prunus padus*)	Yangpyeong, GG	OQ326727	OQ318477
	NIBRFGC000509939	Plant (*Lindera obtusiloba*)	Yangpyeong, GG	OQ326728	OQ318478
	NIBRFGC000509940	Plant (*Sorbus alnifolia*)	Yangpyeong, GG	OQ326729	OQ318479
	NIBRFGC000509941	Plant (*Rubus crataegifolius*)	Yangpyeong, GG	OQ326730	OQ318480
	NIBRFGC000509942	Plant (*Tilia mandshurica*)	Yangpyeong, GG	OQ326731	OQ318481

Table 1. (Continued)

Species	Strain	Source	Origin	NCBI Acc. No.	
				ITS	LSU
L. thermotolerans	NIBRFGC000500468	Plant (*Dendranthema zawadskii* var. *latilobum*)	Gongju, CN	MZ575157	MZ575123
	NIBRFGC000500488	Plant (*Rosa multiflora*)	Jeonju, JB	OQ326713	OQ318463
	NIBRFGC000506493	Mushroom (*Geastrum* sp.)	Sungju, GB	OQ326719	OQ318469
	NIBRFGC000509938	Plant (*Prunus padus*)	Yangpyeong, GG	OQ326727	OQ318477
	NIBRFGC000509939	Plant (*Lindera obtusiloba*)	Yangpyeong, GG	OQ326728	OQ318478
	NIBRFGC000509940	Plant (*Sorbus alnifolia*)	Yangpyeong, GG	OQ326729	OQ318479
	NIBRFGC000509941	Plant (*Rubus crataegifolius*)	Yangpyeong, GG	OQ326730	OQ318480
	NIBRFGC000509942	Plant (*Tilia mandshurica*)	Yangpyeong, GG	OQ326731	OQ318481
	NIBRFGC000509943	Plant (*Zanthoxylum schinifolium*)	Yangpyeong, GG	OQ326732	OQ318482
	NIBRFGC000509944	Plant (*Corylus heterophylla*)	Yangpyeong, GG	OQ326733	OQ318483
	NIBRFGC000509945	Air & insect	Incheon	OQ326734	OQ318484
	NIBRFGC000509946	Plant (rotten wood)	Gwangju	OQ326735	OQ318485
	NIBRFGC000511147	Plant (*Arisaema amurense* f. *serratum*)	Jeongsun, GW	PP589019	PP589507
	NIBRFGC000511148	Plant (*Rhamnus davurica*)	Jeongsun, GW	PP589021	PP589548
	NIBRFGC000511164	Plant (*Sorbus commixta*)	Yangpyeong, GG	PP589017	
	NIBRFGC000511191	Air & insect	Incheon	PP589020	PP589547
	NIBRFGC000511192	Air & insect	Incheon	PP589018	PP589505
	NIBRFGC000511206	Plant (*Dendropanax trifidus*)	Jeju	PP589023	PP589610
	NIBRFGC000511207	Plant (*Aralia elata*)	Jeju	PP589025	PP589613
	NIBRFGC000511217	Plant (*Hydrangea serrata* var. *acuminata*)	Jeju	PP589026	PP589614
	NIBRFGC000511218	Plant (*Callicarpa mollis*)	Jeju	PP589024	PP589611
	NIBRFGC000512349	Plant (*Nandina domestica*)	Gochang, JB	PP589014	PP589609
	NIBRFGC000512350	Plant (*Persicaria thunbergii*)	Gochang, JB	PP589012	PP589506
	NIBRFGC000512351	Plant (*Dendranthema boreale*)	Gochang, JB	PP589015	PP589365
	NIBRFGC000512352	Plant (*Lactuca indica*)	Gochang, JB	PP589022	PP589305
	NIBRFGC000512353	Plant (*Rhamnus yoshinoi*)	Gochang, JB	PP589016	PP589579
	NRRL Y-8284^{T}	Food (mirabelle-plum conserve)	Russia	NR111334	NG042626
L. waltii	NIBRFGC000136094	Plant (*Chaenomeles sinensis*)	Daejeon	OQ326708	OQ318458
	NIBRFGC000512379	Plant (*Aralia cordata* var. *continentalis*)	Ulleung, GB	PP589011	PP589442
	NRRL Y-8285^{T}	Plant (*Ilex integra*)	Japan	KY104011	NG055070
Kluyveromyces dobzhanskii	CBS 2104^{T}	Insect (*Drosophila pseudoobscura*)	USA	NR138156	KY107999
K. marxianus	NRRL Y-8281^{T}	Unknown	Unknown	NR111251	NG042627

The strains in bold were phenotypical characteristics examined. Type strains are denoted with the superscript 'T'.
CN, Chungcheongnam-do; GB, Gyeongsangbuk-do; GG, Gyeonggi-do; GW, Gangwon-do; JB, Jeollabuk-do; JN, Jeollanam-do.

DNA extraction, amplification, and phylogenetic analysis

Species identification was performed by phylogenetic analysis of internal transcribed spacer (ITS) region and domains 1 and 2 (D1/D2) of the large subunit (LSU) rRNA gene. DNA extraction, PCR amplification, and cycle sequencing were performed as previously described [17]. The sequences were edited and aligned using the Geneious® 9 program (Biomatters, New Zealand). A phylogenetic tree was constructed using maximum-likelihood analysis performed with RAxML [18], implemented on the CIPRES web portal [19]. The analysis employed the general time reversible (GTR) model with 1,000 bootstrap replicates.

Morphological and physiological tests

Five representative strains were examined for physiological (carbon source assimilation and oxidation) and biochemical properties following standard protocols [20]. Utilization of major carbon sources was determined using YT plates (Biolog Inc., Hayward, CA, USA). All experiments were conducted in triplicates.

Yeast strains were grown on yeast mold agar media (YMA) separately for 3 and 7 days each at 25℃; cell and colony morphology were observed using Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). Formation of hyphae and pseudohyphae were observed using Dalmau plates, after incubation for 2 weeks on cornmeal agar at 25℃. To induce sporulation, strains were incubated at 25℃ on four types of media—acetate agar (AA), cornmeal agar (CMA), 5% malt extract agar (MEA), and YMA.

RESULTS AND DISCUSSION

We conducted a taxonomic examination of 58 *Lachancea* strains isolated from samples in Korea and identified four distinct species: *L. fermentati*, *L. kluyveri*, *L. thermotolerans*, and *L. waltii* (Table 1). A phylogenetic tree was constructed using concatenated sequences of ITS region and D1/D2 regions of the LSU rRNA gene (Fig. 1). The analysis revealed that the four species formed well-defined phylogenetic clades, each supported by bootstrap values close to 100%, thereby strongly confirming their classification as distinct taxa. Additionally, all strains exhibited high sequence similarity to the type strains in both the ITS and D1/D2 regions, with similarities exceeding 99.5%.

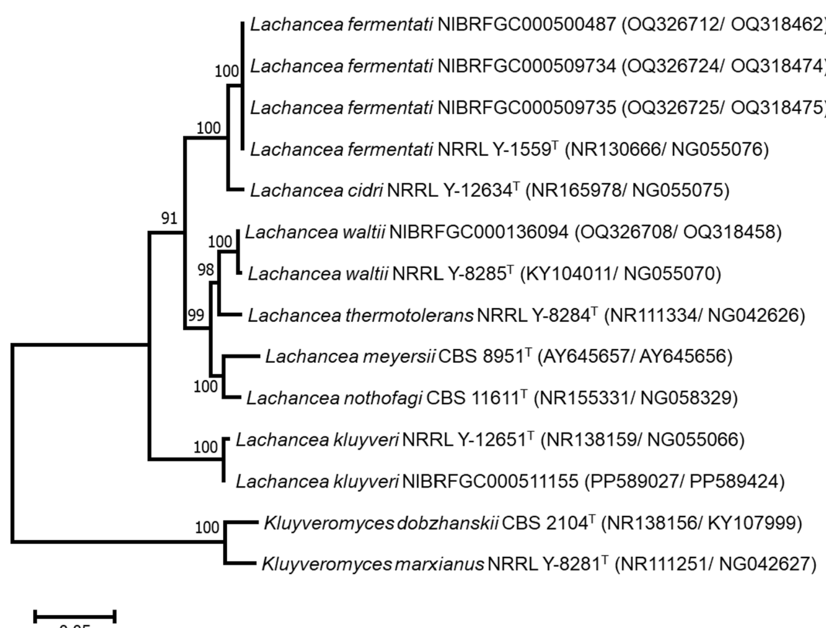

Fig. 1. Phylogenetic tree based on a maximum-likelihood analysis of combined internal transcribed spacer (ITS) and D1/D2 of the large subunit (LSU) rRNA gene sequences to determine the phylogenetic placement of *Lachancea* strains isolated in Korea and related species. Bootstrap values greater than 70% are indicated at the respective nodes. *Kluyveromyces dobzhanskii* and *K. marxianus* were used as outgroups. Strain numbers from culture collections follow the scientific names, and type strains are denoted with the superscript ‘T’. The scale bar represents 0.05 substitutions per site.

The strains also displayed morphological and physiological traits consistent with the type strains of the identified species. Among them, *L. fermentati* was unique in its ability to grow at 40℃, while *L. kluyveri* stood out for its capacity to utilize D-melibiose, a feature absent in the other three species. *L. thermotolerans* was the most frequently identified species, accounting for 23 of the 34 isolates, and was recovered from diverse substrates, including enrichment broths under trees, as well as plants (fruits, flowers, and tree stumps) and mushrooms. In contrast, *L. waltii* was distinguished by its inability to utilize turanose, maltose, or D-galactose (Table 2) and is considered a relatively rare species, with only a few isolates reported globally. Notably, even the CBS culture collection—one of the largest global repositories—contains only four preserved strains of *L. waltii*.

Table 2. Oxidation and assimilation of different carbon sources by three unregistered *Lachancea* yeast species in National Species List of Korea

Carbon sources	*L. fermentati*[a]	*L. kluyveri*[b]	*L. waltii*[c]
Oxidation			
water	-	-	-
acetic acid	-	-	-
formic acid	-	-	-
propionic acid	-	-	-
succinic acid	-	-	-
succinic acid mono-methyl ester	-	-	-
L-aspartic acid	-	-	-
L-glutamic acid	-	-	-
L-proline	-	-	-
D-gluconic acid	-	-	-
dextrin	-	-	-
inulin	-	-	-
D-cellobiose	-	-	-
gentiobiose	-	-	-
maltose	+	+	-
maltotriose	-	-	-
D-melezitose	v	-	-
D-melibiose	-	+	-
palatinose	+	-	-
D-raffinose	-	+	+
stachyose	-	+	+
sucrose	+	+	+
D-trehalose	+	-	-
turanose	+	+	-
N-acetyl-D-glucosamine	-	-	-
α-D-glucose	+	+	+
D-galactose	+	+	-
D-psicose	-	-	-
L-sorbose	-	-	-
salicin	-	-	-
D-mannitol	-	-	-
D-sorbitol	-	-	-
D-arabitol	-	-	-
xylitol	-	-	-

Table 2. (continued)

Carbon sources	*L. fermentati*[a]	*L. kluyveri*[b]	*L. waltii*[c]
Oxidation			
glycerol	-	-	-
tween 80	-	-	-
Assimilation			
water	-	-	-
fumaric acid	-	-	-
L-malic acid	-	-	-
succinic acid mono-methyl ester	-	-	-
bromo-succinic acid	-	-	-
L-glutamic acid	-	-	-
γ-amino-butyric acid	-	-	-
α-keto-glutaric acid	-	-	-
2- keto-D-gluconic acid	-	-	-
D-gluconic acid	-	-	-
dextrin	-	-	-
inulin	-	-	-
D-cellobiose	-	-	-
gentiobiose	-	-	-
maltose	+	+	-
maltotriose	-	-	-
D-melezitose	-	-	-
D-melibiose	-	+	-
palatinose	+	-	-
D-raffinose	v	+	+
stachyose	-	+	+
sucrose	+	+	+
D-trehalose	+	-	-
turanose	+	+	-
N-acetyl-D-glucosamine	-	-	-
D-glucosamine	-	-	-
α-D-glucose	+	+	+
D-galactose	+	+	-
D-psicose	-	-	-
L-rhamnose	-	-	-
L-sorbose	-	-	-
α-methyl-D-glucoside	+	-	-
β-methyl-D-glucoside	-	-	-
amygdalin	-	-	-
arbutin	-	-	-
salicin	-	-	-
maltitol	+	-	-
D-mannitol	-	-	-
D-sorbitol	-	-	-
adonitol	-	-	-
D-arabitol	-	-	-
xylitol	-	-	-
i-erythritol	-	-	-

Table 2. (continued)

Carbon sources	*L. fermentati*[a]	*L. kluyveri*[b]	*L. waltii*[c]
Assimilation			
glycerol	-	-	-
tween 80	-	-	-
L-arabinose	-	-	-
D-arabinose	-	-	-
D-ribose	-	-	-
D-xylose	-	-	-
succinic acid mono-methyl ester plus D-xylose	-	-	-
N-acetyl-L-glutamic acid plus D-xylose	-	-	-
quinic acid plus D-xylose	-	-	-
D-glucuronic acid plus D-xylose	-	-	-
dextrin plus D-xylose	-	-	-
α-D-lactose plus D-xylose	-	-	-
D-melibiose plus D-xylose	-	+	-
D-galactose plus D-xylose	+	+	-
m-inositol plus D-xylose	-	-	-
1,2-propanediol plus D-xylose	-	-	-
acetoin plus D-xylose	-	-	-

Examined strains: [a], NIBRFGC000500487, NIBRFGC000509734, and NIBRFGC000509735; [b], NIBRFGC000511155, [c], NIBRFGC000136094. Growth reactions: +, strong growth; w, weak growth; v, variable; -, no growth.

Of the four *Lachancea* species, three (*L. fermentati*, *L. kluyveri*, and *L. waltii*) have not been previously recorded in Korea. Therefore, here we describe the taxonomic characteristics of these three *Lachancea* species, newly added to the National Species List of Korea (NSLK).

Species description

Lachancea fermentati (H. Nagan.) Kurtzman, FEMS Yeast Res 4(3):240 (2003)

The cells are broadly ellipsoidal after 3 days on YM agar at 25℃, 2.6–5.3 × 3.6–7.3 µm, and usually occur singly or in pairs (Fig. 2). Budding is by multilateral on a narrow base. After 1 week on YM agar at 25℃, colonies are smooth, butyrous, entire to weakly filamentous margin, and tannish-white colored. After 2 weeks of culture on Dalmau plates at 25℃, branched pseudohyphae but not true hyphae are formed. Asci are persistent and each ascus forms one to four spherical ascospores. Ascospores were observed on YM, CMA and MEA after 2 weeks at 25℃.

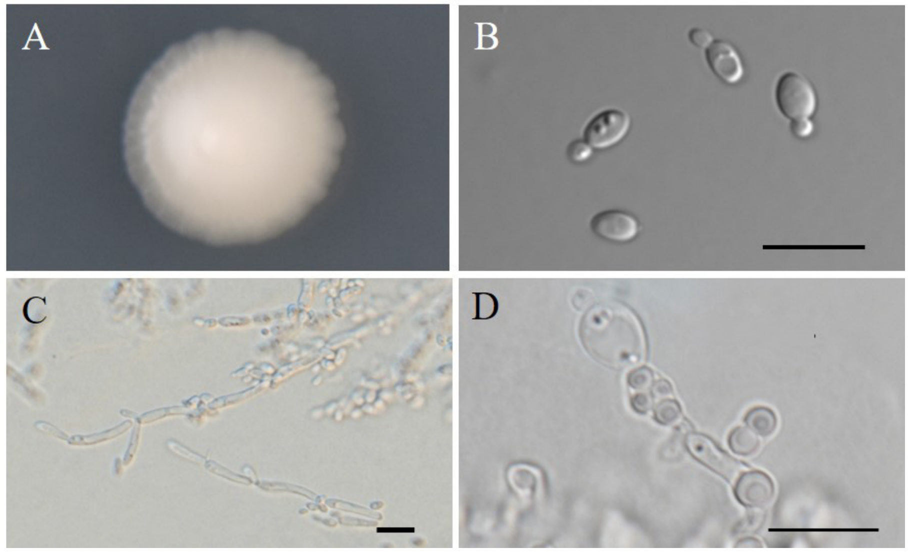

Fig. 2. Morphology of *Lachancea fermentati* NIBRFGC000500487. A: Colony on yeast mold (YM) agar 7 days at 25℃. B: Budding cells on YM agar 3 days at 25℃. C: Pseudohyphae on Dalmau plate with cornmeal agar for 2 weeks at 25℃. D: Ascospores on cornmeal agar 2 weeks at 25℃. Bars, 10 µm.

On the Biolog YT plate, the strains NIBRFGC000500487, NIBRFGC000509734 and NIBRFGC000509735 are positive for the oxidation of maltose, palatinose, sucrose, D-trehalose, turanose, α-D-glucose, and D-galactose. Assimilation of carbon compounds: maltose, palatinose, D-raffinose (variable), sucrose, D-trehalose, turanose, α-D-glucose, D-galactose, α-methyl-D-glucoside, maltitol, and D-galactose plus D-xylose (Table 2). Grow in 50% glucose, but not in 10% NaCl. All strains grow at 40°C, but not at 42°C. Growth occurs in the presence of 0.1% cycloheximide.

Examined strains: NIBRFGC000500487, Jeonju-si, Jeonbuk, Korea, 19 Oct. 2017, isolated from fruits of *Rosa multiflora*; NIBRFGC000509734, Gwangju, Korea, 09 Aug. 2022, isolated from tree stump; NIBRFGC000509735, Gapyeong-gun, Korea, 28 Jul. 2022, isolated from fruits of *Crataegus pinnatifida*.

Remarks: This species appears cosmopolitan and was isolated from diverse habitats such as plants, insects, and even beverages (tequila and kombucha). *L. fermentati* has been reported in the literature as *Saccharomyces montanus* and *Zygosaccharomyces fermentati* in Korea without a taxonomic description [21–23]. *Saccharomyces montanus* was isolated from *Quercus varialilis* [21] and fluid yogurt [22], and *Z. fermentati* was isolated from rotten potato [23]. In addition, one strain (CBS6772) isolated from a spoiled strawberry drink in Korea is being stored in CBS without reference.

Lachancea kluyveri (Phaff, M.W. Mill. & Shifrine) Kurtzman, FEMS Yeast Res 4(3):240 (2003)

The cells are globose to subglobose, 5.2–8.0 × 5.6–8.2 µm, occur singly or in pairs (Fig. 3). Cell division is by budding. Colonies are glossy or dull, cream colored to tan after 1 week on YM agar at 25°C. After 2 weeks of culture on Dalmau plates at 25°C, pseudohyphae may be formed, but true hyphae are not produced. Abundant ascosporulation was observed on acetate, cornmeal, 5% malt extract, YM agars after 3–5 days at 25°C. One to four spherical ascospores were formed in each ascus.

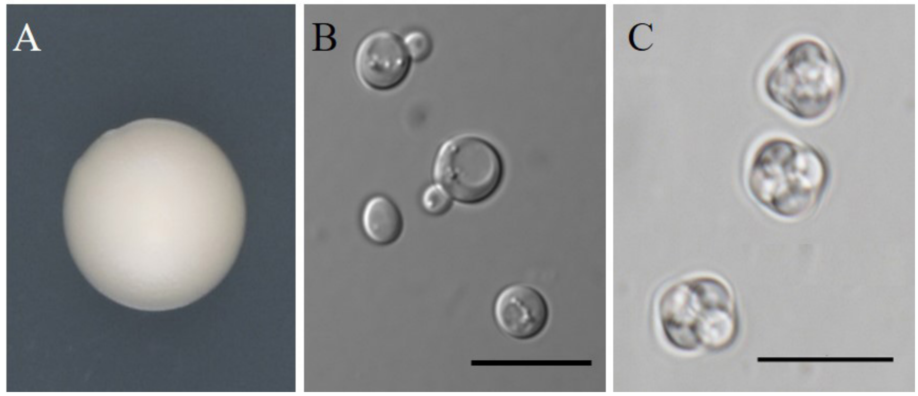

Fig. 3. Morphology of *Lachancea kluyveri* NIBRFGC000511155. A: Colony on yeast mold (YM) agar 7 days at 25℃. B: Budding cells on YM agar 3 days at 25℃. C: Ascospores on cornmeal agar 2 weeks at 25℃. Bars, 10 μm.

On the Biolog YT plate, the strain NIBRFGC000511155 is positive for the oxidation of maltose, maltotriose, D-melezitose, D-melibiose, palatinose, D-raffinose, stachyose, sucrose, D-trehalose, turanose, N-acetyl-D-glucosamine, α-D-glucose, and D-galactose. Assimilation of carbon compounds: maltose, D-melibiose, D-raffinose, stachyose, sucrose, turanose, α-D-glucose, D-galactose, D-melibiose plus D-xylose, and D-galactose plus D-xylose (Table 2). Grow in 50% glucose, but not in 10% NaCl. The maximum temperature for growth is 37°C. Sensitive to 0.01% cycloheximide.

Examined strain: NIBRFGC000511155, Jeongseon-gun, Korea, 14 Sep. 2022, isolated from the cone of *Pinus koraiensis*.

Remarks: The type strain of *L. kluyveri* was isolated from *Drosophila* sp. in the USA [24]. Strains of this species were mainly isolated from environmental samples such as soils, insects, and plants, and it exhibits a wide distribution, considering it was isolated from the USA, Sweden, Russia, and Spain. This species was reported in Korea as *Saccharomyces kluyveri* isolated from *Diospyros kaki* [25] and Meju [26], a raw material used to make Korean soy sauce.

Lachancea waltii (K. Kodama) Kurtzman, FEMS Yeast Res 4(3):240 (2003)

The cells are spherical, subglobose or broadly ellipsoidal after 3 days on YM agar at 25°C, 3.3–5.9 × 3.8–6.8 μm, occur singly or in pairs (Fig. 4). Multilateral budding on a narrow base. Colonies are butyrous, smooth, sometimes umbonate, glossy and cream colored with entire margin after 1 week on YM agar at 25°C. After 2 weeks of culture on Dalmau plates at 25°C, rudimentary pseudohyphae are occasionally formed while septated hyphae are absent. Ascospores are observed on acetate, cornmeal, 5% malt extract, YM agars after 2–4 weeks at 25°C. One to four spherical ascospores are formed from each ascus.

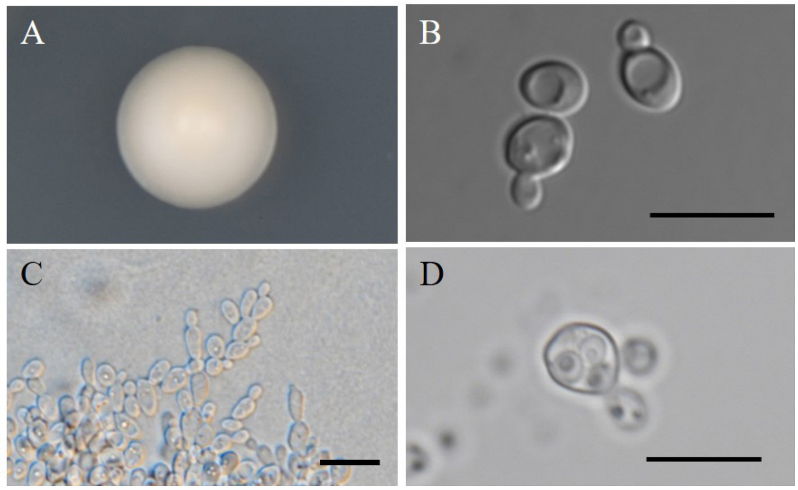

Fig. 4. Morphology of *Lachancea waltii* NIBRFGC000136094. A: Colony on yeast mold (YM) agar 7 days at 25℃. B: Budding cells on YM agar 3 days at 25℃. C: Pseudohyphae on Dalmau plate with cornmeal agar for 2 weeks at 25℃. D: Ascospores on cornmeal agar 2 weeks at 25℃. Bars, 10 µm.

On the Biolog YT plate, the strain NIBRFGC000136094 is positive for the oxidation of D-raffinose, stachyose, sucrose, and α-D-glucose (Table 2). Assimilation of carbon compounds: D-raffinose, stachyose, sucrose, and α-D-glucose. Grow in 50% glucose, but not in 10% NaCl. The strain grows 37°C, but not in 40°C. The strain is sensitive to 0.01% cycloheximide.

Examined strain: NIBRFGC000136094, Daejeon, Korea, 01 Sep. 2013, isolated from unripe fruits of *Chaenomeles sinensis*.

Remarks: *Lachancea waltii* has been isolated from Japan and Canada [27]. Through this study, it has been revealed that this species inhabits Korea. This species was mainly isolated from fruit, oak gall, and black knot of plants (*Ilex integra* and *Prunus virginiana*).

CONFLICT OF INTERESTS

The authors declare no competing interests.

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