Lachancea nothofagi sp. nov., a yeast associated with Nothofagus species in Patagonia, Argentina

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Six strains of a novel yeast species were isolated from Nothofagus species trees in native forests in Patagonia, Argentina. The strains were isolated from bark, fluxes and the ectomycorrhizospheric soil fraction of Nothofagus antarctica, Nothofagus nervosa and Nothofagus pumilio. Analysis of the D1/D2 large-subunit rDNA sequences indicated that the novel species belonged to the genus Lachancea and is closely related to Lachancea meyersii. The name Lachancea nothofagi sp. nov. is proposed to accommodate these strains. The type strain is UWOPS 99-807.3T (=CBS 11611T = NRRL Y-48670T).

The genus Lachancea was proposed by Kurtzman (2003) to accommodate ascomycetous species previously assigned to the genera Zygosaccharomyces, Kluyveromyces and Saccharomyces. Currently, seven species are known, characterized by glucose fermentation, absence of hyphae, 1–4 spherical ascospores, asexual reproduction by multilateral budding and the inability to assimilate nitrate (Kurtzman, 2003; Lee et al., 2009). Within the genus Lachancea, three subclades can be distinguished, one constituted by Lachancea thermotolerans (type species of the genus), Lachancea dasiensis, Lachancea waltii and Lachancea meyersii, the second containing Lachancea cidri and Lachancea fermentati, and the third represented by Lachancea kluyveri (Naumova et al., 2007). Lachancea species have been isolated worldwide in association with plants, plant products or plant-associated insects. Most of the strains have been isolated from the northern hemisphere (Bahamas, Canada, Cayman Islands, Finland, France, Japan, Russia, Spain, USA; Naumova et al., 2007; Lee et al., 2009).

During surveys of the yeast community associated with species of Nothofagus from native forests of Argentinean Patagonia, six glucose-fermenting yeast strains were isolated and assigned to the genus Lachancea based on growth characteristics (carbon assimilation pattern, absence of hyphae, spherical ascospores, multilateral budding and absence of growth on nitrate). Analysis of the D1/D2 domains of the large-subunit rDNA sequences indicated that these strains represent a distinct species closely related to L. meyersii. The name Lachancea nothofagi sp. nov. is proposed to accommodate these strains given their apparent association with Patagonian Nothofagus trees.

Strains UWOPS 99-807.3T and UWOPS 99-808.4 were isolated from sap fluxes of Nothofagus species in 1999 (Table 1) in a forest located at the north end of Mascardí Lake in Nahuel Huapi National Park, where both the tree species Nothofagus dombeyi and Nothofagus antarctica are present. However, the trees were not identified at the time. Sap flux material was suspended in sterile water and streak-inoculated onto YM agar (1% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, 2% agar) supplemented with 100 μg chloramphenicol ml⁻¹.

Three strains were isolated from bark of Nothofagus species in forests where Nothofagus was the predominant tree genus at three different locations in Patagonia (Argentina) in 2007 and 2008 (Table 1): strain CRUB 1792 from N. antarctica (G. Forster) Oerst., at Guillermo Lake in Nahuel Huapi National Park (where this tree species coexists with N. dombeyi and Austrocedrus chilensis); strain CRUB 1793 from N. pumilio (Poeppl. et Endl.) Krasser in a forest from Cerro Moreno in Los Glaciares National Park (also presented Embothrium coccineum and Drimys winteri); and CRUB 1794 from Nothofagus nervosa (Phil.) Dim. et Mil. in the Yuco area of Lanín National Park (mixed forest with Nothofagus obliqua and N. dombeyi). These three strains were isolated using a selective medium containing 1% (v/v) raffinose, 8% (v/v) ethanol and 0.67% (v/v) yeast nitrogen base (Sampaio & Goñi-Colavita, 2008). Tubes with
approximately 10 g bark were incubated at 10 °C until fermentation was observed.

Strain CRUB 1795 was isolated from the ectomycorrhizosphere, that is, the zone of influence of mycorrhizae (Andrade et al., 1997), associated with an adult tree of *N. antarctica* (Table 1) from a forest of the Pampa del Trompul area in Lanin National Park. This is an ecotone habitat between forest and steppe where species of both ecosystems coexist. The isolation medium was MYP agar (0.7 % malt extract, 0.05 % yeast extract, 0.25 % peptone-soytone, 1.5 % agar) supplemented with Rose Bengal (25 μg ml⁻¹) and chloramphenicol (200 μg ml⁻¹). Incubation was at 20 °C.

Physiological characterization of all strains was performed following standard methods (Yarrow, 1998). Glucose fermentation and assimilation tests were all performed at 20 °C. A comparison of physiological characteristics of *L. nothofagi* sp. nov. with those of other recognized species of the genus is presented in Table 2. The responses of *L. nothofagi* sp. nov. and *L. dasiensis* were similar except for growth on 0.01 % cycloheximide and melibiose assimilation, which were negative for *L. nothofagi* sp. nov. and positive for *L. dasiensis*. *L. meyersii* and *L. nothofagi* sp. nov. differed in their ability to assimilate D-galactose.

DNA sequence analysis, DNA extraction, PCR amplification, purification and cycle sequencing were performed according to the protocol described by Sampaio et al. (2001). Comparisons with sequences from GenBank were done using a BLASTN search (Altschul et al., 1997). Sequence alignment and neighbour-joining tree construction (based on 1000 bootstrap iterations) were performed with MEGA version 4 (Tamura et al., 2007).

Sequence comparisons indicated that the novel species belonged to the genus *Lachancea* and was closely related to *L. meyersii*, from which it differs by 11 substitutions in the D1/D2 region of the large-subunit rDNA. *L. meyersii* was isolated from seawater amidst roots of the mangrove *Rhizophora mangle* in the Bahamas (Fell et al., 2004). The phylogenetic placement of *L. nothofagi* sp. nov. is shown in Fig. 1. *L. nothofagi* sp. nov. belongs to the *L. thermotolerans* clade with *L. meyersii* as a sister species. One nucleotide difference was found between CRUB and UWOPS isolates. The polymorphic site appears to be hypervariable within the genus. The adenosine observed in the UWOPS isolates is shared with the type strains of *L. meyersii*, *L. waltii* and *L. thermotolerans*, whereas the guanosine found in the CRUB strains is also found in the type strains of *L. cidri*, *L. fermentati* and *L. kluyveri*. The same position in *L. dasiensis* has a thymidine and an undescribed species, represented by two GenBank deposits (AB087396 and AB087397; K. Ueda-Nishimura, unpublished), has both the adenosine and the guanosine states.

The origin of the six strains of *L. nothofagi* sp. nov. indicates a probable association with *Nothofagus* species in Andean Patagonia. The strains come from three different *Nothofagus* species (*N. nervosa*, *N. antarctica* and *N. pumilio*) with a wide latitudinal range (40–50°S). Five strains of *L. nothofagi* sp. nov. were isolated from fluxes and bark of different

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**Table 1.** Origin of strains of *Lachancea nothofagi* sp. nov. isolated from native *Nothofagus* species forests in Patagonia, Argentina

<table>
<thead>
<tr>
<th>Strain code</th>
<th>GenBank accession no.*</th>
<th>Substrate</th>
<th>Sampling site data</th>
</tr>
</thead>
<tbody>
<tr>
<td>UWOPS 99-807.3³</td>
<td>GQ411403</td>
<td><em>Nothofagus</em> sp. exudate</td>
<td>Mascardi Lake, Nahuel Huapi National Park, October 1999</td>
</tr>
<tr>
<td>UWOPS 99-808.4</td>
<td>–</td>
<td><em>Nothofagus</em> sp. exudate</td>
<td>Mascardi Lake, Nahuel Huapi National Park, October 1999</td>
</tr>
<tr>
<td>CRUB 1792</td>
<td>FJ707476</td>
<td><em>N. antarctica</em> bark</td>
<td>Guillelmo Lake area, Nahuel Huapi National Park, April 2008</td>
</tr>
<tr>
<td>CRUB 1793</td>
<td>FJ707477</td>
<td><em>N. pumilio</em> bark</td>
<td>Cerro Moreno Los Glaciares National Park, January 2008</td>
</tr>
<tr>
<td>CRUB 1794</td>
<td>FJ707478</td>
<td><em>N. nervosa</em> bark</td>
<td>Yuco area, Lanin National Park, December 2007</td>
</tr>
<tr>
<td>CRUB 1795</td>
<td>FJ707479</td>
<td><em>N. antarctica</em> ectomycorrhizosphere</td>
<td>Pampa del Trompul, Lanin National Park, January 2008</td>
</tr>
</tbody>
</table>

*GenBank accession number for D1/D2 large-subunit rDNA sequences.

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**Table 2.** Salient physiological characteristics in the genus *Lachancea*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assimilation of:</strong></td>
<td>D-Galactose</td>
<td>D</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Malrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
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<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Melezitose</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>+</td>
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</tr>
<tr>
<td>Succinate</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DL-Lactate</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Growth on:</strong></td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>0.01% Cycloheximide</td>
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<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>V</td>
</tr>
</tbody>
</table>

Species: 1, *L. nothofagi* sp. nov. (data from this study); 2, *L. meyersii*; 3, *L. dasiensis* (data from Lee et al., 2009); 4, *L. thermotolerans*; 5, *L. waltii*; 6, *L. cidri*; 7, *L. fermentati*; 8, *L. kluyveri*. +, Positive; −, negative; D, delayed growth; V, variable; w, weak. All data, except where indicated otherwise, are from Fell et al. (2004).
Nothofagus species and one came from an adjacent soil sample. Those substrates are influenced by plant exudates, which attract insects and serve as feeding and breeding grounds for them. Plant exudates acquire a complex microbiota that modifies the surrounding environment and microbial composition (Andrews & Harris, 2000). Those facts lead to the suggestion that the yeast could be part of a community associated with insects, possibly drosophilids that visit sap fluxes and bark. Strains of Lachancea species are frequently isolated from tree exudates (Lachance et al., 1995).

Latin diagnosis of Lachancea nothofagi Mestre, Ulloa, Rosa, Lachance et Fontenla sp. nov.


Fig. 1. Neighbour-joining phylogram based on the D1/D2 divergent domains of the large-subunit rDNA sequence of Lachancea nothofagi sp. nov. and its closest relatives. The tree was rooted with the sequence from Kluyveromyces marxianus NRRL Y-8281T (U94924). Bootstrap values (%) were obtained from 1000 iterations. Bar, 1% sequence divergence.

Fig. 2. Asci and ascospores of Lachancea nothofagi sp. nov. strains UWOPS 99-807.3T (a, b) and UWOPS 99-808.4 (c) after 2 weeks at 24 °C on 5% malt extract agar. Asci are formed autogamously (AA) by bud–mother cell conjugation or by conjugation between two separate cells (CA). Asci deliquesce (D) at maturity and released ascospores (RA) tend to agglutinate. Bar, 10 µm.
amylum solubile, D-xylosum, L-arabinosum, D-arabinosum, L-rihamnosum, D-ribosum, ethanolum, erythritolum, ribitolum, galactitolum, salicinum, acidum lacticum, acidum succinicum, acidum citricum, myo-inositol, glucosamine, hexadecanum, methanolum, acetonum nec ethyl acetas. Lysinum assimilantur at non natrium nitricum nec natrium nitrosam. Non-augmentum in 30 °C. UWOPS 99-807.3\textsuperscript{T} (=CBS 11611\textsuperscript{T}=NRRL Y-48670\textsuperscript{3}) typus est.

Description of Lachancea nothofagi Mestre, Ulio, Rosa, Lachance & Fontenla sp. nov.

Lachancea nothofagi (no.tho.fa’gi. N.L. masc. gen. n. nothofagi of Nothofagus species, referring to the genus of tree from which the isolates were obtained).

Yeast cells after 1 week on 5 % malt extract agar (5 %, w/v, malt extract; 1.5 %, w/v, agar) are spherical (mean diameter 4.2 μm). Colonies are white to creamy, with smooth and glistening surfaces and entire margins. Hyphae and pseudohyphae are not formed on any of the culture media tested. Ascospore formation is observed after 2 weeks on 5 % malt extract agar at 24 °C. The asci are formed by conjugation between two cells or autogamously by bud–mother cell conjugation, indicating that the species is homothallic. Ascospores are spherical, 1–2 per ascus (Fig. 2), and are slowly liberated from the mature ascus. Growth at 30 °C is negative. Positive for glucose fermentation after 48 h at 20 °C. Assimilation tests, all carried out at 20 °C, are positive for the following compounds: glucose, raffinose, maltose, sucrose, trehalose, melezitose, glycerol, D-mannitol, D-glucitol, xylitol and lysine. Weak assimilation of galactose is observed for most strains (two strains positive after 48 h at 20 °C). Assimilation tests are negative for the following compounds: sorbose, cellobiose, lactose, melibiose, inulin, soluble starch, D-xyllose, D-arabinose, L-arabinose, rhamnose, D-ribose, ethanol, erythritol, ribitol, galactitol, salicin, lactic acid, succinic acid, citric acid, myo-inositol, glucosamine, hexadecane, methanol, acetone, ethyl acetate, nitrate and nitrite. Growth on 10 % NaCl and on 50 % glucose is variable; growth is positive on yeast nitrogen base without amino acids. Acid production is weak. Not resistant to acetic acid or 0.01 % cycloheximide.

The type strain is UWOPS 99-807.3\textsuperscript{T} (=CBS 11611\textsuperscript{T}=NRRL Y-48670\textsuperscript{3}). Reference strains of L. nothofagi are UWOPS 99-808.4, CRUB 1792, CRUB 1793, CRUB 1794 and CRUB 1795.

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References


