Lindnera rhizosphaerae sp. nov., a yeast species isolated from rhizospheric soil

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Two strains of a novel yeast species were isolated from ectomycorrhizal *Nothofagus pumilio* rhizospheric soil in a native forest of Patagonia, Argentina. Analysis of the D1/D2 large-subunit rRNA gene sequences indicated that the novel species belongs to the recently described genus *Lindnera*. The name *Lindnera rhizosphaerae* sp. nov. is proposed to accommodate these isolates, and the type strain is CRUB 1796^T (=CBS 11400^T =JCM 16499^T).

The genus *Lindnera* was recently proposed by Kurtzman *et al.* (2008) based on analyses of nucleotide sequence divergence in the genes coding for large and small-subunit rRNAs and for translation elongation factor-1 α . This new genus included coenzyme Q-7-forming species, previously assigned to the genera *Pichia* and *Williopsis*. Species of the *Lindnera* clade differ considerably in ascospore morphology (spherical, hat-shaped or saturn-shaped), and the clade includes both hetero- and homothallic species. Species belonging to the genus *Lindnera* ferment glucose and assimilate a variety of sugars, polyols and other carbon sources but fail to assimilate methanol and hexadecane (Kurtzman *et al.*, 2008). A proposal has been presented to rename this genus 'Cyberlindnera' (Minter, 2009) because *Lindnera* is a homonym of a plant genus. Pending acceptance of this proposal, the name *Lindnera* is used provisionally.

During our surveys on the yeast community associated with *Nothofagus pumilio* (Fagaceae) rhizospheric soil from native forests of Argentinean Patagonia, two glucose-fermenting yeast isolates were obtained which, based on growth characteristics, were assigned to the genus *Lindnera* (Kurtzman *et al.*, 2008). Analysis of the D1/D2 variable domain of the large-subunit rRNA gene indicated that these strains represent a distinct species phylogenetically and their closest relative is *Lindnera rhodanensis*. The name *Lindnera rhizosphaerae* sp. nov. is proposed to accommodate these strains, given their occurrence in rhizospheric soil.

Both strains were isolated from a rhizospheric soil fraction of a native forest dominated by ectomycorrhizal *Notho-*

fagus pumilio adult trees on the south-east slope of Cerro Otto in San Carlos de Bariloche (Nahuel Huapi National Park), Argentina, in summer 2008. Two *N. pumilio* adult trees were randomly selected at a distance of more than 15 m apart. Strain CRUB 1796^T (also named OR54) was isolated from a tree in the central forest area with an herbaceous understory. Strain CRUB 1797 (OR47) was isolated from a tree located on the edge of the forest next to a *Chusquea culeou* Desv. (Poaceae) area. Three soil cores (subsamples) from each tree were collected aseptically at 60 cm from the trunk, equidistantly. The superficial layer (2 cm) had previously been removed. Each subsample was processed individually in the laboratory. Root fragments were gently shaken to recover the rhizospheric fraction, which was suspended in a sterile 0.9 % NaCl solution to a final concentration of 1:25 (w/v) and shaken at 250 r.p.m. for 30 min. One hundred microlitres of this suspension was spread on plates containing MYP agar (malt extract 0.7 %, yeast extract 0.05 %, peptone-soytone 0.25 %, agar 1.5 %) supplemented with 25 μ g Rose Bengal ml⁻¹ and 200 μ g chloramphenicol ml⁻¹ and incubated at 20 °C (Mestre *et al.*, 2009).

Physiological characterization of isolates was performed in solid media following standard methods (Yarrow, 1998). DNA extraction was performed using a classical phenol/chloroform/isoamyl alcohol technique adapted from Raeder & Broda (1985). The D1/D2 domain at the 5' end of the large subunit rRNA gene was amplified using primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5'-GGTCCGTGTTTCAAGACGG). Amplification was performed for 35 PCR cycles with annealing at 54 °C, extension at 72 °C for 20 s, and denaturation at 94 °C for 15 s. Fragment amplification and size were confirmed by 1.5 % agarose gel electrophoresis and gels were stained with ethidium bromide. Sequencing of amplicons was carried out using an ET Dynamic

The GenBank/EMBL/DDBJ accession number for the 26S rRNA gene sequences of strains CRUB 1796^T and CRUB 1797 are GQ891688 and GQ891689, respectively.

A supplementary table is available with the online version of this paper.

Terminator kit in a MegaBACE 1000/Automated 96 Capillary DNA sequencer (GE Healthcare). Comparisons with sequences from the international GenBank database (<http://www.ncbi.nlm.nih.gov/>) were done using BLASTN search. Sequence alignment and neighbour-joining tree reconstruction (based on 1000 bootstrap iterations) was performed with MEGA version 4 (Tamura *et al.*, 2007).

Sequence comparisons indicated that the novel species belongs to the genus *Lindnera* and is closely related to *L. rhodanensis*, from which it differs by 39 substitutions in the D1/D2 large-subunit rRNA gene sequence. The sequences obtained from both strains were identical. Phylogenetic placement of *Lindnera rhizosphaerae* sp. nov. is shown in Fig. 1.

The *Lindnera* clade phylogram branching pattern revealed the association of species with similar isolation substrates. Species isolated from insect substrates grouped together; all of them share the capacity to assimilate sucrose, maltose, trehalose, glucitol, mannitol, citrate and, with one exception, melezitose (Table 1). Soil-associated species within the *Lindnera* clade grouped together in the phylogenetic tree, and they lack the ability to assimilate

maltose, trehalose and melezitose (except *Candida vartiovaarae*). *Lindnera rhizosphaerae* sp. nov. and other species isolated from substrates related to rotting bark, trees, orchard environments and plants form a diverse group basal to the other two with variable physiological characteristics. Many species of the genus *Lindnera* seem to be related to decomposing processes (for isolation substrates of *Lindnera* clade strains see Supplementary Table S1 available in IJSEM Online).

Lindnera rhizosphaerae sp. nov. was isolated from the rhizosphere of *Nothofagus* ectomycorrhizal roots. The rhizosphere is a narrow zone of soil extending for a few millimetres from the plant root (and ectomycorrhizal roots) surface into the surrounding soil. This soil fraction is influenced by an interconnected network of roots and fungi. *Nothofagus* trees present high ectomycorrhizal infection values (Diehl *et al.*, 2003). Plant root exudates and fungal metabolites may select specific microbial populations (Botha, 2006; Andrews & Harris, 2000), and *Lindnera rhizosphaerae* sp. nov. is part of the mycorrhizospheric community associated with *Nothofagus* trees.

Fig. 1. *Lindnera* clade. Neighbour-joining phylogram based on the D1/D2 divergent domains of the large-subunit rRNA gene sequence of *Lindnera rhizosphaerae* sp. nov. and its closest relatives. The consensus tree was rooted with *Pichia membranifaciens* NRRL Y-2026^T. Percentage bootstrap values were obtained from 1000 iterations (model used: maximum composite likelihood; missing data: complete deletion). Isolation substrate: ●, insect; □, soil; ■, plant; ▲, others (man, equipment, beer). Strains without data on substrate isolation are unmarked. Bar, 0.05 substitutions per nucleotide position.

Table 1. Physiological characteristics of members of the *Lindnera* clade

All data from Kurtzman (1998) and CBS web page (<http://www.cbs.knaw.nl/>). No available data for strains *Candida* sp. YB-2097, *Candida* sp. Y-7615, *Candida* sp. YB-4088 and *Candida* sp. Y-27267. Gal, D-galactose; Sor, L-sorbose; Ara, L-arabinose; Rham, L-rhamnose; Suc, sucrose; Mal, maltose; Tre, trehalose; Raf, raffinose; Mele, melezitose; Starch, soluble starch; Glucy, D-glucitol; Mann, mannitol; Cit, citrate; NO₃, nitrate; NO₂, nitrite; +, positive; –, negative; w, weakly positive; d, delayed.

Species	Assimilation test															
	Gal	Sor	Ara	Rham	Suc	Mal	Tre	Raf	Mele	Starch	Glucy	Mann	Cit	NO ₃	NO ₂	
Insect subclade																
<i>L. amylophila</i> *	—	—	w, d, —	+	+	+	+	—	+	—	+	+	+	+	—	
<i>L. mississippiensis</i> *	—	+	+	d	+	+	+	—	+	—	+	+	+	—	+	
<i>L. japonica</i> *	—	—	—	+	+	+	+	—	+	—	+	+	+	+	—	
<i>L. fabianii</i> †	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	
<i>L. veronae</i> ‡	—	+	+	—	+	+	+	—	+	+	+	+	+	—	—	
<i>L. americana</i> *	—	—	—	+	+	+	+	—	+	—	+	+	+	+	+	
<i>L. bimundalis</i> *	—	—	d	+	+	+	+	—	+	—	+	+	+	—	+	
<i>C. mycetangii</i> *	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—	
<i>C. maritima</i> †	—	—	d	+	+	+	+	d	+	w, d, +	+	+	+	—	—	
<i>L. euphorbiae</i> *	+	—	+	+	+	+	+	—	+	—	d	d	+	—	—	
<i>L. meyerae</i> *	—	—	—	+	+	+	+	—	—	—	d	d	+	—	—	
<i>L. euphorbiiphila</i> *	—	—	—	—	+	+	+	—	+	—	+	+	+	+	+	
Soil subclade																
<i>L. subsufficiens</i> §	—	—	—	+	+	—	—	+	—	—	d	d	+	+	+	
<i>C. vartiovaarae</i> §	—	—	—	—	+	+	+	—	+	—	+	+	—	+	+	
<i>L. mrakii</i> §	—	—	—	+	—	—	—	—	—	—	+	d	—	+	+	
<i>L. sargentensis</i> §	—	—	—	w, d, +	—	—	—	—	—	—	d	d	—	—	—	
<i>L. saturnus</i> †	—	—	—	w, d, +	+	—	—	+	—	—	+	+	+	+	+	
<i>L. suaveolans</i> §	—	—	—	—	+	—	—	+	—	—	w, d, +	d	—	+	+	
Other strains																
<i>L. lachancei</i> ‡	—	—	—	w, d, +	—	—	—	—	—	—	d	d	—	—	—	
<i>Candida</i> sp. Y-27103	—	—	—	—	w, d, +	+	d	—	+	—	+	+	+	—	—	
<i>L. misumaiensis</i> §	—	—	—	+	—	—	—	—	—	—	+	d	—	+	+	
<i>L. rhodanensis</i> ‡	—	+	—	+	+	+	+	—	+	—	+	+	+	—	—	
<i>L. rhizosphaerae</i> §	—	—	—	—	v	+	—	—	+	—	+	+	—	+	—	
<i>L. petersonii</i> †	—	+	—	+	+	+	+	+	+	—	+	+	+	+	+	
<i>L. jadinii</i> †	—	—	—	—	+	+	+	+	+	—	+	+	+	+	+	
<i>L. maclurae</i> §	+	—	—	—	+	+	+	+	+	—	d	+	+	—	—	

*Insect.

†Others.

‡Plant.

§Soil.

Latin diagnosis of *Lindnera rhizosphaerae* Mestres, Rosa et Fontenla sp. nov.

Cellulae vegetativae globosae aut ovoidae. Pseudomycelium nec mycelium nulla. Glauca colonia, marginis integrae. Ascospores per transformationem ascorum formantur. Asci continentes 4 ascospores petasiformes et rumpentur. Post dies duos glucosum fermentantur at non maltosum, galactosum, raffinsum, lactosum, sucrosum, nec trehalosum. Glucosum, maltosum, melezitosum, D-xylosum, ethanolum, glycerolum, mannitolium, glucitolium, salicinum, acidum lacticum, acetoneum, ethyl acetate, iso-propanol assimilantur, at non galactosum, L-sorbosum, trehalosum, lactosum, melibiosum, raffinsum, amyllum solubile, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, erythritolum, ribitolium,

galactitolium, acidum succinicum, acidum citricum, meso-inositolium, methanolum, hexadecanum, glucosaminum, xylitolium, acidum gluconicum nec N-acetylglucosaminum. Cellobiosum, inulinum et sucrosum asimilantur variabile. Lysinum et natrium nitricum (variabile) assimilantur at non natrium nitrosum. Non augmentum in 30 °C. CRUB 1796^T (=CBS 11400^T = JCM 16499^T) species typica est.

Description of *Lindnera rhizosphaerae* Mestres, Rosa & Fontenla sp. nov.

Lindnera rhizosphaerae (rhi.zo.sphae'rae. Gr. n. *rhiza* root; Gr. n. *sphaera* (Latin transliteration *sphaera*) ball, sphere; N.L. n. *rhizosphaera* the rhizosphere; N.L. gen. n. *rhizosphaerae* from the sphere of the root).

Fig. 2. Micrograph showing cells of strain CRUB 1796^T on 5% malt extract agar after 1 week at 20 °C. RA, released hat-shaped spore; GA, grouped spores; A, asci. Bar, 5 µm.

Morphology

Yeast cells after 3 days on 5% malt extract agar (5% malt extract, w/v; 1.5% agar, w/v) are spheroidal to ovoidal ($4.2\text{--}5.8 \times 3.3\text{--}5.0$ µm) with multilateral budding. Colonies are white with smooth and glistening surfaces and entire margins. Mycelium is not formed on any of the culture media tested.

Spherical asci are formed by conjugation between two cells. The species is homothallic. Ascospore formation is observed after 5 days on 5% malt extract agar at 20 °C. Four hat-shaped ascospores can be seen on early stage cultures, (Fig. 2). Ascospores are released and remain together forming groups or chains.

Physiology

Growth at 30 °C is negative. Glucose fermentation is observed after 48 h at 20 °C; maltose, galactose, raffinose, lactose, sucrose and trehalose fermentation is negative. Assimilates the following compounds at 20 °C: glucose, maltose, melezitose, D-xylose, ethanol, glycerol, D-mannitol, D-glucitol, salicilin, lactic acid, acetone, ethyl acetate, 2-propanol and lysine. Does not assimilate the following compounds at 20 °C: galactose, L-sorbose, trehalose, lactose, melibiose, raffinose, soluble starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, erythritol, ribitol, galactitol, succinic acid, citric acid, *myo*-inositol, methanol, hexadecane, glucosamine, xylitol, gluconic acid, *N*-acetylglucosamine or nitrite. Assimilation of cellobiose, inulin, sucrose and nitrate at 20 °C is variable. Growth is positive on amino-acid-free YNB and negative on 10% NaCl or

50% glucose media. Starch-like compounds are not produced. Acid production is weakly positive. Tolerance of 1% acetic acid and 0.01% cycloheximide is negative.

Strains were isolated from a *Nothofagus pumilio* forest (at 41° 8' 45" S 71° 21' 38" W) and have been deposited at the Centro Regional Universitario Bariloche (CRUB), Bariloche, Argentina. The type strain is CRUB 1796^T (=CBS 11400^T = JCM 16499^T).

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