Spencermartinsiella silvicola sp. nov., a yeast species isolated from rotting wood

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Three strains of a new xylanase-producing yeast species were isolated from rotting wood samples collected in the Atlantic Rain Forest of Brazil. The sequences of the internal transcribed spacer region and D1/D2 domains of the large subunit of the rRNA gene showed that this novel yeast species belongs to the genus Spencermartinsiella, and its closest relatives among recognized species are Spencermartinsiella europaea and Spencermartinsiella ligniputridi. A novel species, named Spencermartinsiella silvicola sp. nov., is proposed to accommodate these isolates. The type strain is UFMG-CM-Y274 (= CBS 13490T). The MycoBank number is MB 813053. In addition, Candida cellulosica is reassigned to the genus Spencermartinsiella as a new combination.

The genus Spencermartinsiella was proposed by Péter et al. (2011) to accommodate a novel yeast species, Spencermartinsiella europaea, isolated from rotten wood collected in different locations in Hungary. This genus belongs to the family Trichomonascaceae, and it is characterized by the production of asci containing a single hemispherical or helmet-shaped ascospore. A multigene sequence analysis showed that Spencermartinsiella is related to the Sugiymaella and Trichomonascus clades (Kurtzman & Robnett, 2013). Dlauchy et al. (2012) described a second species belonging to the genus, Spencermartinsiella ligniputridi, also isolated from rotten wood samples collected in Hungary. Candida cellulosica is the only recognized anamorphic species of the Spencermartinsiella clade (Guo et al., 2012).

During a study of d-xylose-fermenting and xylanase-producing yeasts associated with rotting wood in tropical rain forests of Brazil, three xylanolytic strains of a novel yeast species related to the genus Spencermartinsiella were isolated (Morais et al., 2013b). The sequences of the D1/D2 domains of the large subunit rRNA gene showed that this novel species is most closely related to an undescribed species (Candida sp. NCAIM Y.01937; GenBank accession number GQ340914) in the Spencermartinsiella clade. The D1/D2 sequences of the novel species presented with 100 % identity with the sequences of Candida sp. NCAIM Y.01937 and with 20 differences to that of Spencermartinsiella europaea, the type species of the genus. However, internal transcribed spacer (ITS) sequences showed that the novel species differs by nine substitutions and four indels from Candida sp. NCAIM Y.01937, which suggests that these yeasts may represent different species. Furthermore, the Brazilian isolates either shared identical partial mtSSU rRNA gene sequences or exhibited only minor variability in this region, while the mtSSU rRNA gene sequence of NCAIM Y.01937 was much more divergent. The results of mating experiments did not support the conspecificity of the strains noted above either. In this work, we describe the novel species isolated from rotting wood in Brazil and name it Spencermartinsiella silvicola sp. nov.

The yeast strains were isolated from rotting wood samples collected in the private Natural Heritage Reserve of the Sanctuary of the Caraça, in April 2011. This is an ecological reserve with 11233 ha of Atlantic Rain Forest located in the Serra do Espinhaço (20° 05′ S 43° 28′ W), state of Minas Gerais, south-eastern Brazil (Morais et al., 2013b). Fifty rotting wood samples were collected in sterile plastic...
bags and transported under refrigeration to the laboratory over no more than 24 h. Each wood sample (1 g) was placed, separately, in flasks with 20 ml sterile yeast nitrogen base (YNB)-d-xylose medium [YNB 0.67 % (w/v), d-xylose 0.5 % (w/v) and chloramphenicol 0.02 % (w/v)] or 20 ml sterile YNB-xylan medium [YNB 0.67 % (w/v), xylan 1 % (w/v; Beechwood, Sigma-Aldrich), chloramphenicol 0.02 % (w/v), pH 5.0 ± 0.2], using a two-step enrichment technique, as described by Cadete et al. (2012). When growth was detected, one loopful of each tube was streaked on yeast extract-malt extract agar [YM, glucose 1 % (w/v), yeast extract 0.3 % (w/v), malt extract 0.3 % (w/v), peptone 0.5 % (w/v), agar 2 % (w/v) and chloramphenicol 0.02 % (w/v); Cadete et al., 2012]. The plates were incubated at 25 °C until the yeast colonies developed. The different yeast morphotypes were purified by repeated streaking on YM agar plates and preserved at −80 °C or in liquid nitrogen for later identification. The yeasts were morphologically and physiologically characterized by standard methods (Kurtzman et al., 11).

The ITS region and the D1/D2 domains of the large subunit rRNA gene were amplified by PCR as previously described (Lachance et al., 1999). The amplified DNA was concentrated, cleaned (Wizard Plus SV Minipreps DNA Purification System, Promega) and sequenced in an ABI3130 (Life Technologies) automated sequencing system. The sequences were assembled, edited and aligned with the program MEGA6 (Tamura et al., 2013), which was also used to generate phylogenetic trees. The phylogenetic placement of the novel species was based on the most parsimonious (MP) analysis of the D1/D2 domains of the large subunit rRNA gene alone (Fig. S1, available in the online Supplementary Material) and that of the concatenated sequences of the ITS region and the D1/D2 domains of the large subunit rRNA gene (Fig. 1).

A partial fragment of the mitochondrial small-subunit rRNA gene (mtSSU rRNA) was amplified with the primers 5'-GTGCCAGCGCTGCGGTAGACA and 5'-ATTAAA- CGACATGTCCACTG (Dlauchy et al., 2012), which were also used for sequencing reactions. The GenBank accession numbers of the mtSSU rRNA gene are: KT862883 (UFMG-CM-Y2745), KT862884 (UFMG-CM-Y6154), KT862885 (UFMG-CM-Y616) and KT862886 (NCAIM Y.01937).

**Determination of xylanase activity**

To evaluate xylanase activity, the strains of the novel yeast species were streaked on xylan agar [0.67 % yeast nitrogen base-YNB, 1 % xylan (Beechwood, Sigma–Aldrich) and 2 % agar] and incubated at 25 °C for 48 h. This procedure was repeated twice. After this procedure, the yeast strains were inoculated to an initial optical density at 600 nm (OD600) of 4 in YNB-xylan broth. The xylan used in the medium was autoclaved separately. The yeast cells were cultivated in flasks of 125 ml with 25 ml of medium, at 28 °C, 150 r.p.m. on a rotational shaker for 72 h. The cells were separated by centrifugation, and xylanase activity was assayed in the cell-free supernatant (Lara et al., 2014). All experiments were performed in duplicate.

The xylanases were assayed according to Biely et al. (1980) with a few modifications, as suggested by Lara et al. (2014). The reducing sugars released were determined using the dinitrosalicylic acid method, i.e. spectrophotometrically at 540 nm (Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme

![Figure 1. Phylogenetic placement of Spencermartinsiella silvicola sp. nov. and some related species](image-url)
Species delineation and ecology

The analysis of the ITS and D1/D2 sequences showed that the three strains represent a novel species of the genus Spencermartinsiella (Fig. 1 & Fig. S1). In terms of the pairwise sequence similarity of the D1/D2 sequences, the nearest species are Candida NCAIM Y.01937 and Spencermartinsiella europaea. The D1/D2 sequences of the novel species were identical to that of Candida sp. NCAIM Y.01937; however, this strain differed by nine substitutions and four indels in the sequences of the ITS region from the novel species. According to Daniel et al. (2009), the most often detected intraspecific sequence variability is 0–4 differences in the ITS region, hence, probably they are not conspecific. In addition, two Brazilian strains (UFMG-CM-Y615A and UFMG-CM-Y616) shared identical partial mtSSU rRNA gene sequences, which differed only by 3 substitutions and 2 indels from that of the third Brazilian strain UFMG-CM-Y274T, while the mtSSU rRNA gene sequence of NCAIM Y.01937 was much more divergent. It differed by 26 substitutions and 57–59 indels from the partial mtSSU rRNA gene sequences of the Brazilian strains. The majority of the indels observed were contiguous with the following distribution 47 + 1 + 9 or 49 + 1 + 9, depending on the strains compared. The results of mating experiments did not support the conspecificity of the strains noted above either. The three Brazilian strains formed asci and ascospores if they were mixed in proper combinations, but no sporulation was observed in their pairwise mixtures with strain NCAIM Y.01937.

The novel species differed by 11 substitutions and nine gaps in the D1/D2 sequences from Spencermartinsiella europaea. The ITS and D1/D2 sequences of the three strains of the novel species were identical. The isolates of the novel species were examined after their growth on several common sporulation media (cornmeal agar, dilute V8 agar, 2 % and 5 % malt extract agar and YCB agar supplemented with 0.01 % ammonium sulphate), alone or mixed in pairs, incubated at 15 °C or 25 °C for 28 days. Following 14 days incubation on 2 % malt extract agar at 15 °C, the three strains of the novel species formed ascii with an apical cell if mixed with a strain of the opposite mating type. After 3–4 weeks of incubation a small portion of the ascii contained one hemispheroid or helmet shaped ascospore, which, however seemed to be ellipsoid from the upper view (Fig. 2). The mixed cultures of the designated type strain and any of the two additional strains (UFMG-CM-Y274T X UFMG-CM-Y615A and UFMG-CM-Y274T X UFMG-CM-Y616) proved to be fertile, while no ascosporulation was detected in the mixture of strains, UFMG-CM-Y615A and UFMG-CM-Y616. Strain UFMG-CM-Y615A was designated as the allotype strain of the novel species. Strain NCAIM Y.01937 failed to sporulate with any strain of the novel species. The name Spencermartinsiella silvicola sp. nov., is proposed to accommodate the three Brazilian isolates.

The highest xylanase activity was observed for Spencermartinsiella silvicola sp. nov. UFMG-CM-Y274T (0.17 ± 0.02 U ml⁻¹), while strains UFMG-CM-Y616 and UFMG-CM-Y615 showed very low xylanase activity (0.02 ± 0.01 and 0.03 ± 0.01 U ml⁻¹, respectively). The xylanase activities obtained with these strains were similar to those found for other yeasts (Adsul et al., 2009; Morais et al., 2013a; Lara et al., 2014). The production of xylanases may be an important physiological trait for these yeasts in the colonization of rotting wood. S. silvicola sp. nov. could use the products of xylan degradation as a carbon source.

The three strains of the novel species were isolated from rotting wood samples (Morais et al., 2013b). Strains UFMG-CM-Y274T and UFMG-CM-Y615A were isolated using the YNB-β-xylene medium, and strain UFMG-CM-Y616 using the YNB-xylan medium. The strains came from three different unidentified rotting wood samples collected in an Atlantic Rain Forest site. Candida oleophila, Candida michaelli, Candida orthopsilosis, Schwanniomyces polymorphus and a novel species of Sugiyamaella were co-isolated with this novel species in the rotting wood samples studied (Morais et al., 2013b). The occurrence of this novel species associated with this substrate suggests that Spencermartinsiella silvicola sp. nov. is an inhabitant of rotting wood. The three other species of the Spencermartinsiella clade are also associated with rotten wood (Péter et al., 2011; Dlauchy et al., 2012; Guo et al., 2012), which suggests that this substrate is a possible habitat for species of this genus. With respect to the strains representing undescribed species in the Spencermartinsiella clade, NCAIM Y.01937 and NCAIM Y.01957 were also isolated from rotten wood, while BG090809.6.8.2.24 was recovered from

Fig. 2. A phase-contrast image of an ascosporulating culture of Spencermartinsiella silvicola UFMG-CM-Y274T × UFMG-CM-Y615A. 2 % malt extract agar, 21 days, 15 °C. Bar, 10 μm.
the gut of the Guatemalan passalid beetle. Passalid beetles are associated with rotting logs. Strain FGSFEP was isolated from tanner wastewater, a wood-related substrate, while strains GA1504 and GY44S02 originate from soil.

Spencermartinsiella silvicola sp. nov. can be distinguished from Spencermartinsiella europaea, Spencermartinsiella ligniputridi and Candida cellulosicola by its inability to grow on raffinose as the sole carbon source. The species of the Spencermartinsiella clade with validly published names are positive for the assimilation of this carbon compound. However, sequencing of the ITS region, and the D1/D2 domains, is recommended to confirm the identity of these species.

**Description of Spencermartinsiella silvicola**

Morais, Lara, Oliveira, Peter, Dlauchy & Rosa sp. nov.

Spencermartinsiella silvicola (sil.vi’co.la. L. nom. masc. n. silvicola an inhabitant of woods).

In yeast extract [with 0.5 % (w/v) glucose, 2 % (v/v) broth after 3 days at 25 °C, the cells are spheroid, ovoid, drop-shaped or elongated (1–2 × 2–3 μm). Sediment is formed after 28 days, but no pellicle is observed. On YMA agar after 2 days at 25 °C, colonies are white, opalescent, smooth or folded. Pseudohyphae and true hyphae are present (Fig. 3). The hyphae are often denticulated. The species is heterothallic. Individual strains at 15 °C or 25 °C formed no ascospores after 28 days of incubation. When compatible mating types were mixed, ascosporulation was observed on 2 % malt extract agar at 15 °C within 3 weeks. Ascii are persistent, spheroid (5.5–6.0 μm) to ellipsoid (4.5–6.0 × 6.0–8.0 μm) and usually are formed on lateral hyphae. Each ascus bears an ellipsoid apical cell and some of them contain one hemispheroid or helmet-shaped ascospore, which from an upper view seems ellipsoid (Fig. 2). Fermentation is absent. Assimilation of carbon compounds: glucose, sucrose, melibiose (variable), D-galactose, lactose (variable), D-sorbitose, inulin (variable), maltose, cellobiose, glucosamine (variable), trehalose, melezitose, D-xylene, L-arabinose, D-xylobiose (variable), D-ribose, L-rhamnose, ribitol (variable), ethanol, galactitol (variable), glycerol, erythritol, N-acetyl-D-glucosamine, salicin (variable), D-mannitol, D-glucitol, succinate, citrate (variable), hexadecane (variable), inositol and xylitol. No growth occurs on raffinose, starch, DL-lactate, methanol and gluconate. Assimilation of nitrogen compounds: positive for lysine (variable) and negative for nitrite and nitrate. Growth in amino-acid-free medium is variable. Growth at 37 °C is negative. Growth in 50 % (w/v) glucose is negative. Starch-like compounds are not produced. Growth is positive in 100 mg l−1 cycloheximide.

The type strain accession number of Spencermartinsiella silvicola sp. nov. is UFMG-CM-Y274 (=UFMGCO12.1). It was isolated from rotting wood in the private Natural Heritage Reserve of the Sanctuary of the Caraça, Minas Gerais state, Brazil. It has been deposited in the Collection of Microorganisms and Cells of the Federal University of Minas Gerais (Coleção de Micro-organismos e Células da Universidade Federal de Minas Gerais, UFMG), Belo Horizonte, Minas Gerais, Brazil, as strain UFMG-CM-Y274T, and is permanently preserved in a metabolically inactive state. An ex-type culture has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 13490. Strain UFMG-CM-Y615, one of the opposite mating types of UFMG-CM-Y274T, is the designated allotype. The Mycobank number is MB 813053.

**Novel species combination**

As Candida cellulosicola is well nested in the Spencermartinsiella clade (Fig. 1 & Fig. S1) and the closest match of its D1/D2 sequence among the sequences of the recognized species in the GenBank is Spencermartinsiella europaea, we propose its transfer to the genus Spencermartinsiella as a novel combination.

**Spencermartinsiella cellulosicola** (F.-Y. Bai & X. Guo) Morais, Lara, Oliveira, Peter, Dlauchy & Rosa comb. nov.


Type strain: CBS 11952.

The MycoBank number is MB 814950.
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