Sugiyamaella xylanicola sp. nov., a xylan-degrading yeast species isolated from rotting wood

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Four strains of a novel yeast species were isolated from rotting-wood samples in an Atlantic rainforest site in the state of Minas Gerais, Brazil. These yeasts were obtained from enrichments using yeast nitrogen base (YNB)-d-xylose or YNB-xylan media. The novel yeast species produces bacilliform ascospores typical of the genus Sugiyamaella, and its closest described relative in terms of sequence similarity is Candida (iter. nom. Sugiyamaella) marionensis. The yeast is able to grow in medium with xylan as sole carbon source and produces extracellular enzymes with xylanolytic activities. The novel species Sugiyamaella xylanicola sp. nov. is proposed to accommodate these isolates. The type strain is UFMG-CA-32.1T (=CBS 12683T = CBMAI 1467T).

Xylan is the main hemicellulose component of secondary cell walls in hardwoods and herbaceous plants, constituting about 20–30% of their biomass (Girio et al., 2010). Xylan is a polysaccharide made up of D-xylose units connected by β-1,4-glycosidic bonds (Sjöström, 1993). Hydrolysis of this polysaccharide by xylanases is an important step toward proper use and conversion of lignocellulosic material to value-added products. Xylanases are receiving much attention due to their applications in industry for the potential production of economically valuable products such as xylose and xylo-oligosaccharides (Yoon et al., 2006; Goldman, 2009). Several strains of yeasts (Cryptococcus sp., Sheffersomyces stipitis, Candida ergatensis, Trichosporon cutaneum, etc.) and a yeast-like fungus (Aurorbasidium pullulans) isolated from plant materials have already been reported as potential xylanase producers (Biely et al., 1980; Chávez et al., 2006; Vaz et al., 2011; Carrasco et al., 2012).

Yeasts, filamentous fungi and bacteria capable of hydrolysing the complex carbohydrates present in lignocellulosic substrates are likely to be found in decaying wood. During a study of yeasts associated with rotting wood in tropical rainforests in Brazil, four strains of a novel xylanase-producing yeast species were isolated. Sequences of the D1/D2 domains of the large-subunit rRNA gene showed that this species belongs to the Sugiyamaella clade. The closest relative as determined by pairwise sequence divergence was Candida (iter. nom. Sugiyamaella) marionensis. In this work, we describe the species as Sugiyamaella xylanicola sp. nov.

The yeast strains were isolated from rotting-wood samples collected in the private Natural Heritage Reserve of the Sanctuary of the Caraça. This is an ecological reserve with 11 233 ha of Atlantic rainforest located in the Serra do Espinhaço (20°05’S 43°28’W), Minas Gerais state, southeastern Brazil. The region consists of a mountain complex that constitutes a zone of contact between the ‘Cerrado’ (savannah) and the Atlantic rainforest ecosystems in the south, and a zone of transition from ‘Cerrado’ to Atlantic forest to ‘Caatinga’ ecosystems in the north.

Rotting-wood samples were stored in sterile plastic bags and transported under refrigeration to the laboratory over a period of no more than 24 h. One gram subsamples of each wood sample were placed, separately, in flasks with 20 ml sterile yeast nitrogen base (YNB)-d-xylose medium (6.7 g yeast nitrogen base, 5 g D-xylose and 0.2 g chloramphenicol 1−1) or 20 ml sterile YNB-xylan medium [6.7 g yeast nitrogen base, 10 g xylan (beech wood; Sigma-Aldrich) and 0.2 g chloramphenicol 1−1; pH 5.0 ± 0.2]. D-Xylose, xylan and YNB solutions were sterilized separately. The flasks were incubated at 25 °C on an orbital shaker (New Brunswick) at 150 r.p.m. for 3–10 days. When growth was detected, 0.5 ml aliquots of the culture were transferred to tubes containing 5 ml sterile YNB-d-xylose.
or YNB-xylan medium and the tubes were incubated as described above. After yeast growth, one loopful of culture from each tube was streaked on yeast extract-malt extract (YM) agar (10 g glucose, 3 g yeast extract, 3 g malt extract, 5 g peptone, 20 g agar and 0.2 g chloramphenicol l⁻¹) (Cadete et al., 2012). The plates were incubated at 25 °C until 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 characterized by standard methods (Kurtzman et al., 2011).

The ribosomal cluster region spanning the internal transcribed spacer (ITS), including the 5.8S rRNA gene, and the D1/D2 domains of the large-subunit rRNA gene was amplified by PCR as described previously (Lachance et al., 1999). The amplified DNA was prepared for sequencing by ExoSap-IT (USB-Affymetrix) treatment, which eliminates residual primers, and sequenced in an ABI 3130 (Life Technologies) automated sequencing system at the John P. Robarts Research Institute (London, Ontario, Canada). Sequences were edited with the program Chromas 2.33 (Technelysium) and aligned with the procedures contained in the program MEGAS5 (Tamura et al., 2011), which was also used to generate phylogenetic trees. The tree shown in Fig. 1 was reconstructed by the maximum-likelihood method, using Kimura’s two-parameter substitution model. Evolutionary rate differences were modelled after the gamma distribution with five categories. The final dataset contained 546 aligned positions. The ITS/5.8S rRNA gene regions were excluded from the analysis as many such sequences were not available in GenBank for comparison. Clade consistency was estimated with bootstrap values determined from 100 iterations.

The yeast isolates of the putative novel species were evaluated for xylanase activity. Yeast strains were pregrown in liquid modified YNB-D-xylose medium (6.7 g yeast nitrogen base and 30 g D-xylose l⁻¹), initial pH 5.0, at 30 °C, on an orbital shaker at 150 r.p.m. for 24 h. Cells were harvested by centrifugation and subsequently inoculated at an initial OD₆₀₀ of 3–4 in tubes containing 5 ml modified YNB-D-xylose or YNB-xylan (6.7 g yeast nitrogen base and 10 g xylan l⁻¹) medium. Yeast cells were induced for xylanase production, at 30 °C, on an orbital shaker at 150 r.p.m. for 72 h. Cells were harvested by centrifugation, and cell-free supernatants were used to determine extracellular enzyme activity. All experiments were done in duplicate.

Endo-1,4-β-xylanase (EC 3.2.1.8) was assayed according to Bailey et al. (1992) with a few modifications. The assay mixture consisted of 100 μl culture supernatant and 300 μl of a 10 g xylan (beech wood; Sigma–Aldrich) l⁻¹ suspension in 50 mM acetate buffer (pH 5.5). The mixture was incubated at 50 °C for 30 min followed by immediate chilling on ice. The amount of reducing sugars released was determined using the dinitrosalicylic acid (DNS) method.

![Fig. 1. Maximum-likelihood phylogram based on sequences of the D1/D2 domains of the large-subunit rRNA gene showing the placement of Sugiyamaella xylanica sp. nov. Kimura’s two-parameter transformation was applied. The consistency of the phylogenetic signal was evaluated by bootstrapping from 100 resamplings. Differences in evolutionary rates were fitted to a gamma distribution with five categories. All positions with less than 90% site coverage were eliminated. A total of 546 positions were retained in the final dataset. Bar, 0.05 substitutions per nucleotide position.](http://ijs.sgmjournals.org/2357)
(Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol D-xylose in 1 min under the assay conditions. A standard curve was also generated using 2–10 μmol D-xylose. All enzymic measurements were done in triplicate.

Species delineation, generic assignment and ecology

Analysis of large-subunit rRNA gene D1/D2 sequences showed that the four yeast isolates from rotting wood represent a novel species belonging to the *Sugiyamaella* clade. The novel species forms a poorly supported subclade with several other species (Fig. 1). The branching order suggested by maximum-likelihood, neighbour-joining and maximum-parsimony analyses differed slightly, in spite of the high taxon density in the subclade. In all cases, however, *S. xylanicola* sp. nov. remained distinct from the other taxa included in the analysis. It is hoped that increased character sampling arising from eventual multigene analyses will resolve the matter. Relevant to this is the observation of an unusually large number of synapomorphies subtending the clade that contains *Sugiyamaella americana* and *Candida bullrunensis*. As a result, root placement in this tree was particularly problematic, requiring the presence in the dataset of the large number of outgroup species present in Fig. 1. In terms of pairwise sequence similarity, the nearest species is *Candida marionensis*, with 14 substitutions in the D1/D2 domains and six in the ITS region. Although the latter cannot be regarded as a direct measure of relatedness, it is often the main criterion used in sequence-based identification. The sequences of the four isolates of the novel species were identical. When mixed in pairs on diluted (1 : 9) V8 agar cultures, UFMG-CO-32.1T(h+)
and UFMG-CA-49.3(h−) produced asci with an apical cap cell. Asci were persistent, with a single bacilliform ascospore. The other two isolates (UFMG-CA-14.1 and UFMG-CA-27.2) did not produce ascospores when mixed in pairs with the other cultures or individually on Fowell acetate agar, cornmeal agar, diluted V8 agar, 5 % malt extract agar or yeast carbon base agar supplemented with 0.01 % ammonium sulphate incubated at 15 and 25 °C.

The production of extracellular xylanase activity by *S. xylanicola* strains was evaluated after induction with two different carbon sources, D-xylose and xylan (Table 1). Xylan was the best inducer of xylanase production; D-xylose led to xylanase production by only two strains, UFMG-CO-14.1 and UFMG-CO-32.1T, both showing low xylanase activity (0.03 U ml−1). All *S. xylanicola* strains tested showed higher extracellular xylanase activity under xylan induction (between 0.06 and 0.15 U ml−1), with the highest activity observed with strain UFMG-CO-32.1T. The xylanase activities obtained with these strains are significantly below those found for fungi (80.5 U ml−1) (Shah & Madamwar, 2005), including *A. pullulans* (42.3 U ml−1; Bhadra et al., 2008), and bacteria such as *Streptomyces* species (Nascimento et al., 2003), but similar to those of other yeasts grown under similar conditions (Adsul et al., 2009).

The four strains of *S. xylanicola* were isolated from rotting-wood samples. Two strains, UFMG-CO-14.1 and UFMG-CO-32.1T, were isolated using the YNB-D-xylose medium and the other strains, UFMG-CA-27.2 and UFMG-CA-49.3, were obtained from samples inoculated in the YNB-xylan medium. The strains came from different samples of rotting wood that was widespread in the collection area, and it was not possible to identify a tree species association for the novel species. Isolation from rotting wood and the production of xylanases by this novel yeast suggest that this substrate is its ecological niche and that the assimilation of carbon sources may be independent of the action of other micro-organisms able to degrade polysaccharides such as xylan efficiently. The closest relatives of *S. xylanicola* are species also isolated from rotting wood or from insects inhabiting this substrate (Kurtzman, 2011).

The novel species can be distinguished from *C. marionensis* based on the assimilation of D-arabinose and D-ribose, which is negative for *S. xylanicola* and positive for *C. marionensis*. In addition, the novel species assimilates erythritol, while *C. marionensis* does not. The other species related to *S. xylanicola* (*Candida lignohabitans*, *C. grinbergii* and *C. pinicola*) can be distinguished from the novel species based on the assimilation of D-arabinose, which is negative for the novel species and positive for the other related species.

### Table 1. Xylanase activity in cultures of *S. xylanicola* sp. nov.

<table>
<thead>
<tr>
<th>Strain</th>
<th>D-Xylose</th>
<th>Xylan</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFMG-CA-27.2</td>
<td>0.00</td>
<td>0.06</td>
</tr>
<tr>
<td>UFMG-CA-49.3</td>
<td>0.01</td>
<td>0.12</td>
</tr>
<tr>
<td>UFMG-CO-32.1T</td>
<td>0.03</td>
<td>0.15</td>
</tr>
<tr>
<td>UFMG-CO-14.1</td>
<td>0.03</td>
<td>0.10</td>
</tr>
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Volumetric xylanase activity (U ml−1) was determined in cell-free supernatants after induction with D-xylose or xylan for 72 h at 30 °C.

**Description of *Sugiyamaella xylanicola* Morais, Lachance & Rosa sp. nov.**

*Sugiyamaella xylanicola* [xy.la.ni’co.la. N.L. n. *xylanum* xylan; L. suff. -cola inhabitant, dweller; N.L. n. *xylanicola* an inhabitant of rotting wood (where xylan is one the major polysaccharides), the substrate from which this yeast was first isolated].

In yeast extract (0.5 %)/glucose (2 %) broth after 3 days at 25 °C, the cells are round and ovoid to ellipsoidal (2–3 × 2–5 μm). Budding is multilateral (Fig. 2). Sediment is formed after 1 month, but no pellicle is observed. On YM agar after 2 days at 17 °C, colonies are white, convex, smooth and opalescent. On Dalmau plates after 2 weeks on cornmeal...
agar at 25 °C, abundant pseudomycelium with blastoconidia is formed. Cultures of individual strains grown on Powell acetate, cornmeal agar, diluted (1:9) V8 agar, 5% malt extract agar and yeast carbon base agar supplemented with 0.01% ammonium sulphate incubated at 15 and 25 °C produce no ascospores. When mixed in pairs on diluted (1:9) V8 agar cultures, strains UFMG-CA-49.3 (h) and UFMG-CA-32.1T (h+) produce a single ellipsoidal ascospore (Fig. 2). Fermentation of glucose, galactose (variable) and cellobiose is positive. Assimilation of glucose, galactose, trehalose, cellobiose, salicin, L-sorbos, D-xylene, L-arabinos, erythritol, ribitol, D-mannitol, D-glucitol, succinate, xylitol, D-glucurate and N-acetyl-D-glucosamine is positive. No growth is detected on inulin, sucrose, raffinose, melibioso, lactose, maltose, melezitose, soluble starch, L-rhamnose, D-arabinose, methanol, ethanol, glycerol, galactitol, myo-inositol, DL-lactate, citrate, D-glucosamine or hexadecane. Assimilation of nitrogen compounds: positive for lysine, cadaverine and nitrite and negative for nitrate. Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Growth on YM agar with 10% NaCl is negative. Growth in 50% glucose is negative. Starch-like compounds are not produced. In 100 µg cycloheximide ml⁻¹, growth is positive. Diazonium blue B reaction is negative.

The type strain is UFMG-CA-32.1T, 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 the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands, as CBS 12683T and in the Brazilian Collection of Environmental and Industrial Micro-organisms (Coleção Brasileira de Micro-organismos de Ambiente e Indústria, CBMAI), Campinas, São Paulo, Brazil, as CBMAI 1467T. The designated allotype, UFMG-CA-49.3 (h+) (=CBS 12810 =CBMAI 1554), was also recovered from rotting-wood samples in the same location. The Mycobank number is MB803254.

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References


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Fig. 2. Budding cells (a), pseudohyphae (b), an immature ascus with a small apical cell (c) and a mature ascus (d) with a bacilliform ascospore in a mixed culture of strains UFMG-CA-32.1T and UFMG-CA-49.3 grown on dilute V8 agar after 7 days at 25 °C. Bar, 5 µm.


