

Candida amazonensis sp. nov., an ascomycetous yeast isolated from rotting wood in the Amazonian forest

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Five strains of a novel yeast species were isolated from rotting wood samples collected in an Amazonian forest site in the state of Roraima, northern Brazil. The sequences of the D1/D2 domains of the large subunit of the rRNA gene showed that this species belongs to the *Scheffersomyces* clade and is related to *Candida coipomoensis*, *Candida lignicola* and *Candida queiroziae*. The novel species *Candida amazonensis* sp. nov. is proposed to accommodate these isolates. The type strain of *C. amazonensis* sp. nov. is UFMG-HMD-26.3^T (=CBS 12363^T=NRRL Y-48762^T).

The genus *Scheffersomyces* was proposed by Kurtzman & Suzuki (2010) to include the species *Scheffersomyces segobiensis*, *S. spartinae* and *S. stipitis*. The asexual species *Candida shehatae*, *C. insectosa*, *C. lignosa*, *C. ergatensis*, *C. coipomoensis*, *C. lignicola* and *C. queiroziae* are also members of the *Scheffersomyces* clade (Jindamorakot *et al.*, 2007; Kurtzman, 2011; Santos *et al.*, 2011). *Scheffersomyces stipitis*, *S. segobiensis*, *C. shehatae*, *C. lignosa* and *C. insectosa* are of industrial interest because they ferment D-xylose from biomass to ethanol (Kurtzman, 2011). *C. queiroziae*, *C. coipomoensis* and *C. lignicola* are able to ferment cellobiose. This is also of interest because this sugar is a competitive inhibitor of cellulases and hinders the saccharification process of cellulosic material during bioethanol production (Bezerra & Dias, 2005; Santos *et al.*, 2011).

During a search for novel D-xylose-fermenting yeasts associated with rotting wood in a region of the Amazonian forest in northern Brazil, we isolated five strains of a novel cellobiose-fermenting species. Sequence analyses of the D1/D2 regions of the large subunit (LSU) of the rRNA

gene showed that the novel species belonged to the *Scheffersomyces* clade and was closely related to *C. coipomoensis*, *C. lignicola* and *C. queiroziae*. The name *Candida amazonensis* sp. nov. is proposed for this novel species.

The yeasts were isolated from rotting wood samples collected at a site in the state of Roraima, northern Brazil. This site belongs to a private forest reserve of an estate located in the municipality of Mucajaí (2° 25' N 60° 54' W). The predominant vegetation is characterized as Amazonian forest biome. The climate is hot and humid, with annual precipitation between 1500 and 2100 mm and a mean temperature of 25.6–27.6 °C.

Twenty decayed wood samples were collected in October, 2009. The 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 of each sample was placed separately in flasks with 20 ml sterile D-xylose medium (yeast nitrogen base 0.67 %, D-xylose 0.5 %, chloramphenicol 0.02 %) and 20 ml sterile xylan medium (yeast nitrogen base 0.67 %, xylan 1 %, chloramphenicol 0.02 %, pH 5.0 ± 0.2), respectively. The flasks were incubated at 25 °C on an incubator shaker (New Brunswick) at 150 r.p.m. for 3–10 days. When yeast growth was detected, 0.5 ml aliquots were transferred to tubes containing 5 ml sterile D-xylose or

Abbreviations: ITS, internal transcribed spacer; LSU, large subunit.

The GenBank/EMBL/DDBJ accession number for the gene sequence of the D1/D2 domain of the 26S rRNA gene of strain UFMG-HMD-26.3^T is JF826438.

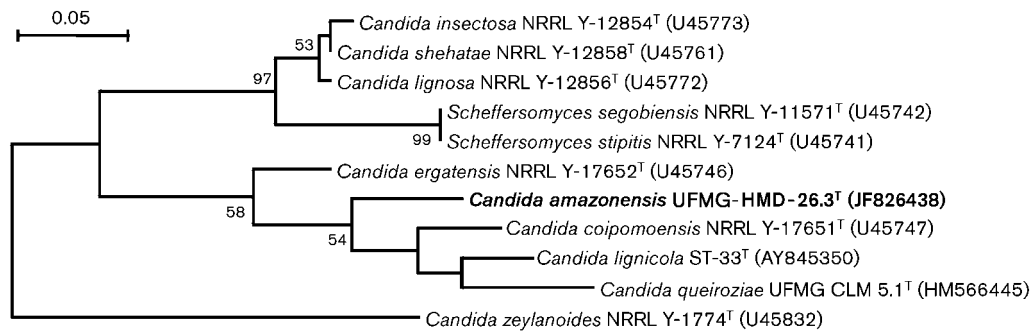


Fig. 1. Phylogenetic placement of *Candida amazonensis* sp. nov. by maximum-parsimony analysis. The bootstrap consensus tree was produced from 1000 iterations using 525 aligned nucleotide positions of the D1/D2 domains of the LSU rRNA gene. The sequence of *Candida zeylanoides* NRRL Y-1774^T was used as the outgroup and all strains analysed were type strains. Bar, 0.05 substitutions per nucleotide position.

xylan medium and the tubes were incubated on an incubator shaker as described above. One loopful of each tube was streaked on D-xylose or xylan agar medium. The plates were incubated at 25 °C until yeast colonies developed (Cadete *et al.*, 2009).

Representatives of the different colony morphotypes were purified by repeated streak inoculation on yeast extract–malt extract agar (YMA) and preserved at –80 °C or in liquid nitrogen for later identification. The yeasts were characterized using standard methods (Kurtzman *et al.*, 2011). Identities were determined by sequencing the D1/D2 domains of the LSU of the rRNA gene. The D1/D2 domains and the internal transcribed spacer (ITS) region of the LSU rRNA gene of the yeast strains were amplified by PCR directly from whole cells as described previously (Lachance *et al.*, 1999). The amplified DNA was concentrated and cleaned on QIAquick PCR columns (Qiagen) and sequenced using an ABI sequencer at the John P. Robarts Research Institute (London, Ontario, Canada). The sequences were assembled, edited and aligned with the program MEGA5 (Tamura *et al.*, 2011). Phylogenetic placement of the strains was based on maximum-parsimony analysis of the sequences of the D1/D2 domains of the LSU rRNA gene. The bootstrap consensus tree was produced from 1000 iterations using 525 aligned nucleotide positions.

The novel species belongs to the *Scheffersomyces* clade and is related to *C. coipomoensis*, *C. lignicola* and *C. queiroziae* (Fig. 1). It differed in the D1/D2 domains by 9 substitutions and 6 gaps from *C. coipomoensis*, 9 substitutions and 7 gaps from *C. lignicola*, and 16 substitutions and 6 gaps from *C. queiroziae*. The D1/D2 and ITS sequences of the five isolates (UFMG-HMD-26.3^T, XMD-24.1, XMD-26.2, XMD-40.2 and XMD-40.3) of the novel species were identical. The isolates, either alone or mixed in pairs, were examined after growth on the most common sporulation media at 17 and 25 °C (cornmeal agar, dilute V8 agar, 5% malt extract agar and yeast carbon base agar supplemented with 0.01% ammonium sulfate, among others), but asci or signs of conjugation were not seen.

The isolation of this novel species from rotting wood suggests that this substrate could be its ecological niche. *C. amazonensis* has not been isolated from rotting wood collected in south-eastern Brazil and could be restricted to the Amazonian region. This novel yeast species and its close relatives form a subclade within the *Scheffersomyces* clade in which all species were isolated from rotting wood (Kurtzman, 2011; Santos *et al.*, 2011); this suggests that these yeasts are adapted to this substrate.

The strains of *C. amazonensis* are physiologically similar to strains of the species *C. lignicola*, *C. coipomoensis* and *C. queiroziae*, but can be distinguished from them based on growth at 37 °C, which is positive for the novel species and negative or weak for the other species (Lachance *et al.*, 2011; Santos *et al.*, 2011). In addition, *C. coipomoensis* and *C. amazonensis* do not produce acid from glucose, whereas *C. lignicola* and *C. queiroziae* exhibit weak acid formation.

Latin diagnosis of *Candida amazonensis* Cadete, Melo, Lopes, Zilli, Vital, Gomes, Lachance & Rosa sp. nov.

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae aut ellipsoideae (2–3 × 2–4 μm). Post unum mensem sedimentum formatur. Cultura in agaro malti post dies 2 (17 °C) parva, convexa, rugosa et candida. In agaro farinae Zea mays post dies 14 pseudohyphae formantur. Glucosum, galactosum, cellobiosum et trehalosum (lente) fermentantur. Glucosum, sucrosom, galactosum, trehalosum, maltosum, melezitiosum (variabile), cellobiosum, salicinum, L-sorbosum, D-xylosum, D-arabiosum (lente), ethanolum (lente), glycerolum, erythritolum, ribitolum, D-mannitolum, D-glucitolum, succinatum, citratum (lente), xylitolum et N-acetyl-D-glucosaminum assimilantur, at non inulinum, raffinolum, melibiosum, lactosum, amyllum solubile, L-rhamnosum, L-arabiosum, D-ribosum, methanolum, galactitolum, myo-inositolum, DL-lactatum, D-gluconatum, D-glucosaminum nec hexadecanum. Ethylaminum, lysinum et cadaverinum assimilantur at non kalium nitricum et natrium nitrosolum. Ad crescentiam vitaminarum externarum necessaria sunt.

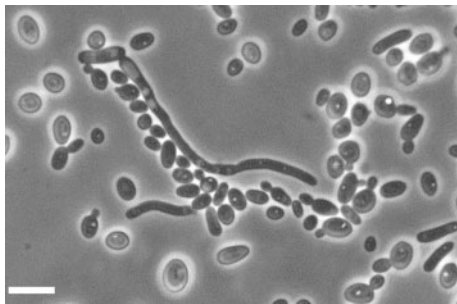


Fig. 2. Phase-contrast micrograph of cells of *Candida amazonensis* sp. nov. grown on yeast nitrogen base with 0.01% ammonium sulfate after 3 days. Bar, 5 μ m.

Augmentum in 37 °C. Habitat materiam Brazil. Typus UFMG-HMD-26.3^T. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 12363^T typus stirps deposita est.

Description of *Candida amazonensis* Cadete, Melo, Lopes, Zilli, Vital, Gomes, Lachance & Rosa sp. nov.

Candida amazonensis (a.ma.zo.nen'sis. N.L. nom. fem. sing. adj., *amazonensis* referring to the region which this yeast was isolated, the Amazonian region).

In yeast extract (0.5%)/glucose (2%) broth after 3 days at 25 °C, cells are ovoid to ellipsoidal (2–3 × 2–4 μ m). Budding is multilateral (Fig. 2). A sediment is formed after a month, but no pellicle is observed. On YMA after 2 days at 17 °C, colonies are white, convex, rough and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, well-developed pseudo-hyphae are present. Fermentation of glucose, galactose, cellobiose and trehalose (slow) is positive. Maltose, sucrose and D-xylose are not fermented. Assimilates the following carbon compounds: glucose, sucrose, galactose, trehalose, maltose, melezitose (variable), cellobiose, salicin, L-sorbose, D-xylose, D-arabinose (slow), ethanol (slow), glycerol, erythritol, ribitol, D-mannitol, D-glucitol, succinate, citrate (slow), xylitol and N-acetyl-D-glucosamine. No growth occurs on inulin, raffinose, melibiose, lactose, soluble starch, L-rhamnose, L-arabinose, D-ribose, methanol, galactitol, myo-inositol, DL-lactate, D-gluconate, D-glucosamine or hexadecane. Positive for assimilation of lysine, ethylamine. HCl and cadaverine, and negative for nitrate and nitrite. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Growth on YM agar with 10% sodium chloride is positive (slow). Growth in 50% glucose/yeast extract (0.5%) is negative. Acid production is negative. Starch-like compounds are not produced. Grows in 100 μ g cycloheximide ml⁻¹. Urease activity is negative. Diazonium Blue B reaction is negative.

The type strain is UFMG-HMD-26.3^T (=CBS 12363^T=NRRL Y-48762^T), isolated from rotting wood in the Amazonian

forest ecosystem in the state of Roraima, Brazil. Four further reference strains were also isolated from the same source. The Mycobank number is MB 563080.

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