Candida materiae sp. nov., a yeast species isolated from rotting wood in the Atlantic Rain Forest

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Three strains of a novel yeast species, *Candida materiae* sp. nov., were isolated from rotting wood in an Atlantic rain forest site in Brazil. Analysis of the sequences of the D1/D2 domains of the large-subunit rDNA showed that this species belonged to the *Spathaspora* clade and was related to *Candida jeffriesii* and *Spathaspora passalidarum*. Unlike *C. jeffriesii* and *S. passalidarum*, *C. materiae* sp. nov. did not ferment xylose. The type strain of *C. materiae* sp. nov. is UFMG-07-C15.1B^T (=CBS 10975^T=CBMAI 956^T).

The genus *Spathaspora* was proposed by Nguyen *et al.* (2006) to accommodate the single species *S. passalidarum*, a xylose-fermenting yeast isolated from the beetle *Odontotaenius disjunctus* in Louisiana, USA. The asexual species *Candida jeffriesii*, *Candida lyxosophila* and *Candida insectamans* are phylogenetically related to *S. passalidarum* (Nguyen *et al.*, 2006). *C. jeffriesii* was isolated from an adult of the beetle *Phrenapates bennetti* collected in Panama. van der Walt *et al.* (1972, 1987) isolated *C. insectamans* from a frass sample of beetle larvae in trees and *C. lyxosophila* from surface woodland soil samples in South Africa, respectively. *C. jeffriesii* and *C. lyxosophila* are also able to ferment D-xylose, whereas *C. insectamans* does not ferment this pentose. During a survey of yeasts associated with rotting wood in Atlantic rain forest in Brazil, representatives of a novel yeast species related to the *Spathaspora* clade were isolated. The isolates were not able to ferment D-xylose and analysis of the sequences of the D1/D2 domains of the large-subunit rRNA gene showed that they were closely related to *C. jeffriesii*. The novel species is described as *Candida materiae* 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 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. The region consists of a mountain complex that constitutes a zone of contact between the 'Cerrado' (savannas) and the Atlantic rain forest ecosys-

tems in the south, and a zone of transition from 'Cerrado' to Atlantic forest to 'Caatinga' ecosystem in the north. The reserve has various floristic formations that include seasonal semideciduous forests, 'high-altitude plateaus' and rupestrian fields (grasslands surrounded by rocky outcrops, as well as shrubs and small trees). Seven decayed wood samples were collected on ecological trails of the Reserve of Caraça in November 2007. 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 carboxymethylcellulose medium (0.67 % yeast nitrogen base, 1 % carboxymethylcellulose, 0.05 % cellobiose and 0.02 % chloramphenicol), and in 20 ml sterile D-xylose medium (0.67 % yeast nitrogen base, 0.5 % D-xylose, 0.02 % chloramphenicol). The flasks were incubated at 25 °C on an incubator shaker (New Brunswick) at 150 r.p.m. for 3–10 days. When growth was detected, 0.5 ml of the cultures was then transferred separately to tubes containing 5 ml sterile carboxymethylcellulose or D-xylose media and the tubes were incubated as described above. One loopfull of solution from each tube was streaked on yeast extract-malt extract agar (YMA; 1 % glucose, 0.3 % yeast extract, 0.3 % yeast malt, 0.5 % peptone, 2 % agar and 0.02 % chloramphenicol). The plates were incubated at 25 °C until yeast colonies developed. The different yeast morphotypes were purified by repeated streaking on YMA plates and preserved at –80 °C or in liquid nitrogen for later identification. The yeasts were characterized by using standard methods (Yarrow, 1998). Fermentation of D-xylose was tested in Durham tubes containing 2 % (w/v) solution of the sugar. The tubes were incubated at 25 °C on an incubator shaker

The GenBank/EMBL/DBJ accession number for the gene sequence of the D1/D2 domain of the 26S rDNA of strain UFMG-07-C15.1B^T is FJ154790.

(New Brunswick) at 80 r.p.m. for 3–20 days. *Candida shehatae* var. *shehatae* CBS 5813, *C. shehatae* var. *insectosa* CBS 4286, *C. shehatae* var. *lignosa* CBS 4705 and *Pichia stipitis* CBS 5773 were used as positive controls for D-xylose fermentation. Preliminary identifications followed the taxonomic keys of Kurtzman & Fell (1998).

The D1/D2 variable domains of the large-subunit rDNA 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 sequence was edited with the program DNAMAN, version 6 (Lynnon BioSoft). Existing sequences for other yeasts were retrieved from GenBank. The CLUSTAL W software (Thompson *et al.*, 1994) was used to align the sequences and construct a neighbour-joining tree with 1000 bootstrap iterations.

Species delineation, classification and ecology

Analysis of the sequences of the D1/D2 region of the large-subunit rRNA gene showed that *Candida materiae* sp. nov. belonged to the *Spathaspora* clade (Fig. 1). In terms of sequence divergence, *C. materiae* sp. nov. differed by 13 substitutions from *C. jeffriesii*, and by 20 substitutions from *S. passalidarum*. Three strains (UFMG-07-C12.1, UFMG-07-C15.1B^T and UFMG-07-C27.1) of the novel species were isolated from samples of rotting wood collected in the Atlantic rain forest of the Reserve of Caraça. These three strains of *C. materiae* sp. nov. were physiologically very similar to *C. jeffriesii* and *S. passalidarum*. However, the novel yeast was not able to ferment D-xylose.

Two strains of *C. materiae* sp. nov. were isolated from yeast nitrogen base plus D-xylose as sole carbon source, whereas the third was isolated from the carboxymethylcellulose medium. However, the strain isolated using the carboxymethylcellulose medium was not able to produce extracellular cellulase (data not shown). The medium contained cellobiose, which can be utilized by *C. materiae* sp. nov. as a sole carbon source. *S. passalidarum* and *C. jeffriesii* are associated with wood-boring beetles that occur throughout the eastern USA and in Panama (Nguyen *et al.*, 2006). The isolation of *C. materiae* sp. nov. from rotting wood

suggests that this yeast was also introduced to this plant material by wood-boring insects that use this substrate.

C. materiae sp. nov. could be distinguished from *C. jeffriesii* based on growth at 37 °C and the fermentation of D-xylose. *C. materiae* sp. nov. gave a positive response for the first physiological test and a negative response for the second. *S. passalidarum* could be easily distinguished from *C. materiae* sp. nov. based on the ability to ferment D-xylose and the production of ascospores by the former species (Nguyen *et al.*, 2006). *C. insectamans* was negative for assimilation of galactose and sucrose (Meyer *et al.*, 1998), whereas the other species of the *Spathaspora* clade are able to use these sugar sources. Isolates of *C. materiae* sp. nov. were examined individually or mixed in pairs on cornmeal, V8, dilute V8, 5% malt extract, yeast carbon base supplemented with 0.01% ammonium sulphate and Gorodkova agars, but asci or signs of conjugation were not observed.

Latin diagnosis of *Candida materiae* Barbosa, Cadete, Gomes, Lachance *et 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 agar malti post dies 2* (17 °C) *candida, convexa, rugosa et opalescens. In agar farinae Zea mays post dies 14 pseudomycelium formantur. Glucosum at maltosum fermentatur. Glucosum, galactosum, L-sorboseum, sucrososum, maltosum, cellobiosum, trehalosum, melezitum, D-xylosum, D-ribosum (lente), glycerolum, erythritolum, ribitolum, D-mannitolum, salicinum (variabile), acidum lacticum (variabile), acidum succinicum, acidum citricum (exiguum), N-acetylglucosaminum (exiguum) et xylitolum (variabile) assimilantur, at non-lactosum, melibiosum, raffinolum, inulinum, amyllum solubile, D-arabinosum, L-arabinosum, L-rhamnosum, ethanolum, galactitolum, glucitolum, meso-inositolum, methanolum, hexadecanum, D-glucosaminum, acetolum, 2-propanolum et ethyl acetum. Ethylaminum, lysinum et cadaverinum assimilantur at non-kalium nitricum et natrium nitrosolum. Ad crescentiam vitamina externa necessaria sunt. Augmentum in 37 °C. Habitat materiam in Brazil. Typus UFMG-07-C15.1B^T. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 10975^T typus stirps deposita est.*

Description of *Candida materiae* Barbosa, Cadete, Gomes, Lachance & Rosa sp. nov.

Candida materiae [ma.te'ri.ae'. L. gen. n. *materiae* of a material, matter, referring to the substrate (wood) where this yeast was found].

In yeast extract (0.5%), glucose (2%) broth after 3 days at 25 °C, the cells are ovoid to ellipsoidal (2–3 × 2–4 µm). Budding is multilateral (Fig. 2). A sediment is formed after a month, but a pellicle is not observed. On YMA after 2 days at 17 °C, colonies are white, convex, rugose and opalescent. In Dalmau plates after 2 weeks on cornmeal

Fig. 1. Neighbour-joining tree showing the placement of *Candida materiae* sp. nov. among related species in the *Spathaspora* clade. Bootstrap values of 50% or above are shown. Bar, 5 substitutions per 100 nucleotides.

Fig. 2. Phase-contrast micrograph of cells of *Candida materiae* sp. nov. strain UFMG-07-C15.1B^T on yeast extract-malt extract agar after 5 days at 22 °C. Bar, 5 µm.

agar, pseudomycelia are formed. Ascospores are not formed. Fermentation of glucose and maltose is positive. The following carbon compounds are assimilated: glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melezitose, D-xylose, D-ribose (slow), glycerol, erythritol, ribitol, D-mannitol, salicin (variable), lactic acid (variable), succinic acid, citric acid (weak), N-acetylglucosamine (weak) and xylitol (variable). No growth occurs on lactose, melibiose, raffinose, inulin, soluble starch, D-arabinose, L-arabinose, L-rhamnose, ethanol, galactitol, glucitol, myo-inositol, methanol, hexadecane, D-glucosamine, acetone, 2-propanol and ethyl acetate. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl, 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 negative. Growth in 50 % glucose/yeast extract (0.5 %) is negative. Starch-like compounds are not produced. In 100 µg cycloheximide ml⁻¹ growth is negative. Urease activity is negative. Diazonium Blue B reaction is negative. The habitat is rotting wood in Atlantic rain forest ecosystem, in the state of Minas Gerais, Brazil.

The type strain of *Candida materiae*, UFMG-07-C15.1B^T, was isolated from rotting wood in Brazil. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as strain CBS 10975^T (=CBMAI 956^T).

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