Candida cellulosicola sp. nov., a xylose-utilizing anamorphic yeast from rotten wood

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Two xylose-utilizing yeast strains isolated from rotten wood collected in the rainforest in different mountains of Hainan province, southern China, were studied. Sequence analysis of the large subunit rDNA D1/D2 domain and internal transcribed spacer region revealed that the strains represent a novel anamorphic yeast species, for which the name Candida cellulosicola sp. nov. is proposed; the type strain is HNX16-2T (=CGMCC 2.3503T=CBS 11952T). Phylogenetically, the novel species was closely related to a xylose-utilizing teleomorphic ascomycetous yeast species Spencermartinsiella europaea in the family Trichomonascaceae, but differed from the latter by 3.0% mismatches in the D1/D2 domain.

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DBJ accession numbers for the ITS region and 26S rDNA D1/D2 domain sequences of strains HNX16-2T and HNX35-3 are HM151013–HM151016.

Forest habitats such as rotten wood and plant litter containing plenty of assimilable carbon compounds are expected to harbour diverse yeasts, including pentose-utilizing or -fermenting species. These yeasts are of potential value in bio-ethanol production from lignocellulose materials. During an ecological survey on microfungi colonizing rotten wood in the rainforest of southern Chile, Dill et al. (1984) and Ramirez & González (1984a, b, c) described a total of 18 novel yeast species (Middelhoven, 2006). Of the six novel methanol-assimilating ascomycetous yeast species from wood material described by Péter et al. (2003), four were isolated from rotten wood. Middelhoven & Kurtzman (2007) identified two novel ascomycetous yeast species from strains isolated from rotten wood. In recent years, new xylose- or cellobiose-fermenting yeast species have been isolated from rotting wood in Brazil and the Atlantic rainforest (Barbosa et al., 2009; Cadete et al., 2009; Santos et al., 2011). In an effort to investigate the biodiversity of yeasts inhabiting rotten wood in the rainforest of Hainan, a tropical island in southern China, a variety of undescribed yeast species were isolated (Wang & Bai, 2009); two of them have been described previously (Wang et al., 2009). A further novel anamorphic ascomycetous yeast species represented by two of the Hainan strains from rotten wood is described in this study.

Yeasts were isolated using the enrichment method selective for xylose-utilizing strains. Rotten wood samples were put in enrichment medium containing: 1% (w/v) yeast nitrogen base (Difco), 2% (w/v) xylose and 200 µg chloromycetin ml⁻¹. After incubation at 25°C for 7–14 days, aliquots (200 µl) of 10⁻¹ to 10⁻³ diluted enrichment medium were spread on agar plates that were prepared using enrichment medium plus 2% agar. After incubation of the plates for 2–5 days at 25°C, yeast colonies with different morphological characters were selected for further study.

Strains HNX16-2T and HNX35-3 were isolated from two different rotten wood samples of broad-leaved trees collected in Wuzhi and Diaoluo Mountains, respectively. The two mountains, which are approximately 30 km apart, are located in Hainan province, southern China. Morphological, physiological and biochemical characteristics were examined according to standard methods commonly used in yeast taxonomy (Yarrow, 1998). Assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase & Suzuki, 1986).

Nuclear DNA was extracted by the method of Makimura et al. (1994). Sequencing of the internal transcribed spacer (ITS) region (including 5.8S rDNA) and the large-subunit rDNA D1/D2 domain, and molecular phylogenetic analysis were performed as described previously (Wu et al., 2006). Reference sequences were retrieved from GenBank (accession numbers are given in Fig. 1). Sequences were selected based on the result of a BLAST search through GenBank with the D1/D2 sequence of strain HNX16-2T as the query and on previous related studies (Kurtzman, 2007; Kurtzman & Robnett, 2007; Péter et al., 2011).

Strains HNX16-2T and HNX35-3 exhibited identical sequences in both D1/D2 and ITS regions, indicating their conspecificity. In the phylogenetic tree constructed from D1/D2 sequences, strain HNX16-2T formed a clade with...
the newly described teleomorphic ascomycetous yeast species *Spencermartinsiella europaea* (Péter et al., 2011) and a few undescribed yeast strains with 100 % bootstrap support (Fig. 1). This clade belongs to the family *Trichomonascaceae* (Kurtzman & Robnett, 2007). In the 572 bp D1/D2 domain sequenced, strain HNX16-2T differed from *S. europaea* NCAIM Y.01817T by 17 (3.0 %) mismatches (14 substitutions and 3 indels) and from other undescribed strains of this clade by 2.3–4.0 % mismatches. In the ITS region, strain HNX16-2T differed from *S. europaea* NCAIM Y.01817T by 16.5 % mismatches and from two other closely related strains, *Candida* spp. GY44502 and GA1504, by 13 % mismatches.

The yeast species closely related to strains HNX16-2T and HNX35-3 generally had a common ecological habitat, i.e. associated with rotten wood. *S. europaea*, the closest relative among the recognized species, was isolated from rotten wood collected in Hungary (Péter et al., 2011). The undescribed strains located in the *Spencermartinsiella* clade were also mostly isolated from rotten wood (G. Péter and C. F. Lee, personal communication). Other close relatives of the *Spencermartinsiella* clade included in the tree (Fig. 1), mostly belonging to members of the *Sugiyamaella* clade, were also isolated from rotten wood or related habitats, such as wood-ingesting insects and insect frass (Kurtzman, 2007; Kurtzman & Robnett, 2007; Suh et al., 2005; Wang et al., 2010).

Strains HNX16-2T and HNX35-3 also possessed the morphological and physiological criteria characterized in the *Spencermartinsiella* and closely related *Sugiyamaella* clades. In addition to cells that reproduced by multilateral budding (Fig. 2a), the novel species formed hyphae and pseudohyphae on various media including malt extract agar, corn meal agar and V8 juice agar. Blastocladia produced on denticulate conidiogenous cells, which are usually observed in species of the closely related genera *Sugiyamaella*, *Spencermartinsiella* and *Trichomonascus* and their anamorphs (Kurtzman & Robnett, 2007; Péter et al., 2011), were also observed in the two strains studied (Fig. 2b). However, the sexual state was not found for single or mixed cultures of the two strains on any of the sporulation media used, including McClary acetate agar, 5 % malt extract agar, corn meal agar and diluted (1 : 4 and 1 : 19) V8 juice agar, which were incubated at 25 °C for 2 months and observed at weekly intervals.

Physiologically, strains HNX16-2T and HNX35-3 were similar to the closely related species in the *Spencermartinsiella* and *Sugiyamaella* clades with positive xylose and cellobiose assimilation reactions but a negative methanol assimilation reaction (Kurtzman, 2007; Péter et al., 2011). However, strains HNX16-2T and HNX35-3 differed from the most closely related species *Spencermartinsiella europaea* in their inability to use L- and D-arabinose and hexadecane and their ability to use inulin as sole carbon source.

The rDNA sequence and phenotypic comparisons made above indicate that strains HNX16-2T and HNX35-3 represent a novel anamorphic yeast species in the *Spencermartinsiella* clade. According to the current taxonomic concept of ascomycetous yeasts (Kurtzman, 1998; Meyer et al., 1998), the name *Candida cellulosicola* sp. nov. is proposed.
Latin diagnosis of Candida cellulosicola F.-Y. Bai et X. Guo sp. nov.


Typus: isolatus ex ligno, HNX16-2T (=CBS 11952T), depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (CGMCC 2.3503T).

Description of Candida cellulosicola F.-Y. Bai & X. Guo sp. nov.

Candida cellulosicola [cell.u.lo.si´co.la. N.L. n. cellulosum cellulose; L. suff. cola (from L. n. incola) inhabitant, dweller; N.L. n. cellulosicola cellulose-dweller, referring to the substrate from which the type strain was isolated].

In YM broth (Yarrow, 1998), after 3 days at 25 °C, cells are oval and ellipsoid, 2.5–8.75 × 2.5–12.5 μm and occur singly, in pairs or in groups (Fig. 2). Budding is multimodal. Blastosporiæ produced on denticulate conidigenous cells are present. After 4 months at 25 °C, sediment is present. On YM agar, after 1 month at 25 °C, the streak culture is white to cream and smooth. The edge is finely lobed and fringed with filament. In Dalmat plate culture on corn meal agar, true hyphae and pseudohyphae are formed after 10 days at 25 °C. Ascospores are not formed. Glucose is not fermented. Glucose, sucrose, maltose, cellobiose, trehalose, galactose, melibiose, raffinose, melezitose, D-xylōse, L-rhamnose, L-sorbosum, lactose (delayed), D-ribose, erythritol, galactitol (delayed), glycerol, D-mannitol, D-sorbitol, ribitol, salicinum (weak), succinate, inulin, D-glucosamine (weak), D-glucitol (weak) and citrate are assimilated; soluble starch, D-lactate, L-arabinose, D-arabinose, inositol, methanol, ethanol, hexadecane, methyl D-glucoside and galactitol are not assimilated. Ammonium sulfate, L-lysine and ethylamine hydrochloride are assimilated; potassium nitrate, sodium nitrite and cadaverine hydrochloride are not assimilated. Growth in vitamin-free medium is positive. Maximum growth temperature is 33 °C. Starch-like compounds are not produced.

The type strain is HNX16-2T (=CGMCC 2.3503T= CBS 11952T), isolated from rotten wood collected from a rainforest, Hainan province, China, in July 2007.

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References


