Candida tibetensis sp. nov. and Candida linzhiensis sp. nov., novel anamorphic, ascomycetous yeast species from Tibet

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Three anamorphic, ascomycetous yeast strains isolated from plant samples collected in Linzhi District, Tibet, China, were revealed as representing two novel species by 26S rRNA gene D1/D2 domain sequence and physiological property comparisons. The names Candida tibetensis sp. nov. and Candida linzhiensis sp. nov. are proposed for these novel species, with XZ 41-6T (=AS 2.3072T = CBS 10298T) and XZ 92-1T (=AS 2.3073T = CBS 10299T) as the respective type strains. D1/D2 sequence analysis showed that C. tibetensis and C. linzhiensis are closely related to Candida carriycola and Candida sequanensis, respectively.

Candida tanzawaensis and Candida sequanensis were among the species of ascomycetous yeasts that were on isolated branches in the phylogenetic trees drawn from 26S rRNA gene D1/D2 domain sequences (Kurtzman & Robnett, 1997, 1998). Recently, more than 20 species with close phylogenetic relationships to C. tanzawaensis were described (Kurtzman, 2001; Suh et al., 2004). Most of the species were isolated from sources associated with insects. Consequently, the C. tanzawaensis clade has been expanded substantially. However, no close relatives of C. sequanensis have been reported.

In an investigation on Tibetan yeast diversity, two novel anamorphic, ascomycetous yeast species were identified. One of them was closely related to C. sequanensis, and the other to Candida carriycola. C. carriycola was described by Kurtzman (2001) as a species weakly associated with the C. tanzawaensis clade.

The yeast strains examined were isolated by an enrichment method using YM medium containing penicillin (100 μg ml⁻¹) and tetracycline (25 μg ml⁻¹) to inhibit the growth of bacteria. Strains XZ 41-6T, XZ 69C3 and XZ 92-1T were isolated from the flowers, leaves and fruit, respectively, of unidentified plants. These samples were collected in July 2004 from Linzhi District, Tibet, at altitudes of 3100–3300 m.

Most of the morphological, physiological and biochemical characteristics were examined according to standard methods (Yarrow, 1998). Assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase & Suzuki, 1986). Extraction, purification and identification of ubiquinones were carried out according to Yamada & Kondo (1973).

Nuclear DNA was extracted by the method of Makimura et al. (1994). The DNA fragment covering the internal transcribed spacer region (including 5.8S rDNA) and the 26S rDNA D1/D2 domain was amplified and sequenced by using a method described previously (Lu et al., 2004). Sequences were aligned with the program CLUSTAL X (Thompson et al., 1997). Molecular phylogenetic analysis was performed by using the methods described by Bai et al. (2002). Reference sequences were retrieved from GenBank under the accession numbers indicated in the tree shown in Fig. 1.

Strains XZ 41-6T and XZ 69C3 have identical D1/D2 and internal transcribed spacer sequences. In the phylogenetic tree drawn from the D1/D2 sequence alignment (Fig. 1), the two strains clustered together with C. carriycola. They differed from C. carriycola by 5-2 % mismatches (25 substitutions and 4 indels) in the D1/D2 domain. Strain XZ 92-1T was closely related to C. sequanensis (Fig. 1). This strain differed from C. sequanensis by 1-8 % (10 substitutions) nucleotide divergence in the D1/D2 domain. Another close relative of strain XZ 92-1T shown in the tree was Candida melibiosaceae, which was recently reinstated as a distinct species by Daniel (2003), with Trichosporon melibiosaceum – a former synonym of Candida fennica (Meyer et al., 1984) – as the basionym.

The novel Candida species described by Kurtzman (2001) and Suh et al. (2004), with the exception of C. carriycola, formed a strongly supported clade with C. tanzawaensis. C. carriycola was described as a species basal to the C.
tanzawaensis clade and weakly associated with the clade (Kurtzman, 2001). However, with the addition of novel species to the existing clades, the present study shows that C. caryicola appears to be more closely related to the clade represented by C. sequanensis than to the C. tanzawaensis clade (Fig. 1).

In agreement with their identical D1/D2 and internal transcribed spacer sequences, strains XZ 41-6T and XZ 69C3 were similar in terms of morphological and physiological characteristics, indicating that they are conspecific. They differed from the phylogenetically closest relative, C. caryicola, in the assimilation reactions with cellobiose, D-xylose, L-arabinose, D-arabinose and D-ribose, and in terms of growth in vitamin-free medium. Strain XZ 92-1T differed from its closest relative, C. sequanensis, in the assimilation reactions with L-sorbose, lactose, melibiose, raffinose, soluble starch, D-ribose, erythritol, methyl a-D-glucoside, hexadecane and L-lysine and in terms of growth in vitamin-free medium.

The molecular and physiological comparison demonstrated that strains XZ 41-6T and XZ 92-1T represent two distinct novel ascomycetous yeast species. As sexual states were not observed in cultures of single strains or in mating tests (between strains XZ 41-6T and XZ 69C3) on various media, including potato dextrose agar, 5 % malt extract agar, yeast carbon base (YCB) agar, cornmeal agar, acetate agar and dilute V8 agar (Yarrow, 1998), they were assigned to the genus Candida Berkhout according to the current taxonomy of yeasts (Kurtzman, 1998; Meyer et al., 1998). Therefore, we propose the names Candida tibetensis sp. nov. and Candida linzhiensis sp. nov. for strains XZ 41-6T and XZ 92-1T, respectively.

**Latin diagnosis of Candida tibetensis Bai & Wu sp. nov.**

Description of Candida tibetensis Bai & Wu sp. nov.

Candida tibetensis (ti.be.te.nis. N.L. fem. adj. tibetensis pertaining to Tibet, the geographical origin of the type strain of the species).

In YM broth, after 3 days at 25°C, the cells are cylindrical to bacilliform, 2.5–2.7×5.0–21.4 μm and occur singly, in pairs, in chains or in branches (Fig. 2a). Budding is multilateral. After 1 month at 25°C, sediment and a climbing ring are present. On YM agar, after 1 month at 25°C, streak culture is butyrous, membranous, cream-coloured, raised, dull, smooth and creased with ridged protuberances; the margin is undulating. In Dalmau plate culture on cornmeal agar, pseudohyphae are formed. Sporulation is not observed. Glucose is fermented, but galactose, sucrose, maltose, lactose and raffinose are not fermented. Glucose, galactose, sucrose, maltose, cellobiose (delayed), trehalose, melezitose, D-xylene, L-arabinose, D-arabinose (delayed), D-ribose (delayed; weak), D-glucosamine, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, methyl α-D-glucoside, salicin (infirme), acidum succinicum (lente), acidum citricum (infirme), et hexadecanum assimilantur at non L-sorbus, lactosum, melibiosum, raffinosum, inulinum, amyllum solubile, L-rhamnosum, methanol, erythritol, galactitol, acidum DL-lacticum nec inositolum. Ammonium sulfatum, ethylaminum, L-lysimum et cadaverinum assimilantur at non natrum nitrosum nec kalium nitricum. Ad cresciantem vitaminae externae non necessariae sunt. Maxima temperatura crescentiae 28°C. Materia amyloidea idophila non formatur. Diazonium caeruleum B non respondens. Ureum non hydrolysatur. Systema coenzymatis Q-9 adest. Typus depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.3072T).

Latin diagnosis of Candida linzihiensis Bai & Wu sp. nov.

In medio liquido YM post dies 3 ad 25°C, cellulae subglobosae (2.7–5.2×3.7–6.0 μm), ovoideae ad elongatae (3.7–4.5×23.6–24.8 μm), singulae, binae vel adhaerentes, blastospora fiunt. Per gammadonem multipolare reproductentes. Post 1 mensum sedimentum et annulus formantur. In agaro YM post 1 mensum ad 25°C, butyrosa, viscidulus, cremea, seminitida, glabra cum radiatis et vittatis tuberculam, margo undulata. In agaro farinaceae Zea mays post dies 7, pseudohyphae et mycelium verum fiunt. Ascosporae non fiunt. Glucosum et galactosum (lente; infirme) fermentantur at non sucrosum, maltosum, lactosum nec raffinosum. Glucosum, galactosum, L-sorbosum (lente), maltosum, cellobiose, trehalosum, lactosum (lente), melibiosum (lente; infirme), raffinosum (lente; infirme), D-xylom, L-arabinosum, D-ribosem (lente),

Fig. 2. Vegetative cells of C. tibetensis XZ 41–6T (a) and C. linzihiensis XZ 92–1T (b) grown in YM broth for 3 days at 25°C. Bars, 10 μm.

Description of Candida linzhiensis Bai & Wu sp. nov.

Candida linzhiensis (lin.zhi.en’sis. N.L. fem. adj. linzhiensis pertaining to Linzhi, the geographical origin of the type strain of the species).

In YM broth, after 3 days at 25 °C, the cells are subglobose (2.7–5.2 × 3.7–6.0 μm) to ovaloid to elongate (3.7–4.5 × 23.6–28.4 μm) and occur singly, in pairs or in groups (Fig. 2b). Elongated cells are straight or curved and may produce small denticles that bear blastoconidia. Budding is multilateral. After 1 month at 25 °C, sediment and a climbing ring are present. On YM agar after 1 month at 25 °C, streak culture is butyrous, viscous, cream-coloured, low and raised, semi-glossy and smooth with faint striations as well as ridged protuberances; the margin is slightly undulating. In Dalmau plate culture on cornmeal agar, pseudohyphae are ridged protuberances; the margin is slightly undulating. Sporulation not observed. Glucose and galactose (delayed; weak) are fermented; sucrose, maltose, lactose and raffinose are not fermented. Galactose, glycerol, lactose, maltose, cellobiose, trehalose, lactose (delayed), melibiose (delayed; weak), raffinose (delayed; weak), D-xyllose, L-arabinose, D-ribose (delayed), ethanol, glyceral, erythritol (delayed), ribitol, D-mannitol, D-glucitol, methyl α-D-glucoside, salicin (delayed) and succinic acid (delayed) are assimilated; sucrose, melezitose, inulin, soluble starch, D-arabinose, L-rhamnose, D-glucosamine, methanol, galactitol, DL-lactic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate, ethylamine hydrochloride and cadaverine dihydrochloride are assimilated; sodium nitrite, potassium nitrate and L-lysine are not assimilated. Grows in vitamin-free medium, but growth is weak and delayed. Maximum growth temperature is 28 °C. Starch-like compounds are not produced. Diazonium blue B reaction is negative. Negative for urease activity. Major ubiquinone type is Q-8.

The type strain, XZ 92-1T (=AS 2.3073T = CBS 10299T), was isolated from a fruit of an unidentified plant collected in Linzhi District, Tibet, in July 2004.

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References


