Candida golubevii sp. nov., an asexual yeast related to *Metschnikowia lunata*

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Two strains of a novel yeast species were isolated from insect frass and a flower in Thailand and Brazil, respectively. The strain from Thailand was isolated from insect frass collected in Than-Tong waterfall, Nong Khai Province, whereas the strain from Brazil was recovered from a flower of *Ipomoea* sp. collected on the banks of the Paraguai River in the state of Mato Grosso do Sul. The sequences of the D1/D2 domains of the large subunit of the rDNA of both strains were identical. This novel species belonged to the *Metschnikowia* clade and was related to *Metschnikowia lunata*. No signs of sporulation were observed for the two strains on various culture media. The novel species, *Candida golubevii* sp. nov., is proposed to accommodate these isolates. The type strain is BCC 8332^T (=CBS 11362^T=NBRC 105679^T).

Metschnikowia lunata is the only described representative of its own subclade within the *Metschnikowia* clade (Mendonça-Hagler *et al.*, 1993). The species was isolated from flowers and plant exudates and differs from other *Metschnikowia* species in forming lunate vegetative cells (Miller & Phaff, 1998). Lachance *et al.* (2003) reported on the isolation of another subclade member (*Candida* sp. UWOPS 02-210.2/MAL-2003) from a flower of *Ipomoea indica* (Convolvulaceae) in Hawaii, but have not published a formal description. In the course of two independent studies of the yeast communities associated with insects and flowers in Thailand and Brazil, two isolates possessing lunate cells were also obtained. Sequencing of the D1/D2 domain of the large subunit rRNA gene showed that these yeasts represent a novel species related to *M. lunata*. The isolates did not produce ascospores when tested on several sporulation media, alone or mixed. In this paper, the novel species is named as *Candida golubevii* sp. nov., in honour of Dr Wladyslav I. Golubev in recognition of his contributions to yeast taxonomy in Russia.

Strain BCC 8332^T (BCC, BIOTEC Culture Collection) was isolated from insect frass collected in Than-Tong waterfall, Nong Khai Province, Thailand, in February of 2001. Strain UFMG-C117A (UFMG, Culture Collection of Micro-organisms and Cells of the Universidade Federal de Minas Gerais) was obtained from a flower of *Ipomoea* sp. (Convolvulaceae) collected on the banks of the Paraguai River near the city of Corumbá, state of Mato Grosso do Sul, within the Pantanal ecosystem, Brazil, in February of 2002. The yeasts were isolated on yeast extract-malt extract agar (YMA; 1 % glucose, 0.5 % peptone, 0.3 % yeast extract, 0.3 % malt extract and 2 % agar) containing 100 mg chloramphenicol l⁻¹. Sampling methods used were as described previously (Rosa *et al.*, 2007). Plates were incubated at room temperature (25 ± 3 °C) for 3–8 days. Each different yeast morphotype was purified and maintained on YMA agar slants or in liquid nitrogen for later identification. The yeasts were characterized by using standard methods (Yarrow, 1998). Identification followed the keys of Kurtzman & Fell (1998).

The nucleotide sequences of the D1/D2 domain of the large subunit rDNA of the strain from Thailand were determined according to procedures described by Jindamorakot

The GenBank/EMBL/DDBJ accession number for the sequence of the D1/D2 domain of the 26S rDNA of strain BCC 8332^T is DQ404455.

et al. (2008). The D1/D2 domains of the large subunit of the rDNA of the Brazilian strain were amplified by PCR directly from whole cells, as described previously (Lachance *et al.*, 1999). The sequence was edited with the program DNAMAN, version 6.0 (Lynnon BioSoft). Existing sequences for other yeasts were retrieved from GenBank. The CLUSTAL W software (Thompson *et al.*, 1994) incorporated in DNAMAN was used to align the sequences and construct a neighbour-joining tree with 1000 bootstrap iterations.

Species delineation and ecology

C. golubevii sp. nov. belongs to the *Metschnikowia* clade and is related to *M. lunata* (Fig. 1). These two species showed 10% divergence in the D1/D2 region of the rDNA large subunit. *C. golubevii* was also related to an undescribed *Candida* species (strain UWOPS 02-210.2/MAL-2003) isolated from a flower of *I. indica* in Hawaii (Lachance *et al.*, 2003), with which it differs by 9% sequence divergence in the same gene region. The D1/D2 sequences of the two isolates of *C. golubevii* were identical.

The habitat specificity of *C. golubevii* was not easily established as one strain was isolated from insect frass in Thailand and the other from a morning-glory flower in Brazil. The species might be associated with floricolous insects. The closest described relative of *C. golubevii*, *M. lunata*, was isolated from a flower of *Vicia cracca* (Fabaceae) in Russia and from an exudate of *Casuarina* sp. (Casuarinaceae) in Japan (Miller & Phaff, 1998), also suggesting an association with the insect–plant interface. The isolation of *C. golubevii* in Thailand and Brazil suggests that it is rare, but widespread in tropical environments.

The two isolates of *C. golubevii* sp. nov. alone or mixed in pairs were examined after growth on the most common

sporulation media at 17 and 25 °C (corn meal agar, dilute V8 agar, 5% malt extract agar, and yeast carbon base agar supplemented with 0.01% ammonium sulphate, among others), but asci or signs of conjugation were not seen.

Latin diagnosis of *Candida golubevii* Rosa, Jindamorakot, Limtong, Nakase, Pagnocca et Lachance sp. nov.

In medio liquido post dies tres cellulae singulae aut binae, cellulae ovoidae aut lunatae ($2\text{--}3 \times 2\text{--}5\ \mu\text{m}$). *Post unum mensem sedimentum formatur. Cultura in agaro multi post dies 2* (17 °C) *parva, convexa, glabra et candida. In agaro farinae Zea mays post dies 14 mycelium nec pseudomycelium non formantur. Glucosi fermentatur. Glucosum, galactosum, L-sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, melezitolum, D-xylosum (lente), D-ribosum (lente), ethanolum (exiguae lente), glycerolum, ribitolum (exiguae lente), D-mannitolum, D-glucitolum, methyl α -D-glucosidum, salicinum, acidum 2-ketogluconicum, acidum succinicum (variabile), xylitolum, acidum D-gluconicum (lente), D-glucosaminum (lente), N-acetylglucosaminum et hexadecanum (lente) assimilantur, at non lactosum, melibiosum, raffinose, inulinum, amyllum solubile, L-arabinosum, D-arabinosum, L-rhamnosum, erythritolum, galactitolum, glucono- δ -lactonum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum citricum, inositolum, acidum D-glucuronicum, acidum D-galacturonicum, L-arabinitolum, propanum-1,2-diolum, butanum-2,3-diolum, nec methanolum. Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum. Ad crescentiam vitamina externa necessaria sunt. Augmentum in 37 °C (variabile). Habitat insectum et flores in Thailand et Brazil. Typus BCC 8332^T (=CBS 11362^T=NBRC 105679^T). In collectione zymotica Centraalbureau voor Schimmelcultures.*

Fig. 1. Neighbour-joining tree showing the placement of *Candida golubevii* sp. nov. among related species in the *Metschnikowiaceae* clade. Bootstrap values of 50% and above are shown. Bar, 0.05 substitutions per nucleotide position.

Fig. 2. Phase-contrast micrograph of budding cells of *Candida golubevii* BCC 8332^T (=CBS 11362^T) on yeast carbon base agar (Difco) with 0.01 % ammonium sulphate after 2 days at 20 °C. Bar, 5 µm.

Description of *Candida golubevii* Rosa, Jindamorakot, Limtong, Nakase, Pagnocca & Lachance sp. nov.

Candida golubevii (go.lu.be'vi.i. N.L. gen. masc. sing. n. *golubevii* of Golubev, in honour of Dr W. I. Golubev for his contributions to yeast taxonomy).

In yeast extract (0.5 %), glucose (2 %) broth after 3 days at 25 °C, the cells are ovoid to lunate (2–3 × 2–5 µm). Budding is multilateral (Fig. 2). A sediment is formed after a month, but no pellicle is observed. On YMA agar after 2 days at 17 °C, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, pseudomycelia or true mycelia are not formed, but chains of budding cells are sometimes present. Fermentation of glucose is positive and galactose variable. Maltose, cellobiose, trehalose, melezitose, D-xylose and methyl α-D-glucoside are not fermented. Assimilation of carbon compounds: glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melezitose, D-xylose (delayed), D-ribose (delayed), ethanol (weak delayed), glycerol, ribitol (weak delayed), D-mannitol, D-glucitol, methyl α-D-glucoside, salicin, 2-ketogluconic acid, succinic acid (variable), xylitol, D-gluconic acid (delayed), D-glucosamine (delayed), N-acetyl-D-glucosamine and hexadecane (delayed) are assimilated. No growth occurs on lactose, melibiose, raffinose, inulin, soluble starch, L-arabinose, D-arabinose, L-rhamnose, erythritol, galactitol, glucono-δ-lactone, 5-ketogluconic acid, DL-lactic acid, citric acid, inositol, D-glucuronic acid, D-galacturonic acid, L-arabinitol, propane-1,2-diol, butane-2,3-diol or methanol. Assimilation of nitrogen compounds: positive for

lysine, ethylamine-HCl and cadaverine, and negative for nitrate and nitrite. Growth in vitamin-free medium is negative; biotin and thiamine are required. Growth in amino-acid-free medium is positive. Growth at 37 °C is variable. Growth on YMA agar with 10 % sodium chloride is positive. Growth in 50 % glucose/yeast extract (0.5 %) is weak to strong. 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. Habitat is insect frass and flower of *Ipomoea* sp. in Thailand and Brazil, respectively.

The type strain is BCC 8332^T (=CBS 11362^T=NBRC 105679^T), isolated from insect frass collected in Than-Tong waterfall, Nong Khai Province, Thailand.

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