Candida bromeliacearum sp. nov. and Candida ubatubensis sp. nov., two yeast species isolated from the water tanks of Canistropsis seidelii (Bromeliaceae)

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Strains belonging to two novel yeast species, Candida bromeliacearum and Candida ubatubensis, were isolated from the bromeliad tank of Canistropsis seidelii (Bromeliaceae) in a sandy coastal plain (restinga) ecosystem site in an Atlantic rainforest of south-eastern Brazil. These species were genetically distinct from all other currently accepted ascomycetous yeasts, based on sequence divergence in the D1/D2 domains of the large-subunit rDNA and in the small-subunit rDNA. The species occupy basal positions in the Metschnikowiaceae clade. The type strains are Candida bromeliacearum UNESP 00-103T (=CBS 10002T = NRRL Y-27811T) and Candida ubatubensis UNESP 01-247R (=CBS 10003T = NRRL Y-27812T).

Yeast isolation and characterization

Three strains each of Candida bromeliacearum and Candida ubatubensis were isolated from water tanks of the bromeliad Canistropsis seidelii in the Picinguaba site in the ‘Serra do Mar’ State Park, in São Paulo State, Brazil (23°22’ 44°48’ W). This State Park contains one of the largest continuous areas of the remaining Brazilian Atlantic Forest in eastern São Paulo State, and is located 230 km from the city of São Paulo. For the isolation of strains belonging to Candida bromeliacearum, the water tanks of six plants were collected during spring (September) 2000, whereas the strains belonging to Candida ubatubensis were isolated during summer (February) 2001, when the water reservoirs of five plants were examined. The plants occurred over a large area of the forest.

The water collected was stirred with a sterile loop and streaked in triplicate on YM agar (1 % glucose, 0.5 % peptone, 0.3 % malt extract, 0.3 % yeast extract, 2 % agar) containing 100 mg chloramphenicol 1 -1 (Trindade et al., 2002). The plates were incubated at 25 °C for 3–5 days, and one of each distinct morphotype was isolated and maintained on YM agar slants at 6–8 °C and at −80 °C. The yeasts were characterized by using standard methods (Yarrow, 1998), and their identification was carried out using the keys of Kurtzman & Fell (1998) and the CD-ROM Yeasts of the World (Boekhout et al., 2002).
DNA sequence analysis

Yeast DNA was extracted and purified according to a protocol recommended for the Genomic Prep. Cells and Tissue DNA isolation kit (Amersham Pharmacia Biotech). The divergent D1/D2 domains of the LSU rDNA were amplified with primers NL1 and NL4 (O’Donnell, 1993). Each PCR was performed with the Ready-To-Go kit (Amersham Pharmacia Biotech) using 1-5 µl solution containing approximately 100 ng DNA, 1-6 µl NLI primer, 1-1 µl NL4 primer (6 pmol each) and 17-8 µl Milli-Q water. The sequence products were resolved in an ABI Prism 377 DNA sequencer (Applied Biosystems) at the Centro de Estudos de Insetos Sociais – UNESP, Rio Claro, São Paulo, Brazil. Alternatively, the DNA was amplified directly from whole cells and sequenced as described by Lachance et al. (1999).

The SSU rDNA was amplified by using this method, with primers SSU1f (5'-TGGAGG-GCAATCTGTCGCACT-3') and SSU2r (5'-ATGATCCTTTCCGGGAGTTCAC-3'). The physiological characteristics of the novel species were examined on Qiagen columns and sequenced using the same primers as for the amplification, plus the primers SSU3f (5'-TGGAGGG-CAGTATCCTGGTCCA-3') and SSU4r (5'-AICTAAGAACGGCCATGCACA-3'). Sequencing was performed with an ABI automated sequencer at the Robarts Research Institute, London, Ontario, Canada. Sequence alignment and tree construction were done with the program DNAMAN 4.1 (Lynnon Biosoft).

Species delineation, classification and ecology

The physiological characteristics of the novel species were typical of those of other species in the Metschnikowiaceae clade, although the absence of growth on galactose and the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of trehalose observed for the weak utilization of 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directly associated with the bromeliad water tank, which has a dynamic influx of a diversity of arthropod and amphibian visitors. As only three isolates of *Candida bromeliacearum* and three of *Candida ubatubensis* were recovered from a collection that comprised 29 isolates, the role of these species in the bromeliad yeast community remains obscure. In a similar approach, Hagler et al. (1993) and Araujo et al. (1998) described the yeast communities of the water tanks of the bromeliad species *Quesnelia quesneliana*, *Quesnelia arvensis*, *Neoregelia cruenta*, *Nidularium procedurum*, *Aechmea nudicaulis* and *Vriesia procer*a occurring in mangrove and sand dune ecosystems. The yeast communities found in those reports and in our study are quite different, as they share only *Candida fansata* and *Candida intermedia*, two widespread, generalistic species.

**Latin diagnosis of Candida bromeliacearum**

Ruivo, Pagnocca, Lachance et Rosa sp. nov.


**Description of Candida bromeliacearum**

Ruivo, Pagnocca, Lachance & Rosa sp. nov.

*Candida bromeliacearum* (bro.me.li.a.ce.a.’rum. N.L. gen. n. *bromeliacearum* of Bromeliaceae, referring to the plant from which the yeast was isolated).

In yeast extract (0.5 %) glucose (2 %) broth after 3 days at 25°C, the cells are ellipsoidal to elongate (3–5 × 4–6 μm), and occur singly, in budding pairs or in short chains. Buds are produced multilaterally (Fig. 2a). On YM agar after 4 days at 25°C, the colonies are white to cream, smooth and butyrous. After 2 weeks in Dalmau plate culture on cornmeal agar, pseudomycelium or true mycelium is not formed. Asci are not formed on common sporulation media. Glucose fermentation is complete after 2–5 days. Assimilation of carbon compounds: glucose, sucrose, trehalose (weak), maltose, melezitose, methyl α-D-glucoside, cellobiose, salicin, L-sorbose, D-xyllose, ethanol, glycerol (variable), erythritol (variable), ribitol, xylitol, D-mannitol, D-glucitol, citric acid (weak and variable), D-gluconate (variable), glucono-δ-lactone, 2-ketogluconic acid, gluconic acid, N-α-acetylglosamin and n-hexadecane (slow) are assimilated. No growth occurs on inulin, raffinose, melibiose, galactose, lactose, starch, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, L-arabinitol, galactitol, myo-inositol, lactic acid, succinic acid, 5-ketogluconic acid, saccharate or D-glucuronate. The following nitrogen compounds are assimilated: cadaverine, lysine and ethylamine; nitrate and nitrite are negative. Growth at 35°C is positive and negative at 37°C. Acid formation on chalk agar is positive. Urease activity and Diazonium blue B reaction are negative. Production of amyloid compounds is negative.

![Fig. 2. Photomicrographs of cells of Candida bromeliacearum strain UNESP 00-103](http://ijs.sgmjournals.org) (a) and *Candida ubatubensis* strain UNESP 01-247R² (b) in yeast extract, glucose broth after 3 days at 25°C. Bars, 10 μm.
Growth on 50% glucose/yeast extract agar is slow. Growth on YM agar with 10% NaCl is positive. Growth in the presence of 1% acetic acid is negative. Growth in the presence of 0-1 and 0-01% cycloheximide is negative.

The type strain, UNESP 00-103T, has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as strain CBS 10002T (= NRRL Y-27811).

**Latin diagnosis of Candida ubatubensis Ruivo, Pagnocca, Lachance et Rosa sp. nov.**


**Description of Candida ubatubensis Ruivo, Pagnocca, Lachance & Rosa sp. nov.**

*Candida ubatubensis* (u.ba.tu.ben’sis. N.L. nom. fem. adj. ubatubensis of Ubatuba, referring to a town near which the yeast was isolated).

In yeast extract (0-5%) glucose (2%) broth after 3 days at 25°C, the cells are spheroidal to ovoid (3-4.5 × 4-6 μm), and occur singly or in budding pairs. Buds are produced multilaterally (Fig. 2b). On YM agar after 4 days at 25°C, the colonies are white to cream, smooth and butyrous. After 2 weeks in Dalmau plate culture on cornmeal agar, pseudomyelum or true mycelium is not formed. Asci are not formed on common sporulation media. Glucose fermentation is complete after 2-5 days. Assimilation of carbon compounds: glucose, galactose, sucrose, trehalose, maltose, melezitose, methyl α-D-glucoside, cellobiose, salicin, L-sorbose, L-rhamnose (weak), D-xyllose, D-ribose, ethanol, glycerol, erythritol, ribitol, xylitol, L-arabininitol, D-manninitol, D-glucitol, D-glucanate, glucono-δ-lactone (variable), N-acetylglicosamine and n-hexadecane are assimilated. No growth occurs on inulin, raffinose, melibiose, lactose, starch, L-arabinose, D-arabinose, methanol, galactitol, myo-inositol, lactic acid, succinic acid, citric acid, 2-ketoglucanonic acid, 5-ketogluconic acid or glucosamine. The following nitrogen compounds are assimilated: lysine, ethylamine and cadaverine; nitrate and nitrite are negative. Growth at 35°C is positive and negative at 37°C. Urease activity and Diazonium blue B reaction are negative. Production of amyloid compounds is negative. Growth on 50% glucose/yeast extract agar is negative. Growth on YM agar with 10% NaCl is positive. Growth in the presence of 1% acetic acid is negative. Growth in the presence of 0-1 and 0-01% cycloheximide is negative.

The type strain, UNESP 01-247R T, has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as strain CBS 10003T (= NRRL Y-27812T).

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**References**


