Candida heliconiae sp. nov., Candida picinguabensis sp. nov. and Candida saopaulonensis sp. nov., three ascomycetous yeasts from Heliconia velloziana (Heliconiaceae)

Carla C. C. Ruivo,1 Marc-André Lachance,2 Carlos A. Rosa,3 Maurício Bacci, Jr1 and Fernando C. Pagnocca1

1Centro de Estudos de Insetos Sociais e Departamento de Bioquímica e Microbiologia, Universidade Estadual Paulista – Unesp, CP 199, Rio Claro, SP, 13506-900, Brazil
2Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada
3Departamento de Microbiologia – ICB, CP 486, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil

Strains belonging to three novel yeast species, Candida heliconiae (four isolates), Candida picinguabensis (three isolates) and Candida saopaulonensis (two isolates), were recovered in the year 2000 from water of flower bracts of Heliconia velloziana L. Emigd. (Heliconiaceae) found in a forest ecosystem site in an Atlantic rainforest of south-eastern Brazil. C. picinguabensis and C. saopaulonensis were nearly identical in morphology and physiology, but sequence divergence in the D1/D2 domain of the large-subunit rDNA indicated that they should be regarded as different species. They belong to the Metschnikowiaceae clade. C. heliconiae had affinities to Pichia mexicana and related species, but was genetically isolated from all currently accepted species in that group. The type strains are C. heliconiae Unesp 00-91C1T (=CBS 10000T = NRRL Y-27813T), C. picinguabensis Unesp 00-89T (=CBS 9999T = NRRL Y-27814T) and C. saopaulonensis Unesp 00-99T (=CBS 10001T = NRRL Y-27815T).

Yeast isolation and characterization

Four strains of C. heliconiae, three of C. picinguabensis and two of C. saopaulonensis were isolated from the water of flower bracts of Heliconia velloziana collected in the Picinguaba area, an Atlantic rainforest site in the ‘Serra do Mar’ State Park in São Paulo State, Brazil (23° 22’ S 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. Collections were made from 14 plants during spring (September) 2000. The water accumulated in the bract was stirred with a sterile loop that was then used to streak-inoculate three plates of YM agar (1% glucose, 0.5% peptone, 0.3% malt extract, 2% agar) containing 100 mg chloramphenicol 1–1 (Trindade et al., 2002). The plates were incubated at 25 °C for 5 days. Selected representative colonies were purified and maintained on YM agar slants at 4 °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 large-subunit rDNA were symmetrically amplified with primers NL-1 and NL-4 (O’Donnell, 1993). Each PCR was performed with the Ready-To-Go kit (Amersham Pharmacia Biotech), according to the manufacturer’s recommendations.

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). Sequence alignment and tree construction were done with the program DNAMAN 4.1 (Lynnon Biosoft).

**Species delineation, classification and ecology**

All strains were examined after growth on common sporulation media, either alone or in pairwise mixtures. Conjugation or ascus formation was not observed. In the absence of a sexual cycle, species delineation relied on sequence divergence. Based on the analysis of the large-subunit rDNA D1/D2 domains, *C. picinguabensis* and *C. saopaulonensis* represent sister species with affinities to the Metschnikowiaeae clade. The sequences of the two taxa differed from each other by 18 substitutions and three gaps, which supports the hypothesis that they represent separate species (Kurtzman & Robnett, 1998). Physiologically, the two species differed only in the assimilation of galactose and the ability to grow in the presence of 10 μg cycloheximide ml⁻¹. The species shown in Fig. 1(a) are representatives of neighbouring clades, chosen to identify the approximate phylogenetic position of the novel species. A reliable connection with any known species within the Metschnikowiaeae clade could not be established, although a weak link with *Metschnikowia* and related *Candida* species found in beetles and other insects of morning glories was apparent. *C. heliconiae* has no clearly identifiable sister species and occupies a basal position in a clade that contains *Pichia mexicana* and related *Pichia* or *Candida* species. The species in Fig. 1(b) were selected to assist in localizing *C. heliconiae* phylogenetically. A weak connection was found with *Candida sinolarantum* and other species known to be associated with the plant–insect interface.

Although the newly described species were isolated from water accumulated in bracts of *Heliconia velloziana*, it cannot be assumed that they are associated only with this plant, as *Heliconia* species are visited by hummingbirds attracted by the flower’s nectar (Stiles, 1975). In addition to the novel species described herein, other yeasts isolated from the same substrates included *Candida azyma*, *Candida boidinii*, *Candida pseudointermedia*, *Candida reisingiae*, *Candida silvae*, *Debaryomyces* spp., *Hanseniaspora uvarum*, *Klyveromyces* sp., *Kodamaea* sp., *Metschnikowia koreensis* and *Metschnikowia* sp., which are often found in flowers (Rosa et al., 1999; Hong et al., 2001). As *C. picinguabensis* and *C. saopaulonensis* have similar physiologies and morphologies, they are expected to occur in similar microhabitats. Our results suggest that these novel species are nectar-inhabiting yeasts.

![Fig. 1. Neighbour-joining dendrograms, based on sequences of the D1/D2 domains of the large-subunit rDNA, depicting the approximate phylogenetic positions of *C. picinguabensis* and *C. saopaulonensis* (a) and *C. heliconiae* (b). Bootstrap values greater than 75 %, determined from 1000 interactions, are shown. Bars, sequence divergence with Kimura’s two-parameter correction. Root placement was based on the inclusion of *Schizosaccharomyces pombe* (NRRL Y-12796). GenBank accession no. U40085; not shown) as an outgroup.

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

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

*Candida heliconiae* (hel.i.co’ni.ae. N.L. gen. n. heliconiae of *Heliconia velloziana*, referring to the plant from which the species was isolated).

In yeast extract (0.5 %) glucose (2 %) broth after 3 days at 25 °C, cells occur singly or in budding pairs. Cells are spheroidal to ovoid (3–5 μm). Buds are produced multilaterally (Fig. 2a). On YM agar after 4 days at 25 °C, colonies are cream-coloured or white, low-convex, smooth and butyrous. After 2 weeks in Dalmau plate culture on cornmeal agar, pseudomycelium or true mycelium is not formed. Glucose fermentation is complete after 2–5 days. The carbon compounds glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, melezitose, D-xylene, L-arabinose (variable), D-arabinose, D-glucosamine (variable), N-acetyl-D-glucosamine, ethanol, glycerol, ribitol, mannitol, glucitol, methyl D-glucoside, D-gluconic acid (slow), salicin (weak) and glucono-δ-lactone are assimilated. No growth occurs on trehalose, lactose, melibiose, raffinose, inulin, starch, D-ribose, L-rhamnose, melezitose, D-xylose, galactitol, DL-lactic acid, succinic acid, citric acid, myo-inositol, N-hexadecane, 2-keto-D-gluconate, 5-keto-D-gluconate or xylitol. Assimilation of nitrogen compounds: L-lysine, ethylamine and cadaverine are positive; nitrate and nitrite are negative. Growth at 35 °C is positive and negative at 37 °C. Acid formation on chalk agar is weak or absent. 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 negative. Growth in the presence of 10 and 100 μg cycloheximide ml⁻¹ is positive. Growth in the presence of 1 % acetic acid is negative.

The type strain, UNESP 00-91C₁ᵀ, was isolated from water accumulated in flower bracts of *Heliconia velloziana* in Brazil. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as strain CBS 10000ᵀ (=NRRL Y-27813ᵀ).

Latin diagnosis of *Candida picinguabensis* Ruivo, Pagnocca, Lachance & Rosa sp. nov.

Trajectum ad Rhenum, sub no. CBS 9999T, typus stirps deposita est.

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

Candida picinguabensis (pi.c.i.n.gua’ben.sis. N.L. fem. adj., picinguabensis pertaining to Picinguaba area, referring to the locality where the species was isolated).

In yeast extract (0-5 %) glucose (2 %) broth after 3 days at 25 °C, cells occur singly or in budding pairs. Cells are spheroidal (3-4-8 µm). Buds are produced multilaterally (Fig. 2b). On YM agar after 4 days at 25 °C, colonies are cream-coloured or white, low-convex, smooth and butyrous. After 2 weeks in Dalmou plate culture on cornmeal agar, pseudomycelium or true mycelium is not formed. Glucose fermentation is complete after 2-5 days. The carbon compounds glucose, L-sorbitose, sucrose, maltose, trehalose, melezitose, D-xyllose, ethanol, ribitol, mannitol, glucitol, methyl D-glucoside, D-gluconic acid (variable), DL-lactic acid (slow), succinic acid (weak), citric acid (weak), n-hexadecane (slow), glucono-D-lactone, 2-keto-D-gluconate and xylitol are assimilated. No growth occurs on galactose, cellobiose, lactose, melibiose, raffinose, inulin, starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, N-acetyl-D-glucosamine, mannitol, glycerol, erythritol, galactitol, salicin, myo-inositol or 5-keto-D-gluconate. Assimilation of nitrogen compounds: L-lysine, ethylamine and cadaverine are positive; nitrate and nitrite are negative. Growth at 37 °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 amylloid compounds is negative. Growth on 50 % glucose/yeast extract agar is slow. Growth on YM agar with 10 % NaCl is negative. Growth in the presence of 100 µg/ml cycloheximide is negative. Growth in the presence of 1 % acetic acid is negative.

The type strain, UNESP 00-99T, was isolated from water accumulated in flower bracts of Heliconia velloziana in Brazil. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as strain CBS 9999T (= NRRL Y-27814T).

Latin diagnosis of Candida saopaulonensis Ruivo, Pagnocca, Lachance & Rosa sp. nov.

In medio liquido post dies tres ad 25 °C, cellulae globosae, singulae aut binae (3-6-5 x 4-7 µm). Cultura in agaro extracta mali et levidinis continente post 4 dies ad 25 °C, albida cremetur et butyrosa. In agaro farinae Zea mays post dies 14 pseudomycelium non formatur. Ascii non formatur.


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

Candida saopaulonensis (sao.pau.lo.nen.sis. N.L. fem. adj., saopaulonensis pertaining to São Paulo State, referring to the Brazilian state where the species was isolated).

In yeast extract (0-5 %) glucose (2 %) broth after 3 days at 25 °C, cells occur singly or in budding pairs. Cells are spheroidal (3-6-5 x 4-7 µm). Buds are produced multilaterally (Fig. 2c). On YM agar after 4 days at 25 °C, colonies are cream-coloured or white, low-convex, smooth and butyrous. After 2 weeks in Dalmou plate culture on cornmeal agar, pseudomycelium or true mycelium is not formed. Glucose fermentation is complete after 2-5 days. The carbon compounds glucose, galactose, L-sorbose, sucrose, maltose, trehalose, melezitose, D-xyllose, ethanol, ribitol, mannitol, glucitol, methyl D-glucoside, D-gluconic acid, DL-lactic acid, succinic acid (weak), citric acid (weak), n-hexadecane, glucono-D-lactone, 2-keto-D-gluconate and xylitol are assimilated. No growth occurs on galactose, cellobiose, lactose, melibiose, raffinose, inulin, starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, N-acetyl-D-glucosamine, mannitol, glycerol, erythritol, galactitol, salicin, myo-inositol or 5-keto-D-gluconate. Assimilation of nitrogen compounds: L-lysine, ethylamine and cadaverine are positive; 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 amylloid compounds is negative. Growth on 50 % glucose/yeast extract agar is slow. Growth on YM agar with 10 % NaCl is negative. Growth in the presence of 100 µg/ml cycloheximide is negative. Growth in the presence of 1 % acetic acid is negative.

The type strain, UNESP 00-89T, was isolated from water accumulated in flower bracts of Heliconia velloziana in Brazil. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures,
Utrecht, the Netherlands, as strain CBS 10001T (= NRRL Y-27815T).

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References


