Candida flosculorum sp. nov. and Candida floris sp. nov., two yeast species associated with tropical flowers

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Two ascomycetous yeast species, Candida flosculorum sp. nov. and Candida floris sp. nov., were isolated from tropical flowers and their associated insects. C. flosculorum was isolated from flower bracts of Heliconia velloziana and Heliconia episcopalis (Heliconiaceae) collected from two Atlantic rain forest sites in Brazil. C. floris was isolated from flowers of Ipomoea sp. (Convolvulaceae) growing on the banks of the river Paraguai in the pantanal ecosystem in Brazil and from an adult of the stingless bee Trigona sp. and a flower of Merremia quinquefolia (Convolvulaceae) in Costa Rica. C. flosculorum belongs to the Metschnikowiaceae clade and C. floris belongs to the Starmerella clade. The type strain of C. flosculorum is UFMG-JL13T (=CBS 10566T=NRRL Y-48258) and the type strain of C. floris is UWO(PS) 00-226.2T (=CBS 10593T=NRRL Y-48255).

Flowers of plant species belonging to the Convolvulaceae and Heliconiaceae families are a rich source of novel yeast species. Most of the novel yeast species isolated from these plants belong to the Metschnikowia, Wickerhamiella and Starmerella clades. In ephemeral flowers of the Convolvulaceae, the yeasts are transported by pollinating and non-pollinating flies, beetles and bees that deposit them in the corolla. In the longer-lasting flowers of the Heliconiaceae, yeasts are probably introduced by a different and more diverse set of animal vectors and they may grow on the sugary compounds present in nectar (Lachance et al., 1998, 1999, 2001; Ruivo et al., 2006; Rosa et al., 2007).

In this paper, we describe two novel yeast species associated with flowers and related insects of these two plant families. The first species was found inhabiting flowers of Heliconia velloziana and Heliconia episcopalis in two Atlantic rain forest sites in Brazil. The second species was isolated from flowers of Ipomoea sp. on the banks of the river Paraguai, Brazil, and from a stingless bee and a flower of Merremia quinquefolia (wood rose) in Costa Rica. Analysis of the sequences of the D1/D2 domains of the large-subunit rDNA showed that these species represent distinct species of the Metschnikowiaceae and Starmerella clades, respectively. These novel species are described as Candida flosculorum sp. nov. and Candida floris sp. nov.

The strains considered in this study are listed in Table 1. Two strains of C. flosculorum were isolated from nectar present in flower bracts of H. velloziana, collected in the Picinguaba area, an Atlantic rain forest site at the Serra do Mar state park in São Paulo state, in September 2000 (Ruivo et al., 2006). Five strains came from flower bracts of H. episcopalis in Parque Estadual do Rio Doce, an Atlantic rain forest site in the state of Minas Gerais, collected in July 2002. Isolates of C. floris were obtained from flowers of Ipomoea sp. collected at the banks of the Paraguai river near the city of Corumbá, in the state of Mato Grosso do Sul, Brazil, in an area of pantanal ecosystem, in February 2002, and from a stingless bee (Trigona sp.) and a flower of Merremia quinquefolia collected in 2000 and 2001, respectively, in Santa Rosa National Park, Costa Rica. The yeasts were isolated on yeast extract-malt extract agar (YMA, 1%
Table 1. Sources of isolation of Candida flosculorum and Candida floris

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Strain number</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. flosculorum</td>
<td>Flower bract of Heliconia episcopalis</td>
<td>UFMG-JL11, UFMG-JL13T (=CBS 10566T), UFMG-JL36, UFMG-JL45, UFMG-JL52</td>
<td>Parque Estadual do Rio Doce Atlantic rain forest, state of Minas Gerais, Brazil</td>
</tr>
<tr>
<td></td>
<td>Flower bract of H. velloziana</td>
<td>UNESP-00-91, UNESP-00-92</td>
<td>Picinguaba Atlantic rain forest site, Parque Estadual da Serra do Mar, state of São Paulo, Brazil</td>
</tr>
<tr>
<td>C. floris</td>
<td>Flowers of Ipomoea sp.</td>
<td>UFMG-C16, UFMG-C37, UFMG-C139, UFMG-C141, UFMG-C144, UFMG-C151</td>
<td>Paraguarí River, pantanal ecosystem, state of Mato Grosso do Sul, Brazil</td>
</tr>
<tr>
<td>Trigona sp. from Merremia quinquefolia</td>
<td>UWO(PS) 00-226.2T (=CBS 10593T)</td>
<td>Santa Rosa National Park, Guanacaste, Costa Rica</td>
<td></td>
</tr>
<tr>
<td>Flower of M. quinquefolia</td>
<td>UWO(PS) 01-159.2</td>
<td>Santa Rosa National Park, Guanacaste, Costa Rica</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Sources of isolation of Candida flosculorum and Candida floris

UFGM, Universidade Federal de Minas Gerais, Brazil; CBS, Centraalbureau voor Schimmelcultures; UWO(PS), Yeast Culture Collection of the Department of Biology, University of Western Ontario, Canada.

Glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract and 2% agar) containing 100 mg chloramphenicol l⁻¹. Sampling methods were as described previously (Lachance et al., 1998; Rosa et al., 2003; Ruivo et al., 2006). Plates were incubated at room temperature (25±3°C) for 3–8 days. Each different yeast morphotype was purified and maintained on YMA slants or liquid nitrogen for later identification. The yeasts were characterized by standard methods (Yarrow, 1998). Identities followed the keys of Kurtzman & Fell (1998).

The D1/D2 variable domains of the large-subunit rDNA were amplified by PCR directly from whole cells as described previously (Lachance et al., 1999). The amplified DNA was concentrated and cleaned on QIAquick PCR columns (Qiagen) and sequenced using an ABI sequencer at the John P. Robarts Research Institute, London, Ontario, Canada. The sequence was edited with the program DNAMAN, version 6.0 (Lynnon BioSoft). Existing sequences for other related yeasts were retrieved from GenBank. The basic local alignment search tool (BLASTN) available at http://www.ncbi.nlm.nih.gov was used for this task. 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, classification and ecology**

Candida flosculorum belongs to the Metschnikowiaceae clade and is phylogenetically related to Candida sp. BCC 7717, an undescribed species isolated from plant materials in Thailand (Fig. 1). These species showed more than 10% sequence divergence in the D1/D2 regions of the 26S rDNA large-subunit. The sequences of the D1/D2 domains of three isolates of C. flosculorum (UFMG-JL13T, UFMG-JL52 and UNESP-00-92) were identical. All isolates of C. flosculorum had identical physiological profiles. C. flosculorum was isolated from two different Heliconia species and may be widely distributed in tropical rain forests. The Heliconia species sampled in our work occur in southeastern Brazil and in Amazonia and H. episcopalis is also used as an ornamental plant in Florida, Hawaii and Costa Rica. Several insects, mainly species of Diptera, feed on the plant leaves and bracts and may be the most important vectors of this yeast. Several species of Merosargus (Diptera) use bracts and flowers of H. episcopalis for
feeding and breeding (J. C. R. Fontenelle, unpublished data). Other possible vectors of the yeast community associated with the nectar of *Heliconia* bracts are species of hummingbirds. In the Parque Estadual do Rio Doce, four species of hummingbirds are known to visit *H. episcopalis* flowers. These include *Glaucis hirsuta* and *Phaethornis idaliae* (A. C. F. Alves & J. C. R. Fontenelle, unpublished data). Three other novel yeast species (*Candida heliconiae*, *Candida picinguabensis* and *Candida saopaulonensis*) were isolated from *H. velloziana* by Ruivo et al. (2006). This result suggests that *Heliconia* species may be an interesting yeast habitat that deserves further study.

*Candida floris* is related to *Candida etschellsii*, as these species showed 5.8% sequence divergence in the D1/D2 region of the rDNA large-subunit. Both species belong to the *Starmerella* clade (Fig. 2). Strain UWO(PS) 00-226.2T differed by two substitutions in the D1/D2 region from the Brazilian strain UFMG-C151 (GenBank accession number EF679791). Our strains of *C. floris* were isolated directly from Convolvulaceae flowers and from a meliponine (stingless) bee captured on such a flower in Brazil and in Costa Rica. An association of *C. floris* with stingless bees that visit Convolvulaceae flowers is not unexpected as these insects are known to be an important source of yeast species in the *Starmerella* clade and are frequent visitors to these flowers. A mutually beneficial interaction may exist between them (Rosa et al., 2003; Pimentel et al., 2005).

Isolates of *C. flosculorum* and *C. floris* were examined after growth, individually or mixed in pairs, on several common sporulation media (cornmeal 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. These species probably occur in nature in the asexual form.

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

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

Candida flosculorum (flos.cu.lo’rum. L. gen. masc. pl. n. flosculorum of little flowers, refers to florets or flowers from where most of the strains were isolated).

In yeast extract-glucose broth (0.5% : 2%, w/v) after 3 days at 25 °C, the cells are ovoid to ellipsoidal (2–3 x 2–5 μm). Budding is multilateral (Fig. 3a). A sediment is formed after a month, but no pellicle is observed. On YMA after 2 days at room temperature, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, pseudomycelia or true mycelia are not formed. Glucose is fermented. The following carbon compounds are assimilated: glucose, sucrose, maltose, L-sorbose, cellobiose, melezitose (slow), D-xylose, glycerol, salicin and xylitol. No growth occurs on galactose, trehalose, inulin, raffinose, melibiose, lactose, methyl α-D-glucoside, soluble starch, L-rhamnose, D-arabinose, L-arabinose, D-ribose, ribitol, ethanol, methanol, 2-propanol, 2-propanol, erythritol, galactitol, D-mannitol, D-glucitol, succinic acid, citric acid, lactic acid, gluconic acid, myo-inositol, glucosamine, N-acetylglucosamine, aceton, ethyl acetate or hexadecane. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl and cadaverine and negative for nitrate and nitrite. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Growth on YMA with 5% sodium chloride is positive, but negative at 10% sodium chloride. No growth in glucose-yeast extract broth (50% : 0.5%, w/v). Starch-like compounds are not produced. No growth with 1000 μg cycloheximide ml⁻¹. Urease activity is negative. Diazonium blue B reaction is negative. The habitat is flower bracts of Heliconia spp. in the habitat of Minas Gerais, Brazil.

The type strain of Candida flosculorum, UFMG-JL13T (=NRRL Y-48258T = CBS 10566T), was isolated from a flower bract of H. episcopalis in the state of Minas Gerais, Brazil.

Fig. 3. Phase-contrast micrograph of budding cells of Candida flosculorum UFMG-JL13T (a) and C. floris UWO(PS) 00-226.2T (b) on yeast carbon base agar with 0.01% ammonium sulphate after 3 days at 22 °C. Bar, 5 μm.

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


Description of Candida floris Rosa, Pagnocca & Lachance sp. nov.

Candida floris (flo.ris. L. gen. masc. n. floris of a flower, from where this yeast was isolated).

In yeast extract-glucose broth (0.5% : 2%, w/v) broth after 3 days at 25 °C, the cells are ovoid to ellipsoidal (2–3 x 2–4 μm). Budding is multilateral (Fig. 3b). A sediment is formed after a month, but no pellicle is observed. On YMA after 2 days at room temperature, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, pseudomycelia or true mycelia are not formed. Glucose fermentation is variable. The following carbon compounds are assimilated: glucose, sucrose, maltose, L-sorbose, cellobiose, melezitose (slow), D-xylose, glycerol, salicin and xylitol. No growth occurs on inulin, raffinose, melibiose, lactose, methyl α-D-glucoside, soluble starch, L-rhamnose, D-arabinose, L-arabinose, D-ribose, ribitol, ethanol, methanol, 2-propanol, 2-propanol, erythritol, galactitol, D-mannitol, D-glucitol, succinic acid, citric acid, lactic acid, gluconic acid, myo-inositol, glucosamine, N-acetylglucosamine, aceton, ethyl acetate or hexadecane. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl and cadaverine and negative for nitrate and nitrite. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Growth on YMA with 5% sodium chloride is positive, but negative at 10% sodium chloride. No growth in glucose-yeast extract broth (50% : 0.5%, w/v). Starch-like compounds are not produced. No growth with 1000 μg cycloheximide ml⁻¹. Urease activity is negative. Diazonium blue B reaction is negative. The habitat is flower bracts of Heliconia spp. in Brazil.

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Diazonium blue B reaction is negative. The habitat is flowers of Ipomoea sp. and M. quinquefolia in Brazil and Costa Rica.

The type strain of Candida floris, UWO(PS) 00-226.2\textsuperscript{T} (=CBS 10593\textsuperscript{T} = NRRL Y-48255\textsuperscript{T}), was isolated from an adult of the stingless bee Trigona sp. from a M. quinquefolia flower in Costa Rica.

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**References**


