Wickerhamiella slavikovae sp. nov. and Wickerhamiella goesii sp. nov., two yeast species isolated from natural substrates

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Two novel yeast species were isolated during three independent studies of yeasts associated with natural substrates in Brazil and Taiwan. Analysis of the sequences of the D1/D2 domains of the large subunit rRNA gene showed that these novel species belong to the Wickerhamiella clade. The first was isolated from freshwater and a leaf of sugar cane (Saccharum officinarum) in Brazil and from leaves of Wedelia biflora in Taiwan. Described here as Wickerhamiella slavikovae sp. nov., it differs by 56 nucleotide substitutions and 19 gaps in the D1/D2 region of the large subunit rRNA gene from Candida sorbophila, the least divergent species. The second species, named Wickerhamiella goesii sp. nov., was isolated from leaves and the rhizosphere of sugar cane collected in Rio de Janeiro, Brazil. The species differs by 54 nucleotide substitutions and nine gaps in the D1/D2 domains from Candida drosophilae, its least divergent relative. The type strains are Wickerhamiella slavikovae sp. nov. IMUFRJ 52096T (=CBS 12417T=DBVPG 8032T) and Wickerhamiella goesii sp. nov. IMUFRJ 52102T (=CBS 12419T=DBVPG 8034T).

The clade Wickerhamiella consists, at the time of writing, of 26 species that include five species with a sexual state and 21 that may eventually be found to form ascospores (Lachance & Kurtzman, 2011; Barbosa et al., 2012; Badotti et al., 2013). Most of these species are highly specialized nutritionally and ecologically, and some of them have a strong association with floricolous insects and plant materials (Lachance & Kurtzman, 2011; Barbosa et al., 2012). In the course of three independent studies of yeasts associated with natural substrates in Brazil and Taiwan, four unidentified yeast strains were found to have almost identical sequences in the D1/D2 domains of the large subunit rRNA gene. The least divergent known sequence was that of Candida sorbophila. Another four unidentified isolates were recovered from leaves and the rhizosphere of sugar cane (Saccharum officinarum) in Rio de Janeiro, Brazil. These isolates, also in the Wickerhamiella clade, exhibited sequence affinity with Candida drosophilae. In this study, we propose the description of these eight strains as representatives of two novel species, Wickerhamiella slavikovae sp. nov. and Wickerhamiella goesii sp. nov., respectively.

Strain UFMG-LD2.04 was isolated from a freshwater sample collected from the Lake de Dentro situated in Ecological State Park of Cantão, state of Tocantins, Brazil, in July 2008. The water sample was filtered on sterile nitrocellulose membranes of 0.45 µm pore size and 47 mm diameter using a Nalgene filtering device. The membrane was placed on the surface of YM agar (0.3 % yeast extract; 0.3 % malt extract; 0.5 % peptone; 1 % glucose; 2 % agar;

Abbreviations: CBS, Centraalbureau voor Schimmelcultures; SIPA, Sistema Integrado de Produção Agroecológica. The GenBank/EMBL/DDBJ accession numbers for the large subunit rRNA gene D1/D2 domain sequences of Wickerhamiella slavikovae IMUFRJ 52096T and Wickerhamiella goesii IMUFRJ 52102T are FJ463264 and JN790617, respectively.

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Candida sp. SJ-1 (EF653272)
Candida sp. 107 (EF141077)
Candida sorbophila NRRL Y-7921\textsuperscript{T} (DQ438229)
Candida infantica CBS11938 (HQ695009)
Candida sp. SN-102 (EF621560)
Candida sp. UWOPS 00-136.3 (AF313356)
Candida musiphila AS2.3479\textsuperscript{T} (EU284104)
Candida spandovensis NRRL Y-17761\textsuperscript{T} (DQ438228)
Candida sp. EVN 1240 CM 134/05 (FR853162)
Candida sergipiensis UFMG-R188\textsuperscript{T} (AF397406)
Candida sp. BG01-7-26-006A-1-1 (AY242275)
Candida BG02-7-18-027A1-2 (AY520352)
Candida kazuoi NBRC 102565\textsuperscript{T} (AB306509)
Candida hasegawae NRBC 102566\textsuperscript{T} (AB306510)
Candida sp. BG02-7-21-004C-1-1 (AY520293)
Wickerhamiella domercqiae NRRL Y-6692\textsuperscript{T} (DQ438240)
Candida bombiphila CBS 9712\textsuperscript{T} (AJ620185)
Candida sp. UWOPS 00-107.1 (AF313369)
Candida sp. UWOPS 00-192.1 (AF313368)
Candida versatilis NRRL Y-6652\textsuperscript{T} (DQ438242)

Wickerhamiella slavikovae IMUFRJ 52096\textsuperscript{T} (FJ463264).

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pH 4), containing chloramphenicol (200 mg l⁻¹). The plate was incubated at room temperature (25 ± 3 °C) for 5 days. The Taiwanese strain, GE15L11, was isolated from leaves of Wedelia biflora by spreading dilutions of a suspension washed from approximately 1 g of leaf vortexed in 10 ml YM broth on acidified YMA and incubating at 25 °C for 3 days as described previously (Chang et al., 2012). Strains IMUFRJ 52096T and IMUFRJ 52097 were isolated from sugar cane leaves at an experimental farm [SIPA – Sistema Integrado de Produção Agroecológica (Integrated System of Agroecological Production)] for organic and nonconventional management in Seropédica, Rio de Janeiro, Brazil, on January 29, 2007. Strains IMUFRJ 52099, IMUFRJ 52100, IMUFRJ 52101 and IMUFRJ 52102 were collected from leaves and the rhizosphere of sugar cane at the same collection site in September of 2007 and January of 2008. The isolation method for these strains was described by Ribeiro et al. (2011). The plates were incubated at room temperature for 3 days. Representatives of different yeast morphotypes were purified by repeated streak-inoculation of YM agar plates and preserved on GYMP (2 % glucose, 2 % malt extract, 0.5 % yeast extract, 0.2 % NaH₂PO₄, H₂O, 2 % agar) slants and at −80 °C or in liquid nitrogen for later identification. Yeast cells were imaged with an Axiosplan 2 microscope equipped with Nomarski differential interference microscopy optics. The yeasts were characterized by standard methods (Kurtzman et al., 2011).

The D1/D2 domains of the large subunit rRNA gene of strain UFMG-LD2.0 were amplified by PCR directly from whole cells as described previously (Lachance et al., 1999), and the PCR was concentrated and cleaned on QIAquick PCR columns (Qiagen) and was sequenced using an ABI sequencer at the John P. Roberts Research Institute (London, Ontario, Canada). Sequencing of the D1/D2 region of the strains isolated in Rio de Janeiro was done according to the procedures described by Ribeiro et al. (2011) and that isolated from Taiwan as described by Lee et al. (2009). The sequences were edited with the program Chromas (2.33; Technelysium) and aligned with the procedures contained in the program MEGA5 (Tamura et al., 2011), which was also used to generate phylogenetic trees. The tree shown in Fig. 1 was reconstructed by the maximum-likelihood method, using the Kimura two-parameter substitution model. Evolutionary rate differences were modelled after the gamma distribution with five categories. The final dataset contained 498 aligned positions. Clade consistency was estimated with bootstrap values determined from 100 iterations.

**Species delineation and ecology**

The sequences of the D1/D2 domains of the large subunit of the rRNA gene showed that the eight strains belong to two different species of the *Wickerhamiella* clade (Fig. 1). The Brazilian strains UFMG-LD2.04, IMUFRJ 52096T, IMUFRJ 52097 and the Taiwanese strain GE15L11 had almost identical D1/D2 sequences and are considered to belong to the same species. Strains UFMG-LD2.04, IMUFRJ 52096T and IMUFRJ 52097 presented identical sequences, but differed from strain GE15L11 by three nucleotides, two of which are a doublet, in the D1/D2 region and four (data not shown) in the ITS region. The novel species differs by 56 nucleotide substitutions and 19 gaps from the D1/D2 domains of *C. sorbophila*, although the basal position of the species relative to the clade that contains *C. sorbophila* precludes the identification of a closest relative. The name *Wickerhamiella slavikovae* sp. nov. is proposed to accommodate these isolates.

The other four strains, IMUFRJ 52099, IMUFRJ 52100, IMUFRJ 52101 and IMUFRJ 52102, were identical in their D1/D2 sequences and for that reason are considered conspecific. They were recovered from the rhizosphere (strains IMUFRJ 52099 and IMUFRJ 52100) and from leaves (strains IMUFRJ 52101 and IMUFRJ 52102) from rhizosphere of sugar cane. The sequences of these isolates differed by 54 nucleotide substitutions and nine gaps in their D1/D2 domains from *C. drosophilae*, but again, the basal position of the novel species does not allow identification of the most closely related species in the clade. The name *Wickerhamiella goesii* sp. nov. is proposed to accommodate these isolates. These non-ascosporic yeasts, one in each of the D1/D2 rRNA gene subclades as can be seen in Fig. 1, were assigned to the genus *Wickerhamiella* rather than *Candida* in accordance with the current version of the Botanical Code that allows only one name for a species.

Isolates of *W. slavikoviae* sp. nov. and *W. goesii* sp. nov. 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.

*W. slavikoviae* sp. nov. can be distinguished from *C. sorbophila* by growth on D-mannitol and D-glucitol, which it is positive for *C. sorbophila* and negative for the novel species. *W. goesii* sp. nov. could be easily distinguished from *C. drosophilae* based on the ability to assimilate maltose, sucrose, trehalose, melezitose and ribitol, which are positive for the former species and negative for the latter.

**Description of Wickerhamiella slavikovae sp. nov.**

*Wickerhamiella slavikovae* (sla.vi.kovae N.L. gen. fem. sing. n. *slavikovae* of Sláviková, referring to Professor Elena Sláviková, in recognition of her contributions to yeast systematics and ecology in Slovakia).

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**Fig. 1.** Maximum-likelihood tree showing the placement of *Wickerhamiella slavikovae* sp. nov. and *Wickerhamiella goesii* sp. nov. in the *Wickerhamiella* clade, based on the D1/D2 domains of the large subunit rRNA gene. Bootstrap values of 50 % and above are shown. Bar, 0.1 substitutions per nucleotide position.
In glucose (2 %), yeast extract (0.5 %) broth after 3 days at 25 °C, cells are ovoid to ellipsoidal, isolated or in pairs (2–3 x 3–6 μm). Budding is multilateral (Fig. 2). Sediment is formed after 1 month, but no pellicle is observed. On YM agar after 2 days at 25 °C, colonies are white, convex, smooth and opalescent. On Dalmau plates after 2 weeks on cornmeal agar, pseudohyphae or true mycelia are not formed. Sexual spores are not observed. Glucose is not fermented. Assimilation of carbon compounds: growth occurs on glucose, galactose, melezitose (variable), L-sorbose, ethanol, glycerol, ribitol (variable), succinate and N-acetyl-D-glucosamine (variable). No growth occurs on inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, soluble starch, cellobiose, salicin, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, erythritol, galactitol, D-mannitol, D-glucitol, myo-inositol, DL-lactate, citrate, D-glucurate, D-glucosamine or hexadecane. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl and cadaverine, negative for nitrate and nitrite. No growth in vitamin-free medium. Growth at 37 °C is variable. Growth on YM agar with 10 % sodium chloride is variable. Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Growth on YM agar with 10 % sodium chloride is positive. Growth in 50 % glucose/yeast extract (0.5 %) is negative. Starch-like compounds are not produced. With 100 μg cycloheximide

**Description of Wickerhamiella goesii sp. nov.**

*Wickerhamiella goesii* (go.es’ii N.L. gen. nom. m. sing. n. goesii of Goes, referring to Professor Paulo de Goes, the founder of the Instituto de Microbiologia Professor Paulo de Goes of the Federal University of Rio de Janeiro in recognition of his contributions to microbiology in Brazil).

In glucose (2 %), yeast extract (0.5 %) broth after 3 days at 25 °C, cells are ovoid, isolated or in pairs (2–3 x 3–4 μm). Budding is multilateral (Fig. 3). Sediment is formed after 1 month, but no pellicle is observed. On YM agar after 2 days at 25 °C, colonies are white, convex, smooth and opalescent. On Dalmau plates after 2 weeks on cornmeal agar, pseudohyphae or true mycelia are not formed. Sexual spores were not observed. Glucose is not fermented. Assimilation of carbon compounds: glucose, galactose, L-sorbose, maltose, sucrose, trehalose (slow), melezitose, D-xylose (weak and variable), ribitol, mannitol, glucitol, xylitol and D-gluconate (variable) are positive. No growth occurs on inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, melezitose, soluble starch, cellobiose, salicin, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, ethanol, glycerol, erythritol, galactitol, myo-inositol, DL-lactate, succinate, citrate, D-glucosamine, N-acetyl-D-glucosamine or hexadecane. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl and cadaverine, negative for nitrate and nitrite. No growth in vitamin-free medium. Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Growth on YM agar with 10 % sodium chloride is positive. Growth in 50 % glucose/yeast extract (0.5 %) is negative. Starch-like compounds are not produced. With 100 μg cycloheximide.

![Fig. 2. Differential interference contrast micrograph of cells of Wickerhamiella slavikovae sp. nov. grown for 3 days on YM agar. Bar, 10 μm.](image1)

![Fig. 3. Differential interference contrast micrograph of cells of Wickerhamiella goesii sp. nov. grown for 3 days on YM agar. Bar, 10 μm.](image2)
ml\(^{-1}\) growth is positive. Urease activity is negative. Diazonium Blue B reaction is negative. Habitat is leaves and rhizosphere of sugar cane (*Saccharum officinarum*).

The type strain is IMUFRJ 52102\(^T\), isolated from leaf of sugar cane collected in Rio de Janeiro, Brazil. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as strain CBS 12419\(^T\), and in the Industrial Yeasts Collection of the Dipartimento di Biologia Applicata, University of Perugia, Italy, as strain DBVPG 8034\(^T\). The MycoBank number is MB 803246.

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**References**


