Metschnikowia cerradonensis sp. nov., a yeast species isolated from ephemeral flowers and their nitidulid beetles in Brazil

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A novel yeast species, Metschnikowia cerradonensis sp. nov., is described from 12 strains isolated from flowers of Ipomoea carnea and from beetles of the genus Conotelus in the Cerrado ecosystem in the region of Jalapão, Tocantins State, Brazil. Analysis of the sequences of the rRNA gene cluster suggested that M. cerradonensis is closely related to Metschnikowia santaceciliae, Metschnikowia continentalis and an undescribed species represented by strain UWOPS 00-154.1. These species mate together but ascospores are very rarely formed, showing that they represent distinct biological species. M. cerradonensis is apparently endemic to the Cerrado ecosystem of the Jalapão area. The type strain of M. cerradonensis is UFMG 03-T67.1T (h+) (=CBS 10409T, =NRRL Y-48067) and the designated allotype is UFMG 03-T68.1 (h−) (=CBS 10410, =NRRL Y-48068).

The large-spored group of the genus Metschnikowia forms a clade with eight recognized teleomorphic species and the anamorphic species Candida ipomoeae (Lachance et al., 2005). The distribution of most of the taxa is regional, suggesting that they arose in time through allopatric speciation (Marinoni & Lachance, 2004). Metschnikowia hawaiiensis is associated with the nitidulid beetle Prosopoeus subaeneus, which is endemic to the island of Hawai‘i, supporting the view that this yeast has speciated in that region (Lachance et al., 1990, 2005). Similarly, Metschnikowia hamakuensis, Metschnikowia kamokouana and Metschnikowia maunuiiana are associated with endemic nitidulid beetles living on various endemic plants on three Hawaiian islands (Lachance et al., 2005). The habitat of Metschnikowia lochheadii and Metschnikowia santaceciliae is in beetles of the genus Conotelus and other insects associated with flowers of Ipomoea (morning glory) species in northwestern Guanacaste Province, Costa Rica (Lachance et al., 2001, 2003). M. lochheadii is also present in insects found in various flowers on the Hawaiian Islands, where it appears to have been introduced. Metschnikowia borealis is restricted to eastern North America, where it is also associated with Conotelus species and Convolvulaceae flowers (Lachance et al., 1998; Marinoni & Lachance, 2004). Metschnikowia continentalis was isolated from Ipomoea flowers and species of the genus Conotelus in Brazil (Lachance et al., 1998), where it has a widespread distribution (C. A. Rosa, unpublished results). Candida ipomoeae is an asexual relative of all these species associated with Conotelus beetles and is found in abundance in all regions where Conotelus species occur, except for localities near the Great Lakes area and northward (Marinoni & Lachance, 2004).

During a study of the yeasts associated with ephemeral flowers in northern Brazil, several strains of a yeast producing large acicular ascospores were isolated from a site in the Cerrado ecosystem. Mating experiments showed that this yeast was capable of mating with M. continentalis. However, ascospores were rarely formed. This and other mating results, in combination with an analysis of rDNA sequences, demonstrated that these isolates represent a novel species in the large-spored group of the genus Metschnikowia. The name proposed for this species is Metschnikowia cerradonensis sp. nov.

Strain collection and characterization

Details of the strains considered in this study are given in Table 1. Collections were made in 2003 and 2005. Flowers of Ipomoea carnea and associated beetles were collected from

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are DQ641236–DQ641243. The Mycobank accession number for Metschnikowia cerradonensis sp. nov. is MB510051 (http://www.mycobank.org).
GCTGCT3
AGTCTCGGGATG3
chloramphenicol l
extract, 0.3 % yeast extract, 2.0 % agar) containing 100 mg
onto YM agar (1.0 % glucose, 0.5 % peptone, 0.3 % malt
was scraped gently with a sterile loop and streak-inoculated
Tocantins State. The nectary region of the
Canguc¸u, near the Javae´s River. These
('Ipucas') of the Lago Verde estate, in the flooded plains
from flowers of
Ipomoea
species in two forest fragments
('ervedouro') in the city of Sa˜o
Delineation and identification of heterothallic, haplontic
Denscrobomyces
species are derived from mating reactions.
Metschnikowia
Denscrobomyces
Yarrow, 1998). Identities were
determined by PCR amplification using the following
regions of the
Saccharomyces
dna templates were prepared as described by de Barros
M. cerradonensis
M. continentalis
ability. Therefore,
gapped positions were excluded, such that 6076 nucleotide
positions out of 7447 were retained.
Two authentic strains of
M. continentalis
and five strains of
M. cerradonensis
sp. nov. were used for PCR fingerprinting,
DNA templates were prepared as described by de Barros Lopes et al. (1998). The primer EI1 (5’CTGGCTTGG-TGTATG3’)
targets intron-splicing sites in hypermutable regions of the
Saccharomyces
genome. PCR assays were
performed as described by the authors. PCR products were
analysed by 1 % agarose gel electrophoresis.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mating type</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>UFMG 03-T67.1T</td>
<td>h+</td>
<td>Conotetes beetle</td>
</tr>
<tr>
<td>UFMG 03-T68.1</td>
<td>h+</td>
<td>Conotetes beetle</td>
</tr>
<tr>
<td>UFMG 03-T62.1</td>
<td>h+</td>
<td>Ipomea carnea flower</td>
</tr>
<tr>
<td>UFMG 03-T64.1</td>
<td>h-</td>
<td>Ipomea carnea flower</td>
</tr>
<tr>
<td>UFMG 03-T64.2</td>
<td>h+</td>
<td>Ipomea carnea flower</td>
</tr>
<tr>
<td>UFMG 03-T215.2</td>
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<td>Conotetes beetle</td>
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<td>UFMG 03-T216.2</td>
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<tr>
<td>UFMG 05-T332.2</td>
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<td>h-</td>
<td>Conotetes beetle</td>
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<td>UFMG 05-T334.1</td>
<td>h+</td>
<td>Conotetes beetle</td>
</tr>
<tr>
<td>UFMG 05-T338.1</td>
<td>h+</td>
<td>Ipomea carnea flower</td>
</tr>
<tr>
<td>UFMG 05-T339.1</td>
<td>h-</td>
<td>Ipomea carnea flower</td>
</tr>
</tbody>
</table>

Ecology and species relationships
Strains of
M. cerradonensis
sp. nov. were isolated from flowers of
I. carnea
and from Conotetes species collected on the margins of a small lake in São Felix do Tocantins. Morning glory flowers sampled in public gardens and streets of São Felix yielded only basidiomycetous yeasts and
Candida azyma.
Samples taken in two other areas of the Cerrado, located approximately 300 km from Jalapão, yielded only
M. continentalis,
suggesting that
M. cerradonensis
might be endemic to the Jalapão region.

The phylogenetic relationships among
M. cerradonensis
and its relatives are shown in Fig. 1. The use of split decomposition instead of the more usual neighbour-joining or other phylogenetic analysis methods was dictated by the fact that different parts of the rDNA cluster would lead to somewhat contradictory phylogenies. Although such inconsistencies would be flagged by low bootstrap values, alternative branching orders would remain unknown. Split decomposition (Huson & Bryant, 2006) displays simultaneously the major trends suggested by the sequence data as parallelograms in which the longer sides join the
better supported hierarchy. In the present case (Fig. 1), the clear interpretation is that *M. cerradonensis*, *M. santaceciliae* and *M. continentalis* are sister species, but that several alternative branching orders are possible among the remaining species. For example, the split decomposition network suggests that *Metschnikowia* sp. UWOPS 00-154.1 and *Candida ipomoeae* are almost as likely to be basal members of the subclade containing the novel species as they are to be sister species with *M. borealis* and *M. hawaiiensis*, respectively. Nonetheless, an affinity of the novel species with *M. lochheadii* and *Metschnikowia* sp. UWOPS 00-154.1 is evident.

Analysis of shorter regions of the rDNA unit, for example the popular D1/D2 LSU domains, has led to somewhat different conclusions about some of these species, including the original belief that *M. continentalis* and *M. borealis* should be viewed as varieties of a single species (Lachance et al., 1998), or that *M. lochheadii* and *Candida ipomoeae* might represent a teleomorph–anamorph pair (Lachance et al., 2001). Both suggestions were later disproved (Marinoni & Lachance, 2004). *M. cerradonensis* differs from *M. santaceciliae* and *M. continentalis* only by two substitutions and 1–2 indels in the D2 domain and from *M. santaceciliae* by one substitution in the D1 domain. The internal transcribed spacer region of *M. cerradonensis* contains several substitutions and is 21 and 30 nt longer than that in *M. santaceciliae* and *M. continentalis*, respectively. Differences from *M. lochheadii* are slightly greater. The species boundaries were established by mixing strains of the above four species plus *Metschnikowia* sp. UWOPS 00-154.1 in every possible combination. Rare single-spored asci were observed on occasion in crosses involving *M. cerradonensis*, *M. santaceciliae* and *M. continentalis*. By contrast, intraspecific crosses gave rise to abundant two-spored asci. Sterile asci are formed in extraspecific mixtures of complementary mating types of all members of the large-spored subclade.

*M. cerradonensis* and its most closely related species were indistinguishable based on growth test profiles. For this reason, PCR amplification with the intron-splicing-site primer was useful in distinguishing *M. continentalis* from *M. cerradonensis*, as both species might possibly occur in the same samples. The two species showed distinct PCR fingerprint profiles (Fig. 2). Conspecific isolates generally produce similar banding profiles (de Barros Lopes et al., 1998; Carreiro et al., 2004; Pimenta et al., 2005). This was the case here, although some variation was detectable within the two species. Ascospore formation in mixtures with the mating types of authentic strains or rDNA sequencing are recommended for definitive identification.

**Latin diagnosis of *Metschnikowia cerradonensis* Rosa, Lachance et Morais sp. nov.**


**Fig. 1.** Split decomposition network of the rDNA sequences of *Metschnikowia cerradonensis* sp. nov. and related species. Only ungapped positions were used in the analysis. Bar, 1 % sequence divergence.

**Fig. 2.** PCR fingerprints of *Metschnikowia continentalis* and *Metschnikowia cerradonensis* sp. nov. strains obtained using the primer EI1. Lanes: 1, 1 kb Plus DNA ladder; 2, *M. continentalis* UFMG 05-T65 (h+); 3, *M. continentalis* UFMG 05-T13 (h+); 4, *M. cerradonensis* UFMG 03-T68.1 (h+); 5, *M. cerradonensis* UFMG 03-T67.1T (h+); 6, *M. cerradonensis* UFMG 05-T338.1 (h+); 7, *M. cerradonensis* UFMG 05-T332.2 (h+); 8, *M. cerradonensis* UFMG 05-T334.1 (h+).
(1.3–1.6 × 60–150 μm). Glucosum fermentatur. Glucosum, sucrosum, galactosum, trehalosum, maltosum, melezitosum, cellobiosum, salicinum (exiguus), L-sorbitosum, D-xylosum, glycerolum (lente et exigu), ribitolum (lente), xylitolum, mannitolum, glucitolum, acidum succinicum, acidum citrimum (lente), acidum gluconicum, N-acetylglucosaminum, ethylacetatus (lente) et hexadecanum assimilantur, at non inulimum, raffinosoph, melibiosum, lactosum, methyl-5-D-glucosidum, amyllum solubile, L-rhamnosum, L-arabinosum, D-arabinosum, D-ribosum, methanolum, 2-propanol, ethanolum, eritritolum, galactitolum, meso-inositolum, acidum lacticum, glucosaminum nec acetonom. Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum. Ad crescendam vitamina externa necessaria sunt. Augmentum in 34 °C, at non 35 °C.

Habitat Conotelus sp. et Ipomoea carnea in Brazil. Typus: UFMG 03-T67.1T (h−). Alloptypus: UFMG 03-T68.1 (h+). In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 10409 et CBS 10410 depositae sunt.

Description of Metschnikowia cerradonensis Rosa, Lachance & Morais sp. nov.

Metschnikowia cerradonensis (cer. ra. do. nen’sis. L. nom. sing. m. adj. cerradonensis of Cerrado, referring to the Cerrado ecosystem, where all known strains of the species were recovered).

On YM agar after 3 days at 25 °C, cells are spherical to ovoidal, occur singly, in parent–bud pairs or in short chains, and measure 2–4 × 3–6 μm. Highly refringent cells as well as very long germ tubes are observed on occasion. After 2 weeks, colonies are of low convexity or are convex, glossy, white, small to medium sized, butyrous or leathery owing to intertwinned tubes. In Dalmau plate cultures on cornmeal agar after 2 weeks, abundant pseudo hyphae are formed in some, but not all, cultures. On yeast carbon base agar at 22 °C, mixtures of cells of complementary mating types give rise to zygotes and asci after 12–24 h. After 3 days, mature ascii containing two acicular ascospores (1.3–1.6 × 40–150 μm) are formed in a persistent ascus that retains more or less conspicuous vestiges of the conjugated parent cells (Fig. 3). Gas production from glucose begins after 1–2 days. Galactose, sucrose, maltose and trehalose are not fermented. Glucose, sucrose, galactose, trehalose, maltose, melezitose, cellobiose, salicin (weak), L-sorbitose, D-xylene, glycerol (slow and weak), ribitol (slow), xyitol, mannitol, glucitol, succinic acid, citric acid (slow), gluconic acid, N-acetylglucosamine, ethyl acetate and hexadecane are assimilated. No growth occurs on inulin, raffinose, melibiose, lactose, soluble starch, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, 2-propanol, ethanol, erythritol, galactitol, myo-inositol, lactic acid, D-glucosamine or acetone. Assimilates the following nitrogen compounds: lysine, ethylamine-HCl and cadaverine; but negative in tests for nitrate and nitrite. No growth in vitamin-free medium, but growth is observed in amino acid-free medium. Grows at 34 °C, but not at 35 °C. Growth on YM agar with 10 % sodium chloride is slow. No growth in 50 % glucose/yeast extract (0.5 %). Starch-like compounds are not produced. No growth in 100 μg cycloheximide ml−1. Urease-negative. Diazonium blue B reaction is negative.

The type strain, UFMG 03-T67.1T (h−) (=CBS 10409T = NRRL Y-48067T), was recovered from Conotelus beetles (Coleoptera: Nitidulidae) associated with flowers of Ipomoea carnea growing in the margins of a small lake of the Jalapão area in the city of São Felix do Tocantins, Tocantins State, Brazil. The designated alloptypus, UFMG 03-T68.1 (h+) (=CBS 10410T = NRRL Y-48068T), was recovered from Conotelus beetles.

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References


