Necrotic tissues of cacti are habitats for several yeast species. The yeasts can be generalists occurring in association with broadly diverse cactus species or specialists found only in certain species, subtribes, or tribes of the Cactaceae (Lachance et al., 1988; Starmer et al., 1990). The most frequent species in necrotic cactus tissue are Pichia cactophila, Candida sonorensis and members of the Sporopachydermia cereana complex (Lachance et al., 1988, 2001; Starmer et al., 1990). These species occur in a wide range of cacti and regions. Other species occur in lower frequencies, or are restricted to a few host plant species or to defined geographical areas (Starmer et al., 1990).

Three cactophilic species of the Starmera clade, Starmera amethionina, Starmera pachycereana and Starmera caribaea, occur in necrotic cactus tissues (Kurtzman, 2011). Phaff et al. (1992) described Pichia caribaea (=Starmera caribaea), a species recovered from necrotic tissue of cacti in the tribe Opuntieae and in columnar cacti of the subtribe Pachycereinae. Isolates of this species were obtained from cacti geographically ranging from southern Texas, USA to Venezuela (Kurtzman, 2011). Starmera caribaea was shown by DNA reassociation to be closely related to Pichia amethionina (=Starmera amethionina), described by Starmer et al. (1978). Pichia amethionina was assigned to two varieties based on ability to assimilate D-mannitol and on ecological associations with two different subtribes of cacti. Kurtzman et al. (2008) elevated the two varieties of Starmera amethionina to the rank of species (Starmera amethionina and Starmera pachycereana) because of their divergent sequences for the D1/D2 large subunit (LSU) and small subunit (SSU) rRNA genes as well as the elongation factor (EF)-1α gene, in addition to their reduced nuclear DNA complementarity (64 %) and decreased fertility in intervarietal crosses. Starmera amethionina is found mainly in cacti belonging to the subtribe Stenocereinae and cannot assimilate D-mannitol. Starmera pachycereana is found predominantly in cacti of the subtribe Pachycereinae and can assimilate D-mannitol. The three cactophilic species have an absolute requirement for organic forms of sulfur, such as the sulfur-containing amino acids L-methionine and L-cysteine. Starmera caribaea, Starmera amethionina and Starmera pachycereana are close relatives, but they are distinct as evidenced by their limited ability to mate and the divergence in the D1/D2 LSU, SSU and EF-1α gene sequences. The organic sulfur requirement is not shared with Candida stellimalicola, a moderate relative,
nor with other species reassigned to the genus Starmera on
the basis of the same sequences, but on less solid grounds
(Kurtzman et al., 2008).

During a survey of yeasts associated with necrotic tissues of
cacti in south-eastern Brazil, we isolated two strains that
were unable to grow on amino acid-free medium. Sequence
analysis of the D1/D2 regions of the large subunit rRNA
gene showed that these strains belong to the Starmera
clad and are closely related to Starmera caribaea. The
two isolates differ from Starmera caribaea by 21 sub-
titutions and two indels in the D1/D2 domains. The novel
species Starmera pilosocereana sp. nov. is proposed to
accommodate these isolates.

Necrotic tissues of Pilosocereus arrabidae (Cacteaceae, Tribe
cereeae) were collected from the Restinga de Jurubatiba
National Park near Macae, Rio de Janeiro State, Brazil
(22°10’S 41°26’W) in December 2012. Samples
(approximately 0.1 g) of decaying stem tissue of cacti
were suspended in 0.9 ml saline and transported to the
laboratory on ice. Aliquots (0.1 ml) of appropriate cellular
dilutions were spread on yeast-malt agar (YMA; glucose
1 %, peptone 0.5 %, malt extract 0.3 %, yeast extract
0.3 % and agar 2 %) supplemented with 100 mg l−1
chloramphenicol and incubated at 25 °C for 2 to 5 days. Representa-
thives of the different colony morphotypes were purified by repeated streak inoculation on YMA and pre-
served at −80 °C for later identification. The yeasts were
characterized using standard methods (Kurtzman et al.,
2011). Single ascospores were isolated from mature ascus
with a Zeiss Axio Scope.A1 microscope equipped with a
micromanipulator. Prior to micromanipulation, the sporul-
culated cultures were treated with a lyticase suspension
(1.5 mg ml−1) for 15 min at 37 °C.

The region spanning the 3′ end of the SSU rRNA gene, the
internal transcribed spacer (ITS), the 5.8S rRNA gene, and
the D1, D2, and partial D3 domains of the LSU rRNA gene
was amplified by PCR directly from whole yeast cells as
described previously (Lachance et al., 1999), using the
primers NS7A and NL5A (Kurtzman & Robnett, 2003).
The amplified DNA was sequenced using an ABI 3730
automated DNA gene analyser (Applied Biosystems) at
the Robarts Research Institute, London, ON, Canada. Identi-

ties were determined by comparing the sequence of the
D1/D2 domains of the LSU rRNA gene. The sequences
were assembled, edited and aligned with the program
MEGAl (Tamura et al., 2013). Phylogenetic placement of
the novel species was based on a maximum-likelihood
analysis of a 783-position alignment of the 3′ end of the
LSU rRNA gene. Bootstrap values were determined from 100
iterations.

Species delineation and phylogenetic placement

The phylogeny based on sequences of the D1/D2 regions of
the rRNA gene showed that the two isolates were closely
related to Starmera caribaea (Fig. 1). The two strains
differed by one substitution in the D1/D2 region and had
identical ITS-5.8S regions. Their status as a distinct species
is supported by 21 substitutions at 15 locations and two
indels of one and three positions, respectively, compared
to Starmera caribaea. Other species in the clade formed
separate subclades and differed by 28 or more substitu-
tions. ITS sequences of the other related species were
not available for identity comparison. We propose the
name Starmera pilosocereana sp. nov. to accommodate
the two isolates of the novel species.

The two isolates of Starmera pilosocereana sp. nov.
(UFMG-CMY-3164 and UFMG-CMY-346) were examined
individually or mixed in pairs on 5 % malt extract and
acetate McClary agars. Only one isolate, UFMG-CMY-
346, produced ascospores on its own. Natural isolates of
the three known cactophilic species of the genus Starmera
are heterothallic, but may occur as haploid or diploid
strains (Kurtzman, 2011). Diploids undergo ascospora-
tion and form two to four hat-shaped ascospores that are
freed through ascus deliquescence (Kurtzman, 2011). We
used the heat-treatment technique described by
Wickerham & Burton (1954) to determine whether the
sporulating strain of Starmera pilosocereana sp. nov.
could represent a diploid. After heat treatment, we
obtained four colonies that only produced spores when
mixed in suitable pairs on McClary’s acetate agar. Strain
UFMG-CMY-346a is a haploid mating type (h−) that
produced ascospores when crossed with strains UFMG-
CMY-316 (h+ and UFMG-CMY-346b (h+). These results
showed that Starmera pilosocereana sp. nov. is heterothallic.
The mating types of the novel species were designated in
reference to the mating types of Starmera caribaea. Ascospores were formed on acetate agar McClary after
2 days at 25 °C. The spores are hat-shaped, and there are
usually four ascospores per ascus (Fig. 2). We also mixed
the strain UFMG-CMY-346a (h−) of the new species with
the mating type h+ of Starmera caribaea (CBS
7694) to test for interspecific mating between these closely
related species. Zygotes and aberrant asci were produced on
acetate agar McClary after 2 days at 25 °C. Malformed
ascospores were produced only after incubation for
7 days (Fig. 3). We performed two independent exper-
iments in which asc from crosses of UFMG-CMY-346a
(h−) and the mating type h+ of Starmera caribaea (CBS
7694) were dissected and the germination of 40 ascospores
was tested. For control purposes, the same procedure was
used to assess the viability of the ascospores resulting
from the cross between the two mating types [strains
UFMG-CMY-316 (h+ and UFMG-CMY-346a (h−)] of
the novel species. The interspecific-cross resulted in
22.5 % viable ascospores whereas the control had 99 %
ascospore viability. This result showing a hybridization
barrier is consistent with a final stage of speciation and
strong reproductive isolation between the two species.
The formation of asci with aberrant ascospores has been
noted by Phaff et al. (1992) in interspecific matings
between Starmera amethionina or Starmera pachycreana

http://ijs.microbiologyresearch.org
and *Starmera caribaea*. When mixed, these species are known to produce ascospores with decreased viability (Starmer et al. 1978; Phaff et al., 1992).

The Melbourne Code (McNeill, 2012) no longer allows the use of two names, one for sexual and the other for asexual taxa, in the case of phylogenetically related species that can be classified in the same genus. The present situation is not entirely straightforward. Whereas the transfer of *Candida stellimalicola* can be effected with confidence, it is not clear that the genus *Starmera* as understood by Kurtzman (2011) will survive the scrutiny of phylogenetic analyses based on intensified taxon and sequence sampling. The phylogeny in Fig. 1 suggests that divergence among species assigned to the genus *Starmera* is comparable to that seen among four representatives of the neighbouring genera *Cyberlindnera* and *Wickerhamomyces*. The patterns seen in a multilocus analysis of these species (Kurtzman et al. 2008) are essentially the same. It is therefore only a matter of time before *Candida berthetii*, *Candida dendrica* and close relatives currently included in the genus *Starmera* will be reassigned to a separate genus. We would therefore be uncomfortable being party to adding confusion to the literature by renaming those two species of the genus *Candida*, only to see them reassigned in the near future.

We propose that the species *Candida stellimalicola* be included in the genus *Starmera* as a new combination. The mention forma asexualis (f.a.) is added to this species as a reminder that a sexual state is not known (Lachance, 2012).

In studies of the yeast communities of columnar cacti and associated insects in the sand dune (‘restinga’) ecosystems of south-eastern Brazil, Rosa et al. (1992, 1994, 1995) and Morais et al. (1994) identified some isolates as *Starmera caribaea* on the basis of growth tests. The isolates came from necrotic stems of *Pilosocereus arrabidae* and *Selenicereus rizzini*, cladodes of *Opuntia* spp., and the insects *Drosophila serido* and *Omalodes marseuli*. It is probable that these strains were in fact representatives of the novel species, *Starmera pilosocereana* sp. nov., proposed in this work. Identification based only on growth tests is not possible as the two species have nearly identical growth response profiles. Unfortunately, the strains collected in those studies have lost their viability in the Yeast Collection of the Universidade Federal do Rio de Janeiro (UFRJ; Allen N. Hagler, personal communication).

*Starmera pilosocereana* sp. nov. was isolated in low frequency in our study. The species occurred only in two

Fig. 1. Phylogram showing the placement of *Starmera pilosocereana* sp. nov. in the genus *Starmera*. Representatives of neighbouring clades in the genera *Wickerhamomyces* and *Cyberlindnera* are included for scale. The tree is the result of a maximum-likelihood analysis conducted with MEGA6 and was rooted by including the sequence for *Eremothecium cymbalariae* NRRL Y-17582T (GenBank accession no. NG_042628; not shown) in the analysis. The general time reversible model was selected based on having the highest likelihood of all models. The data consisted of 783 aligned positions at the 5’ end of the LSU rRNA gene. Bootstrap values $>50\%$ are shown at nodes. The scale bar shows patristic distances.
samples out of 44 collected, with counts of approximately $2 \times 10^3$ c.f.u. g$^{-1}$. Rosa et al. (1992, 1994, 1995) and Morais et al. (1994) reported the occurrence of a similar yeast in approximately 20% of the cactus samples studied at different sites of the sand dune ecosystems of Rio de Janeiro. The prevalent species in their studies were *Pichia cactophila*, *Candida sonorensis*, *Sporopachydemermia cereana* complex and *Clavispora opuntiae*. Differences in geography and host plant species may affect the distribution of the yeast species in the ecosystem and it is possible that *Starmera pilosocereana* sp. nov. represents a minor component of the cactophilic yeast community in the area collected in the present study. The species is probably vectored by insects such as drosophilids and beetles that use the cactus tissues as substrates for oviposition and feeding.

The assimilation of carbon compounds is similar in *Starmera pilosocereana* sp. nov. and *Starmera caribaea*, but the two differ from *Starmera pachy cereana* in their ability to ferment glucose vigorously, and from *Starmera amethionina* in the ability to assimilate D-mannitol. Geographically, *Starmera pilosocereana* sp. nov. appears to be restricted to decaying tissues of *P. arrabidae* and other cacti in the sand dune ecosystems of south-eastern Brazil, whereas *Starmera caribaea* has been reported in necrotic tissue of cacti from southern Texas (USA) to Venezuela (Kurtzman, 2011). *Starmera amethionina* and *Starmera pachy cereana* are found predominantly in Baja California Sur, Mexico, and less frequently in Baja California Norte and the Sonoran desert of Mexico and southern Arizona. *Starmera amethionina* occurs predominantly on cacti of the subtribe Stenocereinae, whereas *Starmera pachy cereana* predominates in cacti of the subtribe Pachy cereinae. However, the geographical distribution of *Starmera pilosocereana* sp. nov. needs be studied in other Brazilian and South America ecosystems.

**Description of *Starmera pilosocereana* Freitas, Barbosa, Sampaio, Lachance & Rosa sp. nov.**

*Starmera pilosocereana* (pi.lo.so.ce.re.a’na, N.L. fem. adj. pilosocereana pertaining to the cactus species in which the yeast species was found).

After 3 days on YM agar at 25 °C, cells are ovoid to ellipsoid (1.5–3.5 × 3.5–9 μm) and occur singly or in pairs. Colonies are cream, convex, smooth and glistening. Asci containing two to four hat-shaped ascospores are formed on McClary’s acetate agar after 2 days at 25 °C (Fig. 2). Ascospores are liberated. Asci are formed directly from diploid cells or after mixing of haploid mating types, although signs of conjugation are not necessarily evident. The species is heterothallic. Glucose is fermented. Glucose, ethanol, glycerol, D-mannitol, DL-lactate, succinate, ethyl acetate and gluconate (weak) are assimilated, but galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, D-ribose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, L-rhamnose, erythritol, ribitol, galactitol, D-glucitol, salicin, citrate, myo-inositol, methanol, hexadecane, D-glucosamine, N-acetyl-D-glucosamine, xylitol, acetone and 2-propanol are not. Lysine is utilized as sole nitrogen source, but not nitrate or nitrite. Growth on amino acid-free medium is negative, requiring an organic form of sulfur such as L-methionine or L-cysteine. Growth at 37 °C is positive, but negative at 40 °C. Growth in the presence of 0.01 % cycloheximide, 50 % glucose or 1 % acetic acid is negative.
Growth on YM agar with 10 % sodium chloride and 5 % glucose is negative. Starch-like compounds are not produced. Acid production from glucose is positive.

The type strain is UFMG-CY-316, isolated from necrotic tissues of Pilosocereus arrabidae in the sand dune ('Restinga') ecosystem of Macaé, Rio de Janeiro, Brazil. It has been deposited in the Collection of Microorganisms and Cells of Federal University of Minas Gerais (Coleção de Micro-organismos e Células da Universidade Federal de Minas Gerais, UFMG), Belo Horizonte, Minas Gerais, Brazil, as strain UFMG-CY-316T (h+), and is permanently preserved in a metabolically inactive state. An ex-type culture has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, as strain CBS 13266T (h+). The designated allotype, from the same locality, is strain UFMG-CY-346a (h-) (=CBS 13265).

The Mycobank number is MB 810683.

New species combination

**Starmera stellimalicola** (M. Suzuki, Nakase & Komagata)


Type strain: CBS 7853.

The Mycobank number is MB 814380.

Acknowledgements

This work was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil (560715/2010-2, 562292/2010-1 and 307015/2013-0), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (APQ-02163-11 and CBB-RDP-2010-1 and 307015/2013-0), Fundação do Amparo à Pesquisa do Rio de Janeiro (FAPERJ), Brazil (560715/2010-2, 562292/2010-1 and 307015/2013-0), FAPESP (2011/06751-2 and 2011/06752-7), and CNPq, Brazil (560715/2010-2, 562292/2010-1 and 307015/2013-0). We are grateful for the collection permits by Instituto Chico Mendes de Conservação da Biodiversidade.

References


