Starmera pilosocereana sp. nov., a yeast isolated from necrotic tissue of cacti in a sandy coastal dune ecosystem

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Two strains of a novel cactophilic yeast species were isolated from the columnar cactus Pilosocereus arribadus in a sand dune ecosystem in Rio de Janeiro, Brazil. Phylogenetic analysis of sequences of the large subunit rRNA gene D1/D2 domains showed that the strains represent a sister species to Starmera caribaea, from which it differs by 21 nt substitutions and two indels. The novel species is heterothallic and the asci are deliquescent with the formation of two to four hat-shaped ascospores. The name Starmera pilosocereana sp. nov. is proposed for the species. The type strain is UFMG-CM-Y316T (=CBS 13266T) and the allotype is UFMG-CM-Y346a (=CBS 13265). The Mycobank number is MB 810683. In addition, Candida stellimalicola belonging to the Starmera clade, is reassigned to Starmera as a new combination.

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 sonorensi 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)-1a 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-1a 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* clade and are closely related to *Starmera caribaea*. The two isolates differ from *Starmera caribaea* by 21 substitutions 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* (Cactaceae, Tribe Cereae) were collected from the Restinga de Jurubatiba National Park near Macaé, 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 decimal 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. Representatives of the different colony morphotypes were purified by repeated streak inoculation on YMA and preserved at −80 °C for later identification. The yeasts were characterized using standard methods (Kurtzman et al., 2011). Single ascospores were isolated from mature asci with a Zeiss Axio Scope.A1 microscope equipped with a micromanipulator. Prior to micromanipulation, the sporulated 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. Identities 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. Bootstraps 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 substitutions. 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-316h 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 ascosporulation 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. Zygoates 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 experiments in which asci 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 amethystina* or *Starmera pachycereana*.
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 marsaei. 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).

**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 pachycereana* 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 pachycereana* 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 pachycereana* predominates in cacti of the subtribe Pachycereinae. 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 ellipsoidal (1.5–3.5 × 3.5–9 μm) and occur singly or in pairs. Colonies are cream, convex, smooth and glistening. Ascospores are liberated. Asci containing two to four hat-shaped ascospores are formed on McClary’s acetate agar after 2 days at 25 °C (Fig. 2). 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-xylene, 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 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-CM-Y316T, 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-CM-Y316T (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-CM-Y346a (h−) (=CBS 13265).

The Mycobank number is MB 810683.

**New species combination**

*Starmera stellimalicola* (M. Suzuki, Nakase & Komagata) Lachance & Rosa f. a., comb. nov.


Type strain: CBS 7853.

The Mycobank number is MB 814380.

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**References**


