Rhodosporidiobolus geoffroeae sp. nov., a basidiomycetous yeast isolated from the waste deposit of the attine ant Acromyrmex lundii

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Abstract

A novel basidiomycetous yeast was isolated from the waste deposit of the attine ant Acromyrmex lundii (Hymenoptera: Formicidae). The field colony was located in Santurce town, Santa Fe province, Argentina. The description of the novel species was based on strain LLU043. Analysis of the D1/D2 domains of the LSU rRNA gene sequences in GenBank demonstrated that strain LLU043, belongs to the Rhodosporidiobolus clade and is closely related to Rhodosporidiobolus lusitaniae and Rhodosporidiobolus colostri with 97% similarity to the two species. The novel species differs from R. lusitaniae and R. colostri in some physiological characteristics such as the lack of assimilation of cellobiose, salicin, succinate, citrate and ethylamine. The name Rhodosporidiobolus geoffroeae sp. nov. is proposed, with LLU043 as the type strain.

Attine ants are limited to the New World [1] and they are known as ‘fungus growing ants’ because they cultivate basidiomycetous fungi (order Agaricales) as a food source for the colony [2, 3]. This amazing symbiosis has been extensively studied [3] and many different groups of microorganisms have been frequently isolated from the colonies [4–6]. Some of them are considered harmful to the symbiosis, such as Escovopsis sp., whereas others are beneficial, such as the actinobacteria [7, 8]. However, the specific role that yeasts can play in this system remains unclear. It is considered that they can be useful to the ants and to their mutualistic fungus, providing nutrients for both [9]. Depending on the ant species, different materials are collected as substrate for fungal growth [10]. A particular group is known as ‘leaf-cutting ants’ because they cut plant material (leaves, flowers, fruits, seeds) which is carried to the nest and prepared for the inoculation of the mutualistic fungi, forming a structure called a ‘fungus garden’ [2]. The ‘fungus garden’ is continuously renovated and the exhausted substrate is discharged in a waste deposit that can be found inside or outside of the nest, depending on the ant species [11, 12]. Acromyrmex lundii from Santa Fe province (Argentina) is a leaf-cutting ant that disposes off exhausted substrate outside the nest [13].

Within the order Sporidiobolales, the family Sporidiobolaceae is divided into three clades: Rhodosporidium, Sporidiobolus and the mixed Rhodosporidium/Sporidiobolus [14, 15]. The genus Rhodosporidiobolus was recently proposed by Wang et al. [16] in order to apply the rule ‘One Fungus=One Name’ [17–19]. This new genus currently includes both the sexual and the asexual forms of species in the lusitaniae clade [16] that were previously classified as Rhodosporidium/Rhodotorula and Sporidiobolus/Sporobolomyces. The genus Rhodosporidiobolus contains nine species, Rhodosporidiobolus fluvialis, R. azoricus, R. microsporus, R. nylandii, R. ruineniae, R. lusitaniae, R. colostri, R. odoratus and R. poonsookiae [15], that have been isolated from the soil, plants, freshwater or litter [20–23].

This study reports on the phenotypic characteristics and the phylogenetic position of strain LLU043, which is considered to represent a novel species belonging to the genus Rhodosporidiobolus, isolated from the waste deposit of the attine ant Acromyrmex lundii (tribe Attini: Formicidae) nest.

Material from the waste deposit of the A. lundii (Hymenoptera: Formicidae) nest was collected in a field in Santurce, Santa Fe province, Argentina (30°10′ 52.40″ S 61°10′ 05.15″ W) during September 2009 and mixed to form a single composite sample. One gram of this sample was homogenized in 9.0 ml of sterile saline solution (0.85%, w/v) and serial dilutions were prepared. Aliquots of 150 μl of each...
dilution were spread (triplicate) on plates containing MYP medium [24] supplemented with chloramphenicol at 150 mg l\(^{-1}\) and pH adjusted to 4. After incubation for 6 days at 20 °C, colonies were isolated and stored in glucose/malt extract/yeast extract/NaH\(_2\)PO\(_4\) (GYMP agar) tubes at 4 °C and in GYMP broth plus 15 % glycerol at −80 °C. Morphological, physiological and biochemical analyses of the isolates were carried out according to Kurtzman et al. [25]. DNA extraction was done as described by Sampaio et al. [26] and PCR amplification was done using the primer pairs ITS1 (5′-TCCGTAAGGTGACCTTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) for the internal transcribed spacer (ITS) region [27] and NL1 (5′-GCATAATCATAAGCTATAGGG-3′) and NL4 (5′-GCGTCCGTGTTTCAAGCGG-3′) for the D1/D2 domains of the LSU rRNA gene [28]. The amplification products were purified using an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). The sequencing reaction was performed with the same primers used during the amplification and ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystem) and purified using 125 mM EDTA, 3 M sodium acetate and ethanol. The resulting products were placed in a 3130 Genetic Analyzer (Applied Biosystems). Sequences were assembled and edited manually with the software BioEdit Sequence Alignment Editor v.7.0.5.3 [29]. Sequences of closely related taxa were downloaded from GenBank (www.ncbi.nlm.nih.gov) and Leucosporidium scottii (CBS 5930\(^T\)/AF070419) was used as an outgroup. For multiple sequence alignment, MAFFT v.7 [30] was used and the analysis of data was performed using MEGA v.6 [31]. For the neighbour-joining method, distances between the sequences were based on the Kimura two-parameter model [32]. Bootstrap analysis was performed to assess the confidence limits of the branching (1000 replicates) [33].

Thirty-nine yeast colonies were recovered from the plates. Twenty-three were ascomycetous yeasts belonging to the genera Candida (eleven), Meyerozyma (eight), Trichomonascus (yeast-like fungus) (four) and the 16 remaining were basidiomycetous yeasts belonging to Papiliotrema (six), Cutaneotrichosporon (six) and Rhodosporidiobolus sp. nov. (four). The four isolates of Rhodosporidiobolus sp. nov. were recovered from four different plates. Sequence analysis of the D1/D2 domains of the LSU rRNA gene sequences and the ITS regions showed that they are identical. The length of the sequences obtained from strain LLU043\(^T\) was 509 bp for the D1/D2 domain (GenBank accession no. KX245044) and 574 bp for the ITS region (KX245045).
Phylogenetic analysis of the D1/D2 domain showed that the novel species belongs to the *Rhodospiridiobolus* clade in which the closest relatives are *R. lusitaniae*, *R. colosti*, *R. poonsookiae* and *R. ruinenia* (Fig. 1). Comparison of the sequences of the D1/D2 domain from strain LLU043\textsuperscript{T} with the sequences of closest related type strains retrieved from the GenBank database showed 97% similarity with *R. lusitaniae* (CBS 7604\textsuperscript{T}/AF070423) and *R. colosti* (CBS 348\textsuperscript{T}/AY372177). In both cases the difference was 3.1% (16 substitutions without indels) in 509 nt whereas the divergence of strain LLU043\textsuperscript{T} with *R. poonsookiae* and *R. ruinenia* was 3.7% (19 substitutions and 19 indels) and 4.7% (24 substitutions and 15 indels) in 509 nt, respectively. Comparing the ITS region between strain LLU043\textsuperscript{T} and *R. lusitaniae* and *R. colosti* revealed 4.5% divergence (26 substitutions and three indels) and 5% divergence (29 substitutions and five indels) in 574 nt respectively.

The comparative results of the physiological and biochemical characterization of strain LLU043\textsuperscript{T} and the four most closely related species are shown in Table 1. Previous studies have described new species of yeasts isolated from the microenvironment surrounding different attine species. They belong to the genera *Cutaneotrichosporon* (former *Cryptococcus haglerorum*, [34]), *Blastobotrys* (former *Symphomyces attinorum*, [35]), *Haglerozyuma* (former *Trichosporon chiarellii*, [36]), *Starmerella* [37] and *Wickerhamomyces* [38]. In addition, novel melanized fungi have been isolated from the cuticle of attine ants such as *Phialophora* and *Ochroconis* species [5, 39].

Based on the analysis of the D1/D2 domain, ITS region, and the morphological, biochemical and physiological properties, we conclude that strain LLU043\textsuperscript{T} isolated from the waste deposit of an *A. lundii* nest represents a novel basidiomycetous yeast species, for which the name *Rhodospiridiobolus geoffroaeae* sp. nov. is proposed. The origin is unknown, but given that it was isolated from waste deposit outside the colony, we can infer that the species is part of the plant tissues discarded, or was vectored by insects that visit the dump.

**DESCRIPTION OF RHODOSPIRIDIOBOLUS GEOFFROAEAE SP. NOV.**

*Rhodospiridiobolus geoffroaeae* (ge.of.froe`ae. N.L. gen. fem. geoffroaeae refering to *Geoffroea*, the plant genus in which field workers of the leaf-cutting ant *Acromyrmex lundii* were foraging during sample collection).

After 6 days at 20°C, cells are cylindrical to bacilliform, 2–3×6–9 μm, occur singly or in pairs and budding is polar (Fig. 2). The streak culture is pink, butyrous, shiny, smooth and with an entire to slightly lobate margin. After 3 weeks no conjugation is observed.

Assimilation of carbon compounds is as follows: glucose, galactose, trehalose, soluble starch (weak), l-sorbose, d-xylose (weak), l-arabinose (slow), ethanol (weak), glycerol (slow), ribitol, galactitol, d-mannitol (weak), d-glucitol, d-glucosinate, saccharate, xylitol (weak), l-arabinitol (weak) and propane 1, 2 diol (slow) are assimilated. Inulin, sucrose, raffinose, melibiose, lactose, maltose, melezitose, methyl α-D-glucoside, celllobiose, salicin, l-rhamnose, D-arabinose, D-ribose, methanol, erythritol, myo-inositol, DL-lactate, sucrose, citrate, D-glucosamine, D-glucosinate, N-acetyl-D-glucosamine, 2-keto-D-glucosinate, 5-keto-D-glucosonate and 2,3-

Table 1. Physiological properties of strain LLU043\textsuperscript{T} (CBS 12828\textsuperscript{T}) that differentiate it from closely related species of the genus *Rhodospiridiobolus*

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Fig. 2. Cell morphology of strain LLU043\textsuperscript{T} after 6 days at 20°C on 5% malt extract agar. Bar, 20 μm.
butanediol are not assimilated. Nitrate and nitrite (weak) are assimilated and growth on vitamin-free medium is positive. No growth occurs on cadaverine, creatinine, L-lysine, ethylamine, 50 % glucose or 10 % NaCl/5 % glucose. No fermentation is detected and no growth occurs in the presence of 0.01 % cycloheximide. Production of starch-like compounds is negative. Reactions with diazomethane blue B and urease are positive. Growth is observed at 25 and 30 °C, but not at 35 °C.

The type strain, LLU043<sup>T</sup> (=CBS 12828<sup>T</sup>=CBMAI 1618<sup>T</sup>), was isolated in September 2009 from the waste deposit of an ant nest of <i>Acromyrmex lundii</i> (Hymenoptera: Formicidae) from Santurce, Santa Fe province, Argentina (30° 10’ 52.40” S 61° 10’ 05.15” W). The MycoBank accession number is MB 817691.

Funding information
The study was supported by funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (CNPq- Proc. 142731/2010-2 and 560.682/2010-7) and a CAPES/PEC-PG Scholarship to V.E.M. (Proc. 142731/2010-2 and 560.682/2010-7) and a CAPES/PEC-PG Scholarship to V.E.M.

Acknowledgements
We are grateful to R. E. Lecuona for use of his laboratory in IMyZA-INTA, Argentina, during the isolation procedures. We also thank A. lozia (the owner of the San Cayetano field, Santurce) and G. J. Masu-\-liosion for their assistance during the fieldwork. We also thank Jonathan Burgess for English review.

Conflicts of interest
The authors declare that there are no conflicts of interests.

Ethical statement
This article does not contain any studies with human participants and/or animals performed by any of the authors. Formal consent is not required for this study.

References


