Bullera vrieseae sp. nov., a tremellaceous yeast species isolated from bromeliads

Melissa Fontes Landell,¹,² Luciana R. Brandão,³ Silvana V. B. Safar,³ Fatima C. O. Gomes,⁴ Ciro R. Félix,¹ Ana Raquel O. Santos,³ Danielle M. Pagani,¹,² Jesus P. Ramos,⁵ Leonardo Broetto,¹ Tami Mott,² Marilene H. Vainstein,⁶ Patricia Valente⁷ and Carlos A. Rosa³

¹Setor de Genética/ICBS, Universidade Federal de Alagoas, Maceió - AL, Brazil
²Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos, Universidade Federal de Alagoas, Maceió - AL, Brazil
³Departamento de Microbiologia, ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte - MG 31270-901, Brazil
⁴Departamento de Química, Centro Federal de Educação Tecnológica de Minas Gerais, Belo Horizonte - MG 30421-169, Brazil
⁵National Reference Laboratory for Tuberculosis, Centro de Referência Professor Hélio Fraga, Escola Nacional de Saúde Pública, Fiocruz - RJ, Brazil
⁶Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre - RS, Brazil
⁷Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal do Rio Grande do Sul, Porto Alegre - RS, Brazil

Two independent surveys of yeasts associated with different bromeliads in different Brazilian regions led to the proposal of a novel yeast species, Bullera vrieseae sp. nov., belonging to the Tremellales clade (Agaricomycotina, Basidiomycota). Analysis of the sequences in the internal transcribed spacer (ITS) region and D1/D2 domain of the LSU rRNA gene suggested affinity to a phylogenetic lineage that includes Bullera miyagiana and Bullera sakaeratica. Six isolates of the novel species were obtained from different bromeliads and regions in Brazil. Sequence analysis of the D1/D2 domains of the large subunit of the rRNA gene showed that the novel species differs from B. miyagiana and B. sakaeratica by 85 and 64 nt substitutions, respectively and by more than 75 nt substitutions in the ITS region. Phenotypically, Bullera vrieseae sp. nov. can be distinguished from both species based on the assimilation of meso-erythritol, which was negative for B. vrieseae sp. nov. but positive for the others, assimilation of D-glucosamine, which was positive for B. vrieseae sp. nov. but negative for B. miyagiana and of L-sorbose, which was negative for B. vrieseae sp. nov. but positive for B. sakaeratica. The novel species Bullera vrieseae sp. nov. is proposed to accommodate these isolates. The type strain of Bullera vrieseae sp. nov. is UFMG-CM-Y379T (BRO443T; ex-type CBS 13870T).

Abbreviations: BI, Bayesian inference; MCMC, Markov Chain Monte Carlo method.

The GenBank/EMBL/DDBJ accession numbers for the ITS region and D1/D2 domain of the LSU rRNA gene sequence of strain UFMG-BRO443T are JX280388 and JX268526, respectively.

Bullera is a polyphyletic and anamorphic genus that has been placed in more than one order and in several clades (Boekhout et al., 2011a). Nowadays, all species are allocated in the subphylum Agaricomycotina, class Tremellomycetes where the majority of species belong to the order Tremellales and few of them are included within the Filobasidiinales and in the Trichosporonales (Boekhout et al., 2011b). Reclassification of the genus Bullera and related genera of basidiomycetous yeasts has been under way and three novel genera, Derxomyces, Hannaella and Mingxiaea were recently proposed (Wang & Bai, 2008; Wang et al., 2011).

The phenotypic characteristic of ballistoconidia formation was the trait used to distinguish the genera Bullera and Cryptococcus; however, some authors report that this trait does not appear to be a significant phylogenetic character and should
Two independent yeast surveys in Minas Gerais (Brazil) and Rio Grande do Sul (Brazil) obtained six yeast isolates (Table 1) with similar physiological profiles, assigned to one novel species of the genus *Bullera*, which we describe as *Bullera vrieseae* sp. nov. The novel species delineation was based primarily on the analysis of the combined sequences of the internal transcribed spacer (ITS) region and D1/D2 region of the LSU rRNA gene, which indicated the strains belong to one species that is phylogenetically separate from all presently recognized species in the genus *Bullera*.

The strains UFMG-CM-Y380, UFMG-CM-Y398 and UFMG-CM-Y379 were isolated from the bromeliads *Vriesea minarum*, collected at the Serra da Piedade, located at the city of Caeté, Minas Gerais state, Brazil (approx. GPS position; 19° 49' 00" S 43° 40' 00" W), in September 2008 and April 2009. Tank water samples were collected aseptically with a sterile pipette, transferred to sterile flasks and transported to the laboratory on ice for processing within 24 h. Aliquots of 0.1 ml of appropriate decimal dilutions were spread on YM agar supplemented with 0.02 % chloramphenicol and 0.0033 % rose Bengal. The plates were incubated at 25 °C for 3–8 days (Safar et al., 2013).

Strains BI23, BI80 and BI251 were isolated from leaves of the bromeliads *Tillandsia gardneri* and *Vriesea friburgensis*. The samples were aseptically collected in April and November 2004 in Itapuã Park, South of Brazil (approx. GPS position; 30° 22' 00" S 51° 04' 00" W). Isolation of these strains was described by Landell et al. (2009, 2010). The samples were spread on YM agar (1 %, w/v, glucose; 0.3 %, w/v, malt extract; 0.3 %, w/v, yeast extract; 0.5 %, w/v, peptone; 2 %, w/v, agar, acidified to pH 4.0 with hydrochloric acid and supplemented with 0.04 % chloramphenicol) plates and incubated at 25 °C for up to 7 days. All yeast isolates were purified by repeated streaking on YM agar plates and preserved at −80 °C or in liquid nitrogen for later identification. The yeasts were characterized by standard methods (Kurtzman et al., 2011).

The region spanning the ITS, the 5.8S rRNA gene and the D1/D2 domain of the large subunit rRNA gene of the UFMG strains were amplified by PCR directly from whole yeast cells as described previously (Lachance et al., 1999). The amplified DNA was sequenced using an ABI BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and an ABI 3730 automated DNA gene analyser (Applied Biosystems) according to the manufacturer’s instructions. DNA from strains BI23, BI80 and BI251 was extracted and purified according to the method of Ramos et al. (2001). The divergent D1/D2 domain of the LSU rRNA gene was amplified with the NL1 and NL4 primers (O’Donnell, 1993) and as described by Kurtzman & Robnett (1998). The ITS region (ITS1, 5.8S rRNA gene and ITS2) was amplified with ITS1 and ITS4 primers and sequenced as described by Péter et al. (2009). The sequences of strains BI23, BI80 and BI251 were obtained using an ABI 3130 automated DNA gene analyser and standard protocols at the facilities of the Instituto Nacional do Cancer, Rio de Janeiro.

The sequences obtained were compared with sequences deposited at the GenBank database (NCBI, National Center for Biotechnology Information) with the basic local alignment search tool (BLAST at http://www.ncbi.nlm.nih.gov), also available from NCBI (Altschul et al., 1990). The ITS and D1/D2 sequences were concatenated with the SequenceMatrix software. To estimate phylogenetic relationships, a neighbour-joining tree was generated that used the Kimura two-parameter to correct for genetic distances (Kimura, 1980) with the MEGA software, version 6 (Tamura et al., 2013). Gaps were excluded from the analysis. The robustness of trees was calculated with 1000 bootstrap pseudoreplicates (Felsenstein, 1985).

To test the reproducibility of the results, Bayesian inference (BI) analysis employing a Markov Chain Monte Carlo method (MCMC) was performed. Before launching BI, the best nucleotide substitution models were determined

---

**Table 1. Localities, substrates of isolation and GenBank sequence accession numbers of strains in this study**

<table>
<thead>
<tr>
<th>Strain no. (sequence accession)</th>
<th>Other designation</th>
<th>Source and locality</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFMG-CM-Y379 (KC169793; FJ828959)</td>
<td>BRO443; CBS 13870</td>
<td>Phytotelmata of <em>Vriesea minarum</em> (Bromeliaceae), Brazil</td>
<td>Apr. 2009</td>
</tr>
<tr>
<td>UFMG-CM-Y380 (KP691956; KP691955)</td>
<td>BRO199</td>
<td>Phytotelmata of <em>Vriesea minarum</em> (Bromeliaceae), Brazil</td>
<td>Sept. 2008</td>
</tr>
<tr>
<td>UFMG-CM-Y398 (KP691957; KP691954)</td>
<td>BRO200</td>
<td>Phytotelmata of <em>Vriesea minarum</em> (Bromeliaceae), Brazil</td>
<td>Sept. 2008</td>
</tr>
<tr>
<td>BI23 (KP691951; EU200784)</td>
<td>Leaf of <em>Vriesea friburgensis</em> (Bromeliaceae), Brazil</td>
<td>Apr. 2004</td>
<td></td>
</tr>
<tr>
<td>BI80 (KP691953; KP691950)</td>
<td>Leaf of <em>Tillandsia gardneri</em> (Bromeliaceae), Brazil</td>
<td>Apr. 2004</td>
<td></td>
</tr>
<tr>
<td>BI251 (KP691956; KP691949)</td>
<td>Leaf of <em>Tillandsia gardneri</em> (Bromeliaceae), Brazil</td>
<td>Nov. 2004</td>
<td></td>
</tr>
</tbody>
</table>

*ITS region.
†D1/D2 region.
with MrModeltest 2.3 (Posada & Buckley, 2004). After calculation of likelihood scores, models were selected according to the Akaike Information Criterion. The general time-reversible evolution model (Rodrı´ guez et al., 1990) was used, including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR + I + G). The alignment was analysed phylogenetically on the CIPRES webportal (Miller et al., 2010) using MrBayes v.3.2.3 (Ronquist et al., 2012). Four MCMC chains were run simultaneously, starting from random trees for 10 000 000 generations. Trees were sampled every 100th generation for a total of 10 000 trees. The first 2500 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala & Yang, 1996) were determined from a majority-rule consensus tree generated with the remaining 7500 trees. Convergence of the log-likelihoods was analysed with TRACER v. 1.6 (Rambaut et al., 2008).

### Phylogenetic Tree

The phylogenetic tree of strain UFMG-CM-Y379<sup>T</sup> inferred from Bayesian analysis of ITS and D1/D2 concatenated nucleotide sequences. The species under study is in bold type. The tree is rooted with *Sporobolomyces roseus*. Bar, substitutions per nucleotide position. Bootstrap values greater than 50 are shown.

![Phylogenetic Tree](image.png)

**Fig. 1.** Phylogenetic tree of strain UFMG-CM-Y379<sup>T</sup> inferred from Bayesian analysis of ITS and D1/D2 concatenated nucleotide sequences. The species under study is in bold type. The tree is rooted with *Sporobolomyces roseus*. Bar, substitutions per nucleotide position. Bootstrap values greater than 50 are shown.
et al., 2014); no lack of convergence was detected. Trees were visualized in FigTree (Rambaut, 2009) and exported to graphics programs. *Sporidiobolus johnsonii* and *Sporobolomyces roseus* were used as outgroups in these analyses.

All sequences generated in the present study were deposited at GenBank and accession numbers are shown in Table 1. Sequence comparisons of the ITS region and the D1/D2 domain of the large-subunit rRNA gene indicated that all strains belong to a novel yeast species within the genus *Bullera* with affinity to *Bullera miyagiana* and *Bullera sakaeratica* (Fig. 1), differing from these species by 85 and 64 nt substitutions in the D1/D2 domain, respectively, and by more than 75 nt substitutions in the ITS region. The substrate for isolation of the three *Bullera* species is associated with living plants, with *B. sakaeratica* isolated from leaves of *Seteria pallide-fusca* and *Urena lobata* in Thailand (Fungsin et al., 2003) and *B. miyagiana* from *Abies firma* collected at Sendai, Japan (Nakase et al., 1990).

Pairwise comparisons between the sequences demonstrated that all the *B. vrieseae* strains had more than 99 % identity in the D1/D2 region (0–6 substitutions and 0–2 indels) and between 98–100 % in the ITS region (0–8 substitutions and 0–3 indels). The Bayesian consensus tree obtained from the ITS and D1/D2 concatenated dataset (Fig. 1) was congruent with the neighbour-joining tree (data not shown) and confirmed our isolates as representatives of a distinct novel species within the genus *Bullera*. Tree probabilities from the Bayesian analysis were calculated from credible sets of trees (3396 trees sampled), where the 50 % credible set contained 60 trees, the 90 % credible set contained 1896 trees, the 95 % credible set contained 2646 trees and the 99 % credible set contained 3246 trees.

*Bullera vrieseae* sp. nov. was isolated from two locations in Brazil (Minas Gerais and Rio Grande do Sul) associated with three different bromeliads (water tank of *Vriesea minarum* and leaves of *Vriesea friburgensis* and *Tillandsia gardneri*) and appeared to be mainly associated with the phyllosphere and phytotelmata of bromeliads. The bromeliads are a rich source of novel yeast species and genera, and several species, including two novel genera have been described from this microhabitat in Brazil (Araújo et al., 2012; Inácio et al., 2008; Ruivo et al., 2005; Landell et al., 2009, 2010, 2014; Safar et al., 2013; Sousa et al., 2014). According to Sousa et al. (2014), the yeast communities associated with bromeliads probably survive using the water and organic detritus present in the leaves and accumulated in the bromeliad tanks.

Phenotypically, *Bullera vrieseae* sp. nov. was similar to *B. sakaeratica* and *B. miyagiana*; however, it could be distinguished from both species based on the assimilation of meso-erythritol, which was negative for *B. vrieseae* sp. nov. but positive for *B. sakaeratica*. The strains were tested alone or in pairs on corn meal agar, malt extract agar, glucose-yeast extract agar and yeast extract malt extract agar and did not show any indication of a sexual reproduction.

**Description of Bullera vrieseae sp. nov.**

*Bullera vrieseae* (vri.e.se’ae. N.L. n. *Vriesea* a botanical genus name; N.L. gen. n. *vrieseae* of *Vriesea*, the bromeliad genus from which the type strain was isolated).

In PDA broth after 5 days at 25 °C, the yeast cells are globose to ovoid and occur singly or in pairs (2.5–3.0 × 3.8–4.7 μm) (Fig. 2). On YM agar after 3 days at 25 °C, colonies are smooth, mucous to butyrous, glistening and cream-coloured. After 3 weeks in Dalmau plate culture on cornmeal agar, pseudohyphae and true hyphae are not formed. Sexual reproduction is not observed. Ballistococidia production is not observed. Fermentation ability is negative. Assimilation of carbon compounds: *D*-glucose, inulin (variable), sucrose (variable), raffinose (variable), melibiose, galactose (variable), lactose, trehalose (variable), maltose, melezitose, methyl-α-D-glucopyranoside, soluble starch (variable), cellobiose, salicin (variable), l-rhamnose (variable), xylose, l-arabinose, D-arabinose (variable), ribose, glycerol (variable), ribitol (variable), xylitol, D-galactitol (variable), mannitol, glucitol (variable), myo-inositol, lactate (variable), succinic acid (variable), citrate (variable), D-gluceric acid, D-glucosamine, N-acetylglucosamine (variable), Tween 20 and Tween 80 are assimilated; no growth occurs on meso-erythritol, L-sorbose, ethanol, methanol, hexadecane, acetone, etc.

![Fig. 2. Cells of strain UFMG-CM-Y379T on YPD broth after 7 days at 25 °C. Bar, 5 mm.](image-url)
ethylacetate and 2-propanol. Assimilation of nitrogen compounds: sodium nitrite (variable), cadaverine and lysine are assimilated; no growth on creatine, creatinine, ethylamine and sodium nitrate. Growth at 30 °C is positive but not growth at 37 °C. No growth is observed on glucose–yeast extract–peptone with 10 % (w/v) sodium chloride or with 50 % (w/v) glucose. Growth on 0.01 % (w/v) cycloheximide is variable but no growth on 0.1 % (w/v) cycloheximide. Production of starch-like compounds is negative. Urease activity is positive. Diazonium blue B reaction is positive.

The type strain is UFMG-CM-Y379T (=UFGM-BRO443T), isolated from the water tank of the bromeliad *Vriesea minarum* in Brazil, deposited in the Collection of Microorganisms and Cells of Federal University of Minas Gerais (Colecção de Micro-organismos e Células da Universidade Federal de Minas Gerais, UFMG), Belo Horizonte, Minas Gerais, Brazil, and is permanently preserved in a metabolically inactive state. Ex-type culture has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 13870T. The Mycobank number is MB 810983.

**Acknowledgements**

The authors thank the Instituto do Cancer, RJ for use of the sequencing facilities, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo a Pesquisa do Estado de Alagoas (FAPPEAL), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, processes numbers 560715/2010-2 and 475378/2013-0), Financiadora de Estudos e Projetos (FINEP, process number APQ-02163-11) for provided financial support.

**References**


Rambaut, A. (2009). FigTree v1.2.3. Institute of Evolutionary Biology, Univ. of Edinburgh. Available at: http://tree.bio.ed.ac.uk/software/figtree.


