Kazachstania bromeliacearum sp. nov., a yeast species from water tanks of bromeliads

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Cultures of a novel nutritionally specialized, fermentative yeast species were isolated from 34 water tanks of five bromeliad species, two mangrove sediment samples and one swamp water sample in Rio de Janeiro, Brazil. Sequence analysis of the D1/D2 domains of the large subunit of the rRNA gene showed that the novel species belongs to the genus Kazachstania. The novel species differs from Kazachstania martiniae by 11 substitutions and 2 gaps in the sequence of the domains D1/D2 of the LSU rRNA gene. The name Kazachstania bromeliacearum sp. nov. is proposed for the novel species. The type strain is IMUFRJ 51496T (=CBS 7996T =DBVPG 6864T =UFMG BR-174T).

Water tanks of bromeliads are dynamic and complex environments inhabited by communities of different organisms including endemic species (Benzing, 1990; Lopez et al., 2009; Whitman, 2000). The presence of trapped water and organic detritus (phytotelmata) in tanks formed in bromeliad leaf rosettes is a major source of nutrients for these organisms and communities associated with the phytotelmata (Richardson et al., 2000). Hagler et al. (1993) isolated, from phytotelmata of the bromeliad Quesnelia quesneliana in the Coroa Grande mangrove, Rio de Janeiro, two cultures that were identified by physiological and morphological tests as Saccharomyces unisporus-like. Araújo et al. (1998) isolated additional strains of the same yeast from water tanks of five bromeliad species in mangroves, rain forest and coastal sand dune ecosystems of Rio de Janeiro. Sequence analysis of the D1/D2 regions of the large subunit of the rDNA gene showed that these strains belong to the genus Kazachstania. The novel species, Kazachstania bromeliacearum sp. nov., is proposed to accommodate these isolates.

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession number for the D1/D2 domain sequence of the large subunit of the rRNA gene of strain IMUFRJ 51496T is HQ412595.
were amplified by PCR directly from whole cells as described previously (Lachance et al., 1999). The sequence was edited with the program MEGA5 (Tamura et al., 2007), which was also used to reconstruct a phylogenetic tree by the maximum-parsimony method, based on 522 aligned positions.

High molecular mass nuclear DNA for optical reassociation experiments was obtained from 24–48 h cultures grown in YEPG (1.0 % yeast extract, 1.0 % peptone, 2.0 % glucose) at 25 °C. Cells were suspended in a sucrose buffer (0.02 M Tris, 0.02M EDTA, 15 % sucrose) together with an equal volume of 0.45–0.50 mm Ø glass beads and were disrupted mechanically using a Bead-Beater Cell Disrupter (Biospec Products). DNA was purified according to Bernardi et al. (1970) with modifications by Price et al. (1978). DNA–DNA reassociation experiments were performed according to the optical method of Kurtzman et al. (1980) using a Gilford 250 spectrophotometer equipped with a model 2527 Gilford thermoprogrammer (Gilford Instruments).

Intact chromosomal DNA for pulsed field gel electrophoresis was prepared as previously reported (Vaughan-Martini et al., 1993). All analyses were performed on a Chef Mapper (Bio-Rad) using gels composed of 1 % agarose (Type II-A, medium EEO; Sigma) in 0.5 × Tris/Borate/EDTA (TBE) buffer. Temperatures of 12–15 °C were maintained throughout the runs. The run time was 68 h with a ramp of 1–5 min at 4.5 V with an angle of 120 degrees.

**Species delineation and ecology**

Although it is clear that *K. bromeliacearum* sp. nov. belongs to the genus *Kazachstania* based on the analysis of the D1/D2 domains of the large subunit rRNA gene, a reliable placement within the genus was not achieved (Fig. 1). Sisterhood with *Kazachstania viticola* was suggested from

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![Fig. 1. Phylogenetic placement of *K. bromeliacearum* sp. nov. by maximum-parsimony analysis of the D1/D2 regions of the large subunit rRNA gene. A bootstrap consensus tree for 100 replicates is shown with bootstrap values greater than 50% only shown. A total of 522 aligned positions were analysed. Bar, 5 changes.](http://ijs.sgmjournals.org)
analyses using other methods such as maximum-likelihood or neighbour-joining, but always with low bootstrap values and a very short internode subsuming the pair of species. Distance-based searches also suggested an affinity of the species with *K. martiniae*. The most closely related sequence in GenBank was that of an unassigned isolate labelled BG02-7-14-003-2-4, which is closely related to *K. martiniae*. The novel species differs from *K. martiniae* by 11 substitutions and 2 gaps and from strain BG02-7-14-003-2-4 by 10 substitutions and 2 gaps in the sequence of the domains D1/D2 of the LSU rRNA gene. CBS culture 7997, which was isolated from the same site but five months later than the type culture, is identical for this sequence (http://www.cbs.knaw.nl/collections/BioMolICS.aspx consulted on 28 October 2002). The current definition of the genus *Kazachstania* (Kurtzman & Robnett, 2003) is based on a phylogeny reconstructed from a concatenation of the small subunit rRNA gene, the ITS rDNA region, parts of the large subunit rRNA gene, the elongation factor 1-alpha, the mitochondrial small subunit rRNA gene, and cytochrome oxidase II. Such an analysis isolates all species often known as *Saccharomyces sensu stricto* as distinct from species assigned to the genus *Kazachstania*, but such a distinction is not possible based on D1/D2 sequences only. Our intent here is purely to demonstrate that strain IMUFRJ 51496T and similar strains are truly representative of a novel species, and not to provide a definitive phylogeny.

Divergence at the level of the ITS-5.8S rDNA region is considerable (in excess of 140 nt differences, depending on the alignment), which attests to the distinct nature of the novel species. However, a credible alignment of the spacers with most other species was not possible as the extent of divergence and the number of indels among congeners are considerable. Some segments found to be unique to *K. bromeliacearum* had no detectable similarity to any sequences in GenBank, whereas other segments had clear similarities with those of *Candida humilis* (AY493349) and *Kazachstania piceae* (FR716598). Any attempt to infer a phylogeny from regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598). Any attempt to infer a phylogeny from these regions other than the 5.8S rRNA gene itself would be futile (FR716598).

DNA reassociation values between the type strain of the novel species and other yeasts of the same clade were as follows: *Kazachstania martiniae* DBVP 6752T, 63%; *Kazachstania transvaalensis* DBVP 6757T, 55%; *Naumovozyma dairenensis* DBVP 6366T, 44%; *Kazachstania kunashirensis* DBVP 6756T, 39%; *Kazachstania spencerorum* DBVP 6746T, 37%; *Kazachstania unispora* DBVP 6368T, 35%; *Kazachstania barnetti* DBVP 6365T, 30%; *Kazachstania exigua* DBVP 6252T, 23%; *Kazachstania servazzii* DBVP 6355T, 18%; *N. dairenensis* DBVP 6357, 31%; *Naumovozyma castellii* DBVP 6298T, 26%; *Lachancea cidri* DBVP 6385T, 28%; and *Lachancea fermentati* DBVP 6297T, 18%. These results support the view that the novel species is closely related to *K. martiniae* although distinct from that species, as seen by an intermediate (63%) DNA–DNA reassociation value. The name *Kazachstania bromeliacearum* sp. nov. is proposed for the novel species.

The 48 cultures of *K. bromeliacearum* were isolated from 34 separate samples of phytotelmata collected from 158 bromeliad tanks, 2 of mangrove sediments and 1 of swamp water from the same sites where the bromeliads were sampled. The size of the ITS region of the rRNA gene, estimated on agarose gels, was the same (approximately 850 base pairs including the primers) in all 22 samples analysed. ITS rDNA sequences were determined for four available strains in different laboratories, resulting in different degrees of completion, although the sequence for the type covered the entire segment from the end of the small subunit rRNA gene to the end of the D2 domain of the large subunit rRNA gene. The length of the ITS regions including the 5.8S rRNA gene was 786 nt measured from the 3’ end of the binding site for primer IT1 (TAGGGGAACCTCGGCCGAAAGGATCAT) and the 5’ end of the binding site for primer NL1, a result that is compatible with those obtained electrophoretically. The four ITS sequences were identical except for a four-nucleotide deletion starting at position 115 for strain DBVP 7105. In addition, two of the more recent isolates, including one from a different location and bromeliad host species (DBVP 7104) from the type culture, had electrophoretic karyotypes, determined by pulsed field gel electrophoresis, that were similar enough to those of the type culture to be considered the same species (Fig. 2). These cultures were mostly from bromeliad tanks that received little sun exposure. The phytotelmata of bromeliads receiving more sunlight are dominated by algal growth and contain few ascomycetous yeasts. Because most isolates of *K. bromeliacearum* sp. nov. were obtained from phytotelmata of different bromeliad species, it can be assumed that the species is associated with this microhabitat. Phytotelmata have a dynamic influx of diverse arthropod and amphibian visitors, and these organisms could act as vectors of *K. bromeliacearum* sp. nov. metapopulations among different plants and for other habitats where the yeast was isolated. In addition to the unique habitat, *K. bromeliacearum* sp.
nov. differs from *K. unispora*, which has the mostly single-sспорed asci and assimilates ethanol, and from *K. martiniae*, *K. transvaalensis*, *K. kunashirensis* and *K. spencerorum* that are all positive for assimilation of trehalose.

**Latin diagnosis of Kazachstania bromeliacearum**

Araújo, Rosa, Freitas, Lachance, Vaughan-Martini, Mendonça-Hagler & Hagler sp. nov.


**Description of Kazachstania bromeliacearum**

Araújo, Rosa, Freitas, Lachance, Vaughan-Martini, Mendonça-Hagler & Hagler sp. nov.

*Kazachstania bromeliacearum* (bro.me lia.ce’a.rum. L. gen. plur. f. n. bromeliacearum of Bromeliaceae, referring to the bromeliads from which most strains of the species were isolated).

In yeast extract (0.5 %), glucose (2 %) broth after 3 days at 25 °C, cells are ovoid to ellipsoid (2–3 × 2–4 μm) and occur singly or in pairs (Fig. 3). Budding is multilateral. A sediment is formed after a month, but no pellicle is observed. On YM agar after 2 days at 17 °C, colonies are white, convex, smooth and opalescent. In Dalmat plates after 2 weeks on cornmeal agar, pseudohyphae or true hyphae are not formed. Unconjugated asc containing one to four globose ascospores are formed after 7 days on Fowell’s acetate agar at 20 °C in freshly isolated cultures. After prolonged storage, ascospores are formed after exposure to 55 °C for 4 min followed by incubation for 2 days at 25 °C in NYMP broth prior to inoculation on acetate agar (Fig. 4). Ascospores are not liberated. Glucose, galactose and occasionally sucrose (type culture is negative) are fermented. Glucose, galactose (variable, type culture is positive), sucrose (rare, type culture is negative), glycerol (rare, type culture is negative), glucono-1,5-d lactone (rare, type culture is delayed weak), lactate (rare, type culture is delayed), succinate (variable, type culture is positive) and ethanol (rare, type culture is negative) are assimilated. Sorbose, glucosamine, ribose, xylose, L-arabinose, D-arabinose, rhamnose, maltose, trehalose, methyl α-D-glucoside, cellubiose, salin, melibose, lactose, raffinose, melezitose, inuline, soluble starch, erythritol, ribitol, D-glucitol, D-mannitolum, galactitol, myo-inositolium, 2-ketogluconate, 5-ketogluconate, citrate, methanol and hexadecane are not assimilated. Ethylamine and cadaverine are assimilated, but L-lysine, sodium nitrate and sodium nitrite are not assimilated. Growth in the absence of vitamins is negative. Growth at 37 °C is variable. Growth on YM agar

![Fig. 3. Budding yeast cells of Kazachstania bromeliacearum sp. nov. CBS 7996 T on GYMP agar after three days at 20 °C. Bar, 20 μm in 4 μm segments.](http://ijs.sgmjournals.org)

![Fig. 4. Ascospores of Kazachstania bromeliacearum sp. nov. CBS 7996 T observed by (a) differential interference and (b) phase-contrast optics. Spores formed after a week at 25 °C on acetate agar, inoculated from a culture heat-shocked for 4 min at 55 °C followed by 2 days of incubation at 25 °C in GYMP broth. Ascospores are about 3 μm in diameter. Bars, 4 μm.](http://ijs.sgmjournals.org)
with 10% sodium chloride is negative. Growth in 50% glucose/yeast extract (0.5%) is negative. Starch-like compounds are not produced. With 100 μg cycloheximide ml⁻¹, growth is negative. Urease activity is negative and the Diazonium Blue B reaction is negative. Inhabits phytotelma in water tanks of shaded bromeliad plants on the south-east coast of Rio de Janeiro, Brazil.

The type strain is CBS 7996T (=IMUFRJ 51496T = UFMG BR-174T = DBVPG 6864T = IFO 10916T), isolated from a water tank of the bromeliad *Quesnelia quesneliana* in Rio de Janeiro, Brazil.

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**References**


