Lachancea mirantina sp. nov., an ascomycetous yeast isolated from the cachaça fermentation process

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In the present work, a novel ascomycete species, Lachancea mirantina sp. nov., isolated from the fermentation process that produces cachaça, a Brazilian spirit, is proposed. Nucleotide sequence analysis of the 26S D1/D2 rDNA locus showed that L. mirantina sp. nov. was genetically related to Lachancea cidri and Lachancea fermentati, although some physiological traits showed remarkable differences. Analysis of the D1/D2 large-subunit rDNA molecular marker showed a clear distinction among all three species, confirming that L. mirantina sp. nov. belongs to a separate taxonomic species in the Lachancea clade. The type strain of Lachancea mirantina sp. nov. is URM 5925T (= CLIB 1160T = CBS 11717T).

Yeast isolation and characterization

The yeast isolate SPC2(7)T (= URM 5925T = CLIB 1160T = CBS 11717T) was isolated from the fermentation process for cachaça production in the distillery Mirante da Cachaca, located in the city of Paudalho (07° 53’ 48” S 35° 10’ 47” W, 69 m above sea-level), Pernambuco State, Brazil. The isolation procedure and fermentation conditions have been reported by Vila Nova et al. (2009) and the strain was characterized by using standard methods (Yarrow, 1998).

DNA sequencing and cladistic analysis

The variable domain D1/D2 of the 26S rRNA gene of strain URM 5925T was amplified using primers NL-1 (5’-GCATACTAAAGGGAGGAAAAG-3’) and NL-4 (5’-GTCGCGTGTCAAGGC-3’), according to de Souza Liberal et al. (2007). The EF1α gene was also sequenced for confirmatory multigene analysis (Kurtzman & Robnett, 2003). Using the phylogenetic species concept, a novel species, Lachancea mirantina sp. nov., isolated from the cachaça fermentation process, is proposed.
Although they were closely related, strains URM 5925T and Lachancea Lachancea were recently reclassified within a new genus, Zygosaccharomyces. Two species were originally considered to be species of the genus Zygosaccharomyces within the clade together with Zygosaccharomyces cidri, which had been isolated in Japan (Vila Nova et al., 2009). However, those two species were recently reclassified within a new genus, Lachancea, that was separated from the genus Zygosaccharomyces by phylogenetic analysis (Kurtzman, 2003). Although they were closely related, strains URM 5925T and IFO 11066 defined a distinct branch in the cluster of what is now designated Lachancea cidri and Lachancea fermentati; this analysis was heavily supported by all clustering methods tested (Vila Nova et al., 2009). Therefore, it is suggested that these isolates represent a novel species of the genus Lachancea.

In view of this possibility, the molecular analysis of strain URM 5925T was extended to include other molecular markers. D1/D2 nucleotide sequence analysis was repeated for strain URM 5925T (GenBank accession no. FJ666084) and 100% similarity to the sequence of strain IFO 11066 was confirmed; compared with the L. fermentati sequence, 17 nt substitutions were observed, which represents 3.5% (17/493) nucleotide divergence. The limit of 1% nucleotide divergence is now widely accepted as a guideline for the delimitation of species for the ascomycetous yeasts (Kurtzman & Robnett, 1998), although some exceptions have been reported (Lachance et al., 2003). In view of this assumption, strains URM 5925T and IFO 11066 belong to a novel species in the genus Lachancea. A similar analysis was performed by sequencing the ITS and EF-1α loci. The ITS locus of strain URM 5925T (accession no. FJ666085.1) showed 94% similarity (4% gaps) to that of L. fermentati (AY046206), 93% similarity (2% gaps) to that of L. cidri (AY046205) and 93% similarity (2% gaps) to that of L. fermentati (AF402076). The cladistic analysis tree of 265 D1/D2 nucleotide sequences included the eight other recently described species of the genus. Recently, three novel species in this genus have been proposed: Lachancea meyersii was isolated from mangrove habitats in the northern Bahamas Islands and can be distinguished from other members of the genus Lachancea by the combined characteristics of lack of growth on galactose and growth on maltose (Fell et al., 2004); Lachancea dasiensis was isolated from leaves and soil in Thailand and was closely related physiologically and phylogenetically to L. thermotolerans and Lachancea waltii (Lee et al., 2009); and Lachancea nothofagi was isolated from trees in native forests in Patagonia, Argentina, and belongs to the L. thermotolerans clade (Mestre et al., 2010). Branch separation was highly supported by the cladistic analysis and showed that L. mirantina sp. nov. now represents the ninth species of this genus (Fig. 1). According to the phylogenetic species concept and the place of isolation, a novel species of the genus Lachancea, Lachancea mirantina sp. nov., is proposed.

**Molecular identification of strain URM 5925T**

Previous cladistic analysis with 26S D1/D2 nucleotide sequences was performed with yeasts isolated from different cachaça fermentation processes. In those analyses, strain URM 5925T was identified and the D1/D2 region of this isolate showed 100% sequence similarity with that of a yeast designated Zygosalachomyces sp. strain IFO 11066 (Vila Nova et al., 2009), which had been isolated in Japan from sake fermentation (data not published). Both URM 5925T and Zygosalachomyces sp. IFO 11066 clustered within the Zygosalachomyces clade together with Zygosalachomyces fermentati and Zygosalachomyces cidri and were originally considered to be species of the genus Zygosalachomyces (Vila Nova et al., 2009). However, those two species were recently reclassified within a new genus, Lachancea, that was separated from the genus Zygosalachomyces by phylogenetic analysis (Kurtzman, 2003). Although they were closely related, strains URM 5925T and IFO 11066 defined a distinct branch in the cluster of what is now designated Lachancea cidri and Lachancea fermentati; this analysis was heavily supported by all clustering methods tested (Vila Nova et al., 2009). Therefore, it is suggested that these isolates represent a novel species of the genus Lachancea.

In view of this possibility, the molecular analysis of strain URM 5925T was extended to include other molecular markers. D1/D2 nucleotide sequence analysis was repeated for strain URM 5925T (GenBank accession no. FJ666084) and 100% similarity to the sequence of strain IFO 11066 was confirmed; compared with the L. fermentati sequence, 17 nt substitutions were observed, which represents 3.5% (17/493) nucleotide divergence. The limit of 1% nucleotide divergence is now widely accepted as a guideline for the delimitation of species for the ascomycetous yeasts (Kurtzman & Robnett, 1998), although some exceptions have been reported (Lachance et al., 2003). In view of this assumption, strains URM 5925T and IFO 11066 belong to a novel species in the genus Lachancea. A similar analysis was performed by sequencing the ITS and EF-1α loci. The ITS locus of strain URM 5925T (accession no. FJ666085.1) showed 94% similarity (4% gaps) to that of L. fermentati (AY046206), 93% similarity (2% gaps) to that of L. cidri (AY046205) and 93% similarity (2% gaps) to that of Lachancea thermotolerans (FJ153217). In the case of the EF-1α locus of strain URM 5925T (GenBank accession no. GQ292877), BLAST results showed 94% similarity (no gaps) to L. fermentati (AF402076). The cladistic analysis tree of 265 D1/D2 nucleotide sequences included the eight other recently described species of the genus. Recently, three novel species in this genus have been proposed: Lachancea meyersii was isolated from mangrove habitats in the northern Bahamas Islands and can be distinguished from other members of the genus Lachancea by the combined characteristics of lack of growth on galactose and growth on maltose (Fell et al., 2004); Lachancea dasiensis was isolated from leaves and soil in Thailand and was closely related physiologically and phylogenetically to L. thermotolerans and Lachancea waltii (Lee et al., 2009); and Lachancea nothofagi was isolated from trees in native forests in Patagonia, Argentina, and belongs to the L. thermotolerans clade (Mestre et al., 2010). Branch separation was highly supported by the cladistic analysis and showed that L. mirantina sp. nov. now represents the ninth species of this genus (Fig. 1). According to the phylogenetic species concept and the place of isolation, a novel species of the genus Lachancea, Lachancea mirantina sp. nov., is proposed.

**Latin diagnosis of Lachancea mirantina Pereira, Costa, Brasiliero, Rosa et Morais sp. nov.**

*In medio liquido post dies 3 cellulae singulae aut binae; cellulae ovoidae aut ellipsoideae (2–4 × 3–5 μm). Cultura in agar multi post dies 5 (28 °C) parva, convexa, glabra et candida. In agar farinæ Zea mays post dies 14, mycelium nec pseudomycelium non formantur. Species homothallica. Asci continentes 1–4 ascosporae globosae. Glucosum, saccharatum et maltosum fermentantur. Glucosum, galactosum (lente), saccharatum, maltosum (lente), trehalosum (lente), raffinosum (lente), inulinum (lente), glycerolum, acidum lacticum (lente), glucitolum, mannotitolum, salicinum et xylitolum (lente) assimilantur, at non L-sorbusum, cellolobiosum, lactosum, melibiosum, melezitosum, amyllum solubile, D-xylolum, L-arabinosinum, D-arabinosinum, D-ribosinum, L-rhamnosinum, ethanolum, erythritolum, ribitolum, galactitolum, acidum succinicum, acidum citricum, myo-inositolum.*
methanolum, hexadecanum, acetona, ethyl acetas, 2-propanolum nec acidum gluconicum. N-Acetylglucosaminum, ethylaminum et cadaverinum assimilantur, at non lysinum, natrium nitricum nec natrium nitrosum. Ad crescentiam vitamina externa necessaria sunt. Augmentum in 30 °C, at non 37 °C. Habitat fermentatus cachaca in Brazil. Typus URM 5925T. In collectione zymotica, Centre International de Ressources Microbiennes, Grignon (sub no. CLIB 1160T) et Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum (sub no. CBS 11717T) typus stirps deposita est.

Description of Lachancea mirantina Pereira, Costa, Brasileiro, Rosa & Morais sp. nov.

Lachancea mirantina (mi.ran.ti’na. N.L. fem. adj. mirantina pertaining to the place of isolation, the distillery Mirante da Cachaça).

In yeast extract (0.5 %)-glucose (2 %) broth after 3 days at 28 °C (± 2 °C), cells are ovoid to ellipsoidal (2–4 × 3–5 μm), with simple budding. On yeast extract-malt extract (YM) agar after 5 days at 28 °C (± 2 °C), yeast colonies are white, convex, smooth and opalescent. After 14 days on cornmeal agar in Dalmau plates, neither pseudomycelium nor true mycelium are produced. After 2 days on agar media with low nitrogen/carbon ratio (e.g. yeast carbon base with 0.01 % ammonium sulfate), conjugated pairs of cells gave rise to asci containing one to four spherical ascospores (Fig. 2), from which single spores were not liberated. The species is homothallic, as conjugation takes place between cells of a single haploid population. Glucose, sucrose and maltose are fermented weakly. D-Glucose, D-galactose (slow), sucrose, maltose (slow), trehalose (slow), raffinose (slow), inulin (slow), glycerol, DL-lactate (slow), D-sorbitol (D-glucitol), D-mannitol, salicin and xylitol (slow) are assimilated. No growth occurs on L-sorbose, cellobiose, lactose, melezitose, soluble starch, D-xyllose, L-arabinose, D-arabinoise, D-ribose, L-rhamnose, ethanol, erythritol, ribitol, galactitol, succinate, citrate, myo-inositol, methanol, hexadecane, acetone, ethyl acetate, 2-propanol or gluconate. Positive for assimilation of the nitrogen compounds N-acetylglucosamine, ethylamine.HCl and cadaverine. Unable to assimilate lysine, like L. cidri (Yarrow, 1998) and L. nothofagi (Mestre et al., 2010). Like L. nothofagi, it cannot assimilate nitrate (Mestre et al., 2010). Growth in amino acid-free medium is positive. Grows at 30 °C, but not at 37 °C. Does not grow on YM agar with 10 % sodium chloride or on 50 % glucose (50 %)-yeast extract (0.5 %) medium. Starch-like compounds are not produced. Negative for growth in 10 mg cycloheximide ml⁻¹, urease activity and the diazonium Blue B reaction.

The type strain is URM 5925T (=CLIB 1160T =CBS 11717T), isolated from the cachaca fermentation process.

Fig. 1. Maximum-likelihood tree recovered in a neighbour-joining framework with a GTR+I and Distance corrected with Kimura two-parameter model of sequence evolution, with all characters within the D1/D2 26S dataset in the clade Lachancea. Values above the branches represent support values from Distance analysis with 1000 bootstrap replicates. Values below the branches represent maximum-likelihood (1000 replicates) bootstrap percentages. Two species of the genus Kluyveromyces were used as the outgroup. Bar, 0.05 substitutions per site.

Fig. 2. Vegetative cells and asci containing one to four spherical ascospores of Lachancea mirantina URM 5925T after 3 days of incubation on yeast carbon base medium supplemented with 100 mg ammonium sulfate l⁻¹ at 28 °C (± 2 °C). Arrow indicates the halter structure typical of members of the Zygosaccharomyces/Lachancea clade. Bar, 10 μm.
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References


