Saturnispora quitensis sp. nov., a yeast species isolated from the Maquipucuna cloud forest reserve in Ecuador

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A single strain, CLOCA-10-114T, representing a novel yeast species belonging to the genus Saturnispora was isolated from the fruit of an unidentified species of bramble (Rubus sp.), collected from the Maquipucuna cloud forest reserve, near Quito, in Ecuador. Sequence analyses of the D1/D2 domains of the large-subunit rRNA gene and ribosomal internal transcribed spacer region indicated that the novel species is most closely related to the recently described species Saturnispora gosingerensis, isolated from the fruiting body of a mushroom collected in Taiwan, and Saturnispora hagleri, a Drosophila-associated yeast found in Brazil. The name Saturnispora quitensis sp. nov. is proposed to accommodate this strain; the type strain is CLOCA-10-114T (=CBS 12184T=NCYC 3744T).

The yeast genus Saturnispora is characterized by teleomorphic species that typically produce one to four spheroidal ascospores ornamented with an equatorial ledge (i.e. saturn-shaped) and have a fairly restricted physiological profile (Kurtzman, 1998). The genus is well-supported by phylogenetic analyses based on multigene sequence analysis of the small-subunit and large-subunit (LSU) rRNA genes, and genetic analyses based on multigene sequence analysis of the (Kurtzman, 1998). The genus is well-supported by phylogenetic analyses based on multigene sequence analysis of the small-subunit and large-subunit (LSU) rRNA genes, and genetic analyses based on multigene sequence analysis of the (Kurtzman, 1998).

During a pilot study to survey yeast diversity found in the Maquipucuna cloud forest nature reserve, located 50 miles north-west of Quito, Ecuador (0° 03' 09” N 78° 41’ 06” W; 1668 m above sea-level), CLOCA-10-114T was isolated together with more than 70 other yeast strains. Sequence analysis of the D1/D2 domain of the LSU rRNA gene identified the isolates as belonging to 25 different species of the genera Barnettozyma (1 isolate), Candida (6), Hanseniaspora...
(2), Lachancea (1), Lodderomyces (1), Metschnikowia (2), Pichia (3), Rhodotorula (1), Saccharomyces (1), Saturnispora (1), Trichosporon (2), Wickerhamomyces (3) and Yarrowia (1). Strain CLQCA-10-114T was isolated from the fruit of an unidentified species of bramble (Rubus sp.) and, based on its physiology and ability to produce saturn-shaped ascospores, was identified as a representative of the genus Saturnispora (Kurtzman, 1998). Subsequent sequence analyses of the LSU D1/D2 domain and ribosomal internal transcribed spacer (ITS) region established that this strain belongs to a genetically distinct and hitherto undescribed species closely related to S. hagleri and the recently described S. gosingensis (isolated in Taiwan) and S. serradocipensis (isolated in south-eastern Brazil) (Canelhas et al., 2011). The name Saturnispora quitensis sp. nov. is proposed in recognition of the location in Ecuador where it was first found.

The variable D1/D2 domain of the LSU rRNA gene and ribosomal ITS region were amplified by PCR directly from whole yeast cell suspensions as described previously by James et al. (1996). The LSU D1/D2 domain was amplified and sequenced using primers NL1 and NL4 (O’Donnell, 1993). The ITS region was amplified using primers ITS5 and ITS4, and sequenced using these primers as well as internal primers ITS2 and ITS3 (White et al., 1990). PCR products were checked by agarose gel electrophoresis, purified and concentrated using QIAquick PCR purification columns (Qiagen), and sequenced using a Life Technologies 3730XL sequencer at The Genome Analysis Centre, Norwich, UK. Sequence traces were edited and consensus sequences were generated using the program SEQMAN version 7 (DNASTAR). The LSU D1/D2 sequences were compared pairwise using a FASTA similarity search (Pearson & Lipman, 1988) and were aligned with sequences of closely related taxa using the multiple alignment program CLUSTAL W (Thompson et al., 1994), included in the software package DNAMAN version 5.1.5 (Lynnon BioSoft). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with the Jukes–Cantor distance measure. Confidence limit values were estimated from bootstrap analyses of 1000 replicates (Felsenstein, 1985).

Phylogenetic analysis based on LSU D1/D2 sequences showed that CLQCA-10-114T was placed on a separate branch within the Saturnispora clade (Fig. 1). In terms of pairwise sequence similarity, CLQCA-10-114T displayed 0.8 % divergence (4 nt substitutions) with S. gosingensis, 1.1 % divergence (3 nt substitutions and two indels) with S. hagleri and 1.3 % divergence (5 nt substitutions and one indel) with S. serradocipensis. Indeed, along with S. dispora (2.6 % divergence; 12 nt substitutions and two indels), S. zarzensis (3.9 % divergence; 18 nt substitutions), Candida agrestis ES3M03 (3.3 % divergence; 16 nt substitutions and one indel) and Saturnispora sp. GJ5M11 (3.3 % divergence;
17 nt substitutions), these taxa form a distinct and separate species subgroup within the *Saturnispora* clade (Fig. 1). Despite the close phylogenetic relationship based on LSU D1/D2 sequences (0.8–1.3% sequence divergence), strain CLQCA-10-114, *S. gosingensis* GA4M05T (=MRC-2010aT), *S. hagleri* UFMG-55T and *S. serradocipensis* UFMG-DC-198T (=MRC-2010bT) can be readily distinguished from one another by ITS sequencing.

Although only partial ITS1 sequences currently exist for *S. gosingensis* GA4M05T, *S. hagleri* UFMG-55T and *S. serradocipensis* UFMG-DC-198T, all three strains still differ from CLQCA-10-114 by 6 nt substitutions and two indels, 3 nt substitutions and two indels, and by 1 nt substitution and one indel, respectively. In the ITS2 region, for which there are complete sequences available, the variation is far more pronounced: *S. gosingensis* GA4M05T differs by 17 nt substitutions and one indel; *S. hagleri* UFMG-55T by 28 nt substitutions and four indels; and *S. serradocipensis* UFMG-DC-198T by 35 nt substitutions and five indels. In fact, the overall size of the ITS2 region varies quite markedly between the four *Saturnispora* strains ranging from 110 bp (UFMG-55T to 133 bp (CLQCA-10-114)). Separate pairwise ITS alignments between *S. quitensis* CLQCA-10-114 and the other three strains are shown in Supplementary Fig. S1 (available in IJSEM Online).

A similar situation exists between the closely related species pair of *S. ahearnii* and *S. besseyi* (formerly *Pichia besseyi*) (Fig. 1). On the basis of LSU D1/D2 sequence divergence, the two taxa appear to be conspecific, as they differ from one another by only two (single nucleotide) indels. However, when Kurtzman (2006) compared the ITS sequences of the type strains (*S. ahearnii* NRRL Y-7555T and *S. besseyi* NRRL YB-4711T), currently the only known representatives of these species, far greater sequence diversity was detected. In the ITS1 region, the two strains differ by four substitutions and eight indels, and in the ITS2 region, by five substitutions and seven indels (Kurtzman, 2006). When compared against the level of ITS sequence variation observed between three different strains of *S. saitoi*, Kurtzman (2006) reported that *S. ahearnii* and *S. besseyi* (*P. besseyi*) had three times as many ITS substitutions relative to the number found among the three *S. saitoi* strains. Based on these results, he concluded that *S. ahearnii* and *S. besseyi* represent closely related species rather than divergent members of the same species (Kurtzman, 2006).

At present, it is difficult to generalize on the ecological niches of these four closely related species, as only a small number of strains currently exist (*S. gosingensis*, 1 isolate; *S. hagleri*, 6, *S. quitensis*, 1; *S. serradocipensis*, 2) (Morais et al., 2005; Canelhas et al., 2011; data from this study). However, based on those isolates that have been identified, *S. quitensis* appears to be most similar to *S. hagleri* and *S. serradocipensis* as all three species have been found in the neotropics. *S. hagleri* has been isolated from two different species of *Drosophila* (*D. cardiae* and *D. fascioloide*) collected in an Atlantic rainforest site in Brazil (Morais et al., 2005), *S. quitensis* from a bramble fruit collected in a cloud forest site in Ecuador, and *S. serradocipensis* from leaf detritus in a tropical stream in south-eastern Brazil (Canelhas et al., 2011). In contrast, the solitary strain of *S. gosingensis* so far identified was isolated from the fruiting body of a mushroom (*Coprinus* sp.) collected in Taiwan (Canelhas et al., 2011). In their species description of *S. hagleri*, Morais et al. (2005) noted that of the six identified strains, four were recovered from the crops of *D. cardiae*. This led the authors to suggest that this yeast may colonize tropical fruits and substrates regularly visited by these flies and utilized as a food source. Although only a single strain of *S. quitensis* has so far been isolated, it seems plausible to speculate that, like *S. hagleri*, additional strains of *S. quitensis* could, in future, be isolated from *Drosophila* flies and other insects that visit and feed upon tropical fruits found in Maquipucuna and other neotropical regions.

Physiologically, *Saturnispora* yeasts are almost indistinguishable from one another (Kurtzman, 1998, 2006). As reported previously, species separation is restricted to a small number of growth characteristics, specifically the assimilation of glycerol, ribitol and trehalose, as well as the ability to form a pellicle on the surface of liquid media (Morais et al., 2005). *S. quitensis*, like *S. gosingensis*, *S. hagleri* and *S. serradocipensis* assimilates both ribitol and trehalose, and is unable to form a pellicle (Morais et al., 2005; Canelhas et al., 2011). The only growth characteristic that appears to be discriminatory is the assimilation of glycerol. *S. hagleri* and *S. serradocipensis* both assimilate this carbon source, but this is a variable characteristic for *S. gosingensis* (Morais et al., 2005; Canelhas et al., 2011). In the case of *S. quitensis*, no growth was observed, even after prolonged incubation (4+ weeks). Thus, in view of the fact that these four species are physiologically almost indistinguishable from one another, we strongly recommend that LSU D1/D2 and, in particular, ITS sequencing are used to reliably determine species identity.

**Latin diagnosis of *Saturnispora quitensis* James, Cadet, Bond, Carvajal et Roberts sp. nov.**

*In medio liquido post dies duo cellulae globosae aut ovoidae (4–7 × 5–8 μm), cellulae singulae et aggregatae, per gemmationem multipolare reproducentes. Post unum mensem sedimentum formatur. Pseudomycelium nec mycelium non formatur. Post dies octo in agar glucose et extracti levidinis asci formatur. Ascii stipes. Glucosum fermentatur at non galactosum, maltosum, lactosum, raffinosum nec sucrosum. Glucosum, trehalosum, ethanolum, ribitolium, mannotolum, glucitolium, acidum lacticum et acidum succinicum assimilantur at non galactosum, L-sorbitosum, sucrorum, maltosum, cellobiosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, methanolium, glycerolium, erythritolum, methyl 2-D-glucosidum, salicinum, acidum citricum nec inositolum. Ethylaminum et cadaverinum assimilantur at non kalium nitricum. Crescit in 30 °C, at non crescit in 37 °C. Non crescit in 10% NaCl/5% glucosum nec 50%
glucosum. Materia amyloidea non formantur. Non crescit in 0.01 % cycloheximido. Typus stirps CLQCA-10-114T (=CBS 12184T=NCYC 3744T).

Description of Saturnispora quitensis sp. nov.

Saturnispora quitensis (qui.ten’sis. N.L. fem. adj. quitensis of or belonging to Quito, the capital of Ecuador, near where this strain was isolated).

Cells are spheroidal to ovoid (4–7 × 5–8 μm) and occur singly or in groups after growth in YM broth for 2 days at 25 °C. Budding is multilateral. Sediment is formed after 1 month, but no pellicle is observed. Pseudomyelia or true mycelia are not formed. After 8 days on agar media with a low nitrogen/carbon ratio (i.e. yeast carbon base with 0.01 % ammonium sulphate), conjugated cells give rise to asci containing a single ascus containing two ascospores, one of which is ornamented with an equatorial ledge (i.e. saturn-shaped) (Fig. 2a and b). Ascospores are not liberated. Conjugation takes place between individual cells and, more commonly, between cells and their buds (Fig. 2a).

Fig. 2. Saturnispora quitensis sp. nov. CLQCA-10-114T. (a) Photomicrograph of cells grown on yeast carbon base supplemented with 0.01 % ammonium sulphate after 8 days incubation at 20 °C. Note the vegetative cells, conjugation between three separate cells with their respective buds, and asci with two ascospores. Bar, 5 μm. (b) Transmission electron micrograph of a single ascus containing two ascospores, one of which is ornamented with an equatorial ledge. Bar, 1 μm.

Glucose is fermented. Galactose, maltose, lactose, raffinose and sucrose are not fermented. Glucose, trehalose, ethanol, ribitol, D-mannitol, D-glucitol, lactic acid and succinic acid are assimilated. No growth occurs on galactose, L-sorbose, sucrose, maltose, cellobiose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylene, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, glycerol, erythritol, methyl α-D-glucoside, salicin, citrate or inositol. Positive for ethylamine hydrochloride and cadaverine, but negative for nitrate. Growth is observed at 30 °C, but not at 37 °C. No growth is observed on YM agar with 10 % NaCl or on 50 % glucose/0.5 % yeast extract. No growth is observed in the presence of 50 % (w/v) glucose. Starch-like compounds are not produced. No growth with 100 μg cycloheximide ml⁻¹.

The type strain is CLQCA-10-114T (=CBS 12184T=NCYC 3744T), isolated from the fruit of an unidentified species of bramble (Rubus sp.), Maquipucuna, Ecuador.

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References


