Saturnispora hagleri sp. nov., a yeast species isolated from *Drosophila* flies in Atlantic rainforest in Brazil

Paula B. Morais,¹ Marc-André Lachance² and Carlos A. Rosa³

Correspondence

Carlos A. Rosa
carlosa@icb.ufmg.br

¹Laboratório de Microbiologia Ambiental e Biologia, Campus Universitário de Palmas, Fundação Universidade Federal do Tocantins, Palmas, Tocantins, 77010-154, Brazil

²Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7

³Departamento de Microbiologia – ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil

Six strains representing a novel yeast species belonging to the genus *Saturnispora* were isolated from two species of the *Drosophila fasciola* subgroup (*Drosophila repleta* group) in an Atlantic rainforest site in Rio de Janeiro State, Brazil. Four strains were isolated from crops and one from external parts of *Drosophila cardinae*. The other strain was isolated from external parts of *Drosophila fascioides*. Analysis of the D1/D2 large-subunit rDNA sequences indicated that the novel species is closely related to *Saturnispora dispersa*. The name *Saturnispora hagleri* sp. nov. is proposed to accommodate these strains. The type strain is UFMG-55^T (= CBS 10007^T = NRRL Y-27828^T).

The ascomycetous yeast genus *Saturnispora* is well supported by phylogenetic analysis based on analysis of the D1/D2 domains of the large-subunit rDNA sequence. The genus comprises four recognized species, *Saturnispora ahearnii*, *Saturnispora dispersa*, *Saturnispora saitoi* and *Saturnispora zaruensis*. *Candida diversa* is an anamorph of this genus. Species of *Saturnispora* are characterized by the production of spheroidal ascospores with an equatorial ledge. The four recognized species have a narrow physiological profile. Most were isolated from plant exudates, rhizosphere or soil (Liu & Kurtzman, 1991; Kurtzman & Fell, 1998; Kurtzman & Robnett, 1998; Suzuki & Nakase, 2002).

During a survey of yeasts associated with four species of the *Drosophila fasciola* subgroup (*Drosophila repleta* group) in the tropical Atlantic rainforest of Brazil, six strains of a yeast whose physiology and ascospore morphology are typical of the genus *Saturnispora* were isolated and identified as representing a *Saturnispora* species (Morais *et al.*, 1995). Analysis of the D1/D2 domains of the large-subunit rDNA sequence indicated that these strains represent a genetically distinct species closely related to *S. dispersa*. The novel species is named as *Saturnispora hagleri* sp. nov., in honour of Professor Allen N. Hagler in recognition of his contributions to yeast taxonomy and ecology in Brazil.

The origins of the strains studied here are listed in Table 1. The samples were collected from one site of the Atlantic rainforest on the island of Ilha Grande, Rio de Janeiro State, between November 1991 and November 1992. The Ilha Grande site is situated west of the village of Abraão, in Ilha Grande State Park Forest Reserve. Methodologies for *Drosophila* collection and isolation of yeast strains were as described by Morais *et al.* (1995). The yeasts were characterized by standard methods (Yarrow, 1998) and identification followed the keys of Kurtzman & Fell (1998).

The D1/D2 variable domains of the large-subunit rDNA were amplified by PCR directly from whole cells as described by Lachance *et al.* (1999). The amplified DNA was concentrated and cleaned on QIAquick PCR columns (Qiagen) and sequenced using an ABI sequencer at the John P. Roberts Research Institute, London, Ontario, Canada. The sequence was edited with the program DNAMAN, version 4.1 (Lynnon BioSoft). Existing sequences for other yeasts were retrieved from GenBank. We used CLUSTAL W software (Thompson *et al.*, 1994) to align the sequences and to construct a neighbour-joining tree based on 1000 bootstrap iterations.

Sequence comparisons indicated that the novel species belongs to the genus *Saturnispora* and is most closely related to *S. dispersa* (Fig. 1), from which it differs by 12 substitutions and three gaps in the D1/D2 large-subunit rDNA sequence. *S. dispersa* had been isolated from exudates of *Quercus* sp. (Kurtzman & Fell, 1998). Isolation of the new strains from *Drosophila* flies in Atlantic rainforest suggests

Published online ahead of print on 1 March 2005 as DOI 10.1099/ij.s.0.63673-0.

The GenBank/EMBL/DDBJ accession number for the 26S rDNA sequence of UFMG-55^T is AY762363.

Table 1. Origin of strains of *Saturnispora hagleri* sp. nov. isolated from Ilha Grande State Forest Park, Rio de Janeiro State, Brazil

Abbreviations: UFMG, Culture Collection of the Universidade Federal de Minas Gerais, Brazil; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA.

Strain(s)	Origin	<i>Drosophila</i> species
UFMG-55 ^T (= CBS10007 ^T = NRRL Y-27828 ^T), UFMG-56, UFMG-57 and UFMG-58	Crops	<i>D. cardinae</i>
UFMG-59	External parts	<i>D. cardinae</i>
UFMG-60	External parts	<i>D. fascioloides</i>

that the yeast is associated with tropical substrates visited by these insects for feeding. Four of the strains were isolated from crops of *Drosophila cardinae*, suggesting that the yeast is utilized as food by the fly. The other two strains were isolated from external parts of *D. cardinae* and *Drosophila fascioloides*, respectively. These fly species belong to the *D. fasciola* subgroup of the *D. repleta* group. Unlike the majority of the desert-inhabiting *D. repleta* group species, members of the *D. fasciola* subgroup prefer humid areas of the neotropical region. The latter subgroup is the dominant section of the *D. repleta* group found in rainforests (Wasserman, 1982; Vilela *et al.*, 1982). Morais *et al.* (1995) showed that the feeding niche of the *D. fasciola* subgroup was narrower than that of other forest-inhabiting flies and that the diet overlap between the *D. fasciola* subgroup and other forest *Drosophila* species was low. The six new strains were isolated only from *D. fasciola* subgroup flies in Atlantic rainforests, suggesting that the yeast colonizes fruits or other substrates visited by these flies. All flies of the *D. fasciola* subgroup were captured near epiphytic cacti on tree branches. Sene *et al.* (1977) suggested that at least *Drosophila onca* (a species belonging to the *fasciola* subgroup) breeds in stems of *Rhypsales* cactus. In other extensive collections of yeasts associated with *Drosophila* species in Atlantic rainforest sites in Brazil, *S. hagleri* was not detected (Morais *et al.*,

1992, 1995, 1996; Pimenta, 2001). These results suggest an association between *S. hagleri* and the substrates visited by *D. cardinae* and *D. fascioloides* in forests.

Separation of *Saturnispora* species is based only on a few growth characteristics, in particular the assimilation of trehalose and the ability to form a pellicle on the surface of liquid growth media. *S. hagleri* assimilates trehalose and does not form a pellicle. The physiological separation of *S. hagleri* from *S. dispora* or *S. zaruensis* is difficult as the three species have nearly identical physiological profiles. *S. hagleri* showed weak growth on glycerol as the carbon source whereas the other two species did not grow on this substrate. However, confirmation of identity based on D1/D2 sequencing is recommended.

Latin diagnosis of *Saturnispora hagleri* Morais, Lachance & Rosa sp. nov.

*In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (2–3 × 2–5 µm). Post unum mensem sedimentum formatur. Cultura in agar multi post dies 14 (17 °C) parva, convexa, glabra et candida. In agar farinae Zea mays post dies 14 mycelium nec pseudomycelium non formantur. Post dies unus in agar glucosi et extracti levidinis asci formantur. Asci stabiles. Glucosum et trehalosum (variabile) fermentantur. Glucosum, trehalosum, ethanolum, glycerolum (lente), ribitolum, mannitolum, glucitolum, acidum lacticum et acidum succinicum assimilantur, at non galactosum, L-sorbosum, maltosum, sucrosus, raffinose, D-ribosum, inulinum, melibiosum, lactosum, melezitolum, cellobiosum, salicinum, amyllum solubile, L-rhamnosum, D-xylosum, L-arabinosum, D-arabinosum, methanolum, erythritolum, galactitolum, meso-inositolum, acidum citricum, 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, xylitolum, acetolum, ethyl acetat, hexadecanum, nec acidum gluconicum. Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosus. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 30 °C, at non 37 °C. Habitat congregationem *Drosophila fasciola* in Brazil. Typus UFMG-55. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 10007 typus stirps deposita est.*

Fig. 1. Neighbour-joining phylogram based on the D1/D2 divergent domains of the large-subunit rDNA sequence of *Saturnispora hagleri* sp. nov. and its closest relatives. Percentage bootstrap values were obtained from 1000 iterations. All strains shown are type strains. Bar, 5% sequence divergence.

Description of *Saturnispora hagleri* sp. nov.

Saturnispora hagleri (hag'le.ri. N.L. gen. masc. sing. n. *hagleri* in honour of Professor Allen N. Hagler for his contributions to yeast taxonomy and ecology in Brazil).

Cells are ovoid to ellipsoidal ($2\text{--}3 \times 2\text{--}5\ \mu\text{m}$) after growth in yeast extract (0.5%), glucose (2%) broth for 3 days at 25 °C. Budding is multilateral. Sediment is formed after 1 month, but no pellicle is observed. On YM agar after 2 days at room temperature, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, pseudomycelia or true mycelia are not formed. After 2 days on agar media with a low nitrogen/carbon ratio (e.g. yeast carbon base with 0.01% ammonium sulfate), conjugated pairs of cells give rise to asci containing one or two spheroidal ascospores (Fig. 2). Ascospores are not liberated. The species is homothallic, as conjugation takes place between cells, and between cells and their buds of a single haploid population. Glucose and trehalose (variable) are fermented. Glucose, trehalose, ethanol, glycerol (slow), ribitol, D-mannitol, D-glucitol, lactic acid and succinic acid are assimilated. No growth occurs on galactose, L-sorbose, maltose, sucrose, raffinose, D-ribose, inulin, melibiose, lactose, melezitose, cellobiose, salicin, soluble starch, L-rhamnose, D-xylose, L-arabinose, D-arabinose, methanol, isopropanol, 2-propanol, erythritol, galactitol, myo-inositol, citric acid, 2-ketogluconate, glucosamine, N-acetylglucosamine, xylitol, acetone, ethyl acetate, hexadecane or gluconic acid. Positive for lysine, ethylamine hydrochloride and cadaverine, but negative for nitrate and nitrite. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth observed at 30 °C but not at 37 °C. No growth observed on YM agar with 10% sodium chloride or on 50% glucose/yeast extract (0.5%). Starch-like compounds are not produced. No growth with 100 μg cycloheximide ml^{-1} . Urease activity is negative. Diazonium blue B reaction is negative. Found in substrates visited by the *Drosophila fasciola* (*D. repleta* group) subgroup in Atlantic rainforests in Brazil.

Fig. 2. Phase-contrast micrograph of cells of *Saturnispora hagleri* sp. nov. grown on yeast nitrogen base with 0.01% ammonium sulfate after 3 days. Note vegetative cells, conjugation between mother cell and the bud, and asci with one or two ascospores. Bar, 5 μm .

The type strain, UFMG-55^T (=CBS 10007^T=NRRL Y-27828^T), was isolated from the crop of a *D. cardinae* fly, Rio de Janeiro State, Brazil.

Acknowledgements

This work was funded by the Programa Norte de Pesquisas e Pós-Graduação do Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil (PNOPG-CNPq), CNPq – Brazil (process no. 477528/03-1), and the Natural Science and Engineering Research Council of Canada (M.-A. L.).

References

- Kurtzman, C. P. & Fell, J. W. (1998). *The Yeasts, a Taxonomic Study*. Amsterdam: Elsevier.
- Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* **73**, 331–371.
- Lachance, M. A., Bowles, J. M., Starmer, W. T. & Barker, J. S. F. (1999). *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian *Hibiscus* flowers. *Can J Microbiol* **45**, 172–177.
- Liu, Z. W. & Kurtzman, C. P. (1991). Phylogenetic relationships among species of *Williopsis* and *Saturnospora* gen. nov. as determined from partial rRNA sequences. *Antonie van Leeuwenhoek* **60**, 21–30.
- Morais, P. B., Hagler, A. N., Rosa, C. A., Mendonça-Hagler, L. C. & Klaczko, L. B. (1992). Yeasts associated with *Drosophila* in tropical forests of Rio de Janeiro, Brazil. *Can J Microbiol* **38**, 1150–1155.
- Morais, P. B., Rosa, C. A., Hagler, A. N. & Mendonça-Hagler, L. C. (1995). Yeast communities as descriptors of habitat use by the *Drosophila fasciola* subgroup (*repleta* group) in Atlantic rain forests. *Oecologia* **104**, 45–51.
- Morais, P. B., Rosa, C. A., Abranches, J., Mendonça-Hagler, L. C. & Hagler, A. N. (1996). Yeasts vectored by *Drosophila quadrum* (*Calloptera* group) in tropical rain forests. *Rev Microbiol* **27**, 87–91.
- Pimenta, R. S. (2001). *Levantamento de leveduras em dois fragmentos de mata atlântica no estado de Minas Gerais*. Masters dissertation, Departamento de Microbiologia – UFMG, Belo Horizonte, Brazil (in Portuguese).
- Sene, F. M., Paganelli, C. H. M., Pedroso, L. G., Garcia, E. & Palombo, C. R. (1977). Local natural de criação de *Drosophila onca* Dobzhansky and Pavan, 1943. *Cien Cult* **29** (Suppl.), 716 (in Portuguese).
- Suzuki, M. & Nakase, T. (2002). A phylogenetic study of ubiquinone-7 species of the genus *Candida* based on 18S ribosomal DNA sequence divergence. *J Gen Appl Microbiol* **48**, 55–65.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Vilela, C. R., Pereira, M. A. Q. R. & Sene, F. M. (1982). Preliminary data of the geographical distribution of *Drosophila* species within morphoclimatic domains in Brazil. II. The *repleta* group. *Cien Cult* **35**, 66–70.
- Wasserman, M. (1982). Evolutionary speciation in selected species groups: the *repleta* species group. In *The Genetics and Biology of Drosophila*, pp. 33–56. Edited by M. Ashburner, H. L. Carson & J. N. Thompson. New York: Academic Press.
- Yarrow, D. (1998). Methods for the isolation and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 77–100. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.