Candida bombiphila sp. nov., a new asexual yeast species in the *Wickerhamiella* clade

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Two yeast strains were isolated from a bumblebee and bumblebee honey. The strains were almost identical in their D1/D2 domain of the large-subunit rDNA and their physiological abilities. In both respects the strains resembled *Wickerhamiella domercqiae*. On the basis of these data, it is proposed that the strains represent a novel species with the name *Candida bombiphila* sp. nov. The type strain is CBS 9712^T (=NRRL Y-27640^T=MH268^T).

Two yeast strains were isolated from a bumblebee and the honey pots in a bumblebee nest. The strains resembled *Wickerhamiella domercqiae* physiologically and in their sequences in the D1/D2 domain of the large-subunit rDNA. The genus *Wickerhamiella* was first described on the basis of the single homothallic species, *W. domercqiae* (van der Walt & Liebenberg, 1973), and later expanded to include four species from flowers and associated insects (Lachance *et al.*, 1998, 2000). Together with a number of asexual species in the genus *Candida*, the genus *Wickerhamiella* forms a loose monophyletic clade of species that exhibit a relatively low value of DNA relatedness in the D1/D2 region (Lachance *et al.*, 1998). Most species in the clade are associated with insects (Lachance *et al.*, 2000). We now describe *Candida bombiphila* sp. nov.

The strains were isolated from the proboscis of a *Bombus terrestris* queen and from the honey pots in a nest of *Bombus pascuorum* bees. Isolation was performed by direct plating of the honey and by stroking the proboscis of a *B. terrestris* queen on YM agar (Yarrow, 1998) supplemented with chloramphenicol (100 mg l⁻¹). The yeasts were purified and characterized using standard methods (Yarrow, 1998). Physiological tests were performed using replica plating (Lachance, 1987). Sexual cross-reactivity was evaluated by mixing actively growing cultures on YM agar, 10% (w/w) malt-extract agar and on restricted growth medium (Yarrow, 1998). The mixtures were incubated at room temperature and observed periodically with an Axiophot microscope (Zeiss). Images were recorded digitally by the means of a Mega Fire electronic camera (Intas) and

optimized for brightness and contrast with the PHOTOSHOP software package (Adobe).

The D1/D2 domain of the large-subunit rDNA was amplified from whole cells and then sequenced as previously described by Lachance *et al.* (1999). Known sequences for other species were retrieved from GenBank. The DNAMAN package (Lynnon Biosoft) was used to edit and align the sequences with the CLUSTAL W algorithm (Thompson *et al.*, 1994), and to construct trees with the neighbour-joining algorithm.

Latin diagnosis of *Candida bombiphila* Brysch-Herzberg & Lachance sp. nov.

Cultura in extracto malti post dies 3 ad 25 °C cellulae globosae ad ellipsoideae (2.7–4.0 × 1.9–2.5 µm), singulae aut binae. Cultura in agaro malti post dies 10 ad 25 °C crenea et butyrosa. Pseudomycelium et mycelium verum formantur. Glucosum fermentatur. L-Sorboseum, D-ribosum, glycerolum, mannitolium, glucitolium, acidum succinicum, acidum citricum, acidum malicum (lente), acidum gluconicum, glucono-Δ-lactonum assimilantur, at non inulinum, sucrosum, raffinoseum, melibioseum, galactoseum, lactoseum, trehaloseum, maltoseum, melizitosum, methyl α-D-glucosidum, amyllum, cellobioseum, salicinum, L-rhamnoseum, D-xyloseum, L-arabinoseum, D-arabinoseum, methanolium, ethanolium, erythritolum, ribitolium, xylitolium, galactitolium, inositolium, acidum lacticum, 2-keto-D-gluconatum, D-glucosaminum, N-acetyl-D-glucosaminum, acidum glucuronicum nec hexadecanum. Natrium nitricum, natrium nitrosum, ethylaminum, lysinum et cadaverinum assimilantur. Ad crescentiam vitaminarum necessariae sunt. Augmentum ad 37 °C. Habitat Bombus spp. in Germania.

Typus in collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 9712^T deposita est.

Published online ahead of print on 27 February 2004 as DOI 10.1099/ij.s.0.63139-0.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are AJ620185 and AJ620186.

Fig. 1. (a) Differential interference contrast micrograph of budding cells of *C. bombiphila* (CBS 9712^T) after 3 days in malt-extract broth (5%, w/w) at 25 °C. (b) Bright-field micrograph of pseudomycelium after 1 day on YM agar. (c, d) Bright-field (c) and phase-contrast (d) micrographs of true mycelium after 10 days on malt agar (10%, w/w) at 25 °C. Bars: 10 µm (a, d); 20 µm (b, c).

Description of *Candida bombiphila* Brysch-Herzberg & Lachance sp. nov.

Candida bombiphila (bom.bi'phi.la. N.L. nom. fem. adj. *bombiphila* *Bombus*-loving, in reference to the source of isolation, bumblebees).

In 5% (w/w) malt extract after 3 days at 25 °C, the cells are ovoid, single or in parent-bud pairs and 2.7–4.0 µm × 1.9–2.5 µm (Fig. 1a). On malt agar (10%, w/w) after 10 days at 25 °C, colonies are cream-coloured, butyrous, convex to umbonate, with a smooth and glossy surface and an entire edge. Occasionally the colonies are convoluted with a lobate edge. Pseudomycelium is formed after 1 day on YM agar at 25 °C (Fig. 1b). True mycelium is formed after 2 weeks on malt agar (10%, w/w) (Fig. 1c, d). Sexual activity has not been observed. Glucose is fermented weakly. Carbon compounds L-sorbose, D-ribose, glycerol, mannitol, glucitol, succinic acid, citric acid, malate (slow), D-gluconate and glucono-Δ-lactone are assimilated; no growth occurs on inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose, maltose, melezitose, methyl α-D-glucoside, starch, cellobiose, salicin, L-rhamnose, D-xylose, L-arabinose, D-arabinose, methanol, ethanol, erythritol, ribitol, xylitol, galactitol, inositol, glucuronate, DL-lactic acid, 2-keto-D-gluconate, D-glucosamine, N-acetyl-D-glucosamine or hexadecane. Nitrate, nitrite, ethylamine, lysine and cadaverine are assimilated. Diazonium blue B reaction is negative. Growth on vitamin-free medium is negative. Growth on amino-acid-free medium is positive. Growth at 37 °C is positive. Acid production on chalk agar

Fig. 2. Neighbour-joining dendrogram of *Wickerhamiella* and related *Candida* species based on the D1/D2 variable domains of the large-subunit rRNA gene. Numbers on nodes indicate percentage bootstrap values for 1000 iterations. Accession numbers are shown. The scale bar shows the proportional sequence divergence.

is slow. Gelatin liquefaction is negative. Casein hydrolysis is weak. Starch formation is negative. Growth on 60 % glucose/yeast extract agar is positive. Growth on YM agar with 5 % (w/w) NaCl is slow and growth on 10 % (w/w) NaCl is negative. No growth was observed in the presence of 0.01 % cycloheximide.

The habitat is bumblebees and their honey provisions. The type strain has been deposited in the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, as strain CBS 9712^T (=NRRL Y-27640^T=MH268^T). It was isolated from the proboscis of a *Bombus terrestris* queen in early spring. A second strain, CBS 9713 (=X316.5), was isolated from the honey provision in a nest of *Bombus pascuorum* bees in the summer. Both strains were isolated in the New Botanical Garden of Philipps University, Marburg, Germany.

Species delineation and identification

The D1/D2 sequence of the large-subunit rDNA of the type strain differs from the most closely related species, *W. domercqiae*, by 57 substitutions and three gaps (Fig. 2). Strain CBS 9713 (AJ620186) differs from the type strain by one substitution and one gap. Kurtzman & Robnett (1998) showed that in most cases distinct species differ by 1 % or more in these sequences; we conclude that our strains represent a novel, well-separated species. The origin and significance of strains with the designation UWOPS in Fig. 2 have been discussed by Lachance *et al.* (2001). They were included in the analysis for comparison and will be described as part of other studies. The sequence for strain NRRL Y-17858 was deposited in GenBank by Kurtzman & Robnett (1998).

C. bombiphila can be differentiated from *W. domercqiae* by the inability to grow on ethanol, 2-ketogluconate and 10 % (w/w) NaCl, by its growth at 37 °C and by the ability to ferment glucose. *C. bombiphila* can be separated from the rest of the clade by the combination of acid production on chalk agar and the ability to grow at 37 °C. The formation of true mycelium is unique to the *Wickerhamiella* clade. In view of the variability observed in growth characteristics of yeasts in general and the growing number of described species with similar nutritional profiles, definitive

identification of the species should rely on determination of the D1/D2 sequence.

Acknowledgements

We thank R. Fischer for the supply of material for the genetic work and P. Frenzel for access to the microscope. This work was funded by the Deutsche Bundestiftung Umwelt (M.B.-H.) and the Natural Science and Engineering Research Council of Canada (M.-A.L.).

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