The yeast genus *Starmerella* gen. nov. and *Starmerella bombicola* sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow

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Seven strains of a heterothallic haploid yeast species were isolated from flowers of *Calystegia sepium* (hedge bindweed, Convolvulaceae) and associated sap beetles of the genus *Conotelus*. Conjugation was observed between some of the isolates and the type strain of *Candida bombicola*, resulting in evanescent asci with one ascospore with a convoluted surface. The sequences of the D1/D2 variable domain of the large subunit of the rDNAs of three strains differed by only one or two bases from that of the type strain. The new genus *Starmerella*, with the single species *Starmerella bombicola*, is proposed to accommodate the teleomorph of *C. bombicola*. The designated isotype is strain UWO(PS)97-118¹ (h⁻; CBS 8451¹).

Keywords: *Candida bombicola*, teleomorph, *Starmerella*, rDNA

INTRODUCTION

Candida (syn. *Torulopsis*) *bombicola* was isolated from the honey of *Bombus* sp. (bumble-bee) by Spencer *et al.* (1970). *C. bombicola* produces a sophorose lipid with surfactant properties (Kim *et al.*, 1997) and has been studied for its potential biotechnological applications (Cooper & Paddock, 1984; Ito & Inoue, 1982; Kim *et al.*, 1997).

In a study of the yeast community associated with flowers of Convolvulaceae and associated insects, we isolated seven strains of a heterothallic haploid yeast species producing a single ascospore with a convoluted surface. Determination of the nucleotide sequence of the D1/D2 variable region at the 5' end of the large-subunit rDNA showed that these strains are closely related to *C. bombicola*. The type strain of the latter formed asci and ascospores when mixed with compatible strains of the new species. Based on these results, we propose the new genus *Starmerella* to accommodate the sexual stage of *C. bombicola*, in honour of William T. Starmer, in recognition of his contributions to the ecology and evolution of yeasts associated with plants and insects.

The GenBank accession numbers for the sequences in this paper are AF046041 and AF046042.

METHODS

Yeast isolation and identification. Yeasts were isolated from flowers of the hedge bindweed *Calystegia sepium*, and an associated nitidulid beetle of the genus *Conotelus*. The collections were made in July 1997 on the campus of the University of Western Ontario, London, Ontario, Canada, and in a suburb of Syracuse, NY, USA. The nectary region of flowers was scraped gently with a sterile loop and streak-inoculated onto acidified YM agar (1.0% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, 2.0% agar, acidified with HCl to pH 3.7). Nitidulid beetles were allowed to walk in plates of the same medium for 30–60 min before being removed. Yeasts were purified and characterized by the standard methods of van der Walt & Yarrow (1984) with a few additional tests (Lachance *et al.*, 1988). Identities were verified in the keys of Kreger-van Rij (1984) and Barnett *et al.* (1990). Sexual cross-reactivity was evaluated by mixing pairs of actively growing cultures on GY agar (1% glucose, 0.01% yeast extract, 2% agar).

Microscopy. Phase-contrast microscopy using a Leitz Ortholux microscope was performed on fresh cells spread over a thin agar slab. Images were recorded on Polaroid type 55 film. For scanning electron microscopy, material from a 2-d-old mixed culture of compatible mating types on GY agar was suspended in sterile water and deposited over a Nuclepore membrane (0.45 µm) on agar. This material was fixed in 2.5% glutaraldehyde in 0.3 M cacodylate buffer, pH 7.0, for at least 15 min, rinsed twice in cacodylate buffer, and dehydrated for 15 min in 2,2-dimethoxypropane lightly acidified with HCl. The material was critical point dried,

sputter-coated with gold for 5 min, and observed with a Hitachi S4500 Field Emission scanning electron microscope (Surface Science Western). Images were recorded electronically.

Electrophoretic karyotyping. Preparations of intact chromosomes were made by the method of Gardner *et al.* (1993), which does not require wall lytic enzymes. Karyotypes were obtained using contour-clamped homogeneous electric field electrophoresis (Bio-Rad CHEF DRII). Electrophoresis was carried out in a 0.8% agarose gel in $1 \times$ TBE buffer under the following conditions: 20 h at 120 V with linear ramping of 100–200 s, 25 h at 110 V with linear ramping of 450–600 s and 24 h at 100 V with linear ramping of 800–900 s.

rDNA sequence analysis. DNA was extracted according to Lachance (1990). The D1 and D2 domains of the large-subunit rDNA were amplified using primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG) and NL4 (5'-GGTCCGTGTTTCAAGACGG) (O'Donnell, 1993). PCR amplification was conducted following the instructions provided by the supplier of *Taq* polymerase (Boehringer Mannheim), in the presence of 1.5 mM $MgCl_2$, in a Perkin-Elmer System 2400 cycler. The mixture was denatured for 2 min at 95 °C, and then subjected to 35 cycles at 95 °C for 15 s, 53.7 °C for 25 s and 72 °C for 20 s, with a final extension for 5 min at 72 °C. The amplified DNA was concentrated and cleaned by ultrafiltration in MicroCon 100 concentrators (Amicon) and sequenced in an ABI sequencer at the John P. Robarts Research Institute, London, Ontario. The sequences were edited with the program DNAMAN, version 3.2 (Lynnon BioSoft) and compared with the sequences in GenBank.

RESULTS AND DISCUSSION

Generic assignment, phylogeny and ecology

Seven yeast strains were isolated from bindweed flowers and associated sap beetles (Table 1). Crosses between compatible mating types gave rise to asci containing one spheroidal ascospore with a convoluted wall and a membranous basal ledge (Fig. 1). The strains were haploid and had two mating types. Although these characteristics suggested an affinity with the genus *Wickerhamiella*, the large-subunit rDNA sequences were similar to that of *C. bombicola* (GenBank U45705; Kurtzman & Robnett, 1997). For this reason, the strains were crossed with the type strain of *C. bombicola*. Strains of one mating type (h^-) conjugated with the type strain and gave rise to asci and ascospores, confirming that they represent the same biological species. A single substitution was found between the sequences of two London strains and the type strain of *C. bombicola*. The Syracuse isolate differed by two substitutions from the others. These differences were not correlated with differences in mating intensity, but the nutritional profile of the Syracuse strain exhibited some variation compared to the others, as specified below. The sequence variation is consistent with the observation, by Kurtzman & Robnett (1997), that members of a single biological species of yeast show no more than two nucleotide substitutions in the D1/D2 domains. The electrokaryotypes of the type strain of *C. bombicola* and three

strains from bindweeds were invariant (Fig. 2). Each apparently has two large chromosomes.

The new genus *Starmerella* is proposed to accommodate the sexual stage of *C. bombicola* because this species is not closely related to any known ascospore-producing yeast genus (Kurtzman & Robnett, 1997, Fig. 2). *C. bombicola* forms a cohesive clade with *Candida etchellsii*, *Candida apicola*, *Candida bombi*, *Candida floricola*, *Candida stellata* and *Candida lactis-condensi*. A sister clade comprises five other *Candida* species. These yeasts may in the future be shown to be haploid mating types of other *Starmerella* species. Interestingly, most were isolated from insects associated with flowers. Physiologically similar, most yeasts in the 'Starmerella clade' are fermentative and utilize few carbon compounds. All are osmotolerant. This indicates a specialization towards a common niche, and a possible association with nectar-feeding insects as their principal habitat.

Latin diagnosis of the genus *Starmerella* gen. nov.

Multiplicatio vegetativa gemmatione multilateralis. Cellulae ovoidae ad ellipsoidae. Pseudomycelium et mycelium verum nullum. Asci gemini. Ascosporae singulae globosae, cum ora, parietibus asperibus, ad maturitatem ex summis ascis liberantur et inter se agglutinant. Fermentatio fieri potest. Ad Saccharomycetaceae pertinens. Species typica: Starmerella bombicola Rosa & Lachance sp. nov.

Description of the genus *Starmerella* gen. nov.

Starmerella (Star.mer.el'la. L. nom. f. n. *Starmerella* named in honour of William T. Starmer, in recognition of his major contributions to the ecology and evolution of yeasts associated with plants and insects).

The genus belongs to the family Saccharomycetaceae. Vegetative reproduction is by multilateral budding. Vegetative cells are ovoid to ellipsoidal. Asci are conjugated and form one spheroidal ascospore with a convoluted surface and a membranous basal ledge. Asci are evanescent and the ascospores are released terminally and tend to agglutinate. Pseudomycelia or true mycelia are not formed. The single species is fermentative. Type species: *Starmerella bombicola* Rosa & Lachance sp. nov.

Latin diagnosis of *Starmerella bombicola* Rosa & Lachance sp. nov.

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (1–2 × 2–4 µm). Post unum mensem annulus et sedimentum formantur. Cultura in agarō multi post dies 14 (17 °C) parva, convexa, glabra, candida et butyrosa. In agarō farinae Zea mays post dies 14 mycelium nec pseudomycelium non formatur. Post dies unus, cellulae stirpium interfertiliū mixtarum in agarō glucosi et extracti levidinis tubi junctionis et asci

Table 1. List of strains of *Starmerella bombicola* used in this study

Strain no.*	Host	Locality	Mating type
CBS 6009 ^T (NRRL-Y-17069 ^T)	<i>Bombus</i> sp.	Pincher Creek, Alberta, Canada	h ⁺
UWO(PS)97-105	<i>Conotelus</i> sp.	University of Western Ontario, Canada	h ⁺
UWO(PS)97-107	<i>Conotelus</i> sp.	University of Western Ontario, Canada	h ⁺
UWO(PS)97-112	<i>Conotelus</i> sp.	University of Western Ontario, Canada	h ⁺
UWO(PS)97-114	<i>Calystegia sepium</i>	University of Western Ontario, Canada	h ⁺
UWO(PS)97-118 ^I (CBS 8451 ^I)	<i>Calystegia sepium</i>	University of Western Ontario, Canada	h ⁻
UWO(PS)97-119	<i>Calystegia sepium</i>	University of Western Ontario, Canada	h ⁻
UWO(PS)97-211.1	<i>Calystegia sepium</i>	Syracuse, NY, USA	h ⁺

*T, Holotype; I, designated isotype.

Fig. 1. (a) Phase-contrast micrographs of maturing asci of *S. bombicola* showing conjugated cells and an empty ascus. (b-d) Scanning electron micrographs of *S. bombicola* showing ascospores with agglutination mucilage and membranous ledge (c) and a convoluted surface (d). Bar, 1 µm.

juncti formantur. Post dies tres, asci ascospora una liberant. Glucosum, sucrosum et raffinsum (variabile) fermentantur. Sucrosum (aliquando exigue), galactosum (variabile et exigue), raffinsum (variabile), L-sorbozum (aliquando lente aut exigue), ethanolum (lente aut exigue), glycerolum (saepe lente), mannitolum, glucitolum (saepe lente), acidum succinicum (variabile), acidum citricum (variabile), acidum gluconicum (varia-

Fig. 2. Electrokaryotypes of *S. bombicola* strains. See Methods for conditions. The chromosomal DNAs of *Saccharomyces cerevisiae* (Bio-Rad) are shown as standards, where the largest three bands represent molecules of 2.2, 1.6 and 1.1 Mb, respectively.

bile) et glucono- δ -lactonum (*variabile et exigue*) assimilantur at non inulinum, melibiosum, lactosum, trehalosum, maltosum, melezitosum, methyl α -D-glucosidum, cellobiosum, salicinum, amyllum solubile, L-rhamnosum, D-xylosum, L-arabiosum, D-arabiosum, D-ribosum, methanolum, 1-propanolum, 2-propanolum, 1-butanolum, erythritolum, ribitolum (*lente aut exigue*), xylitolum (*lente*), galactitolum, meso-inositolum, acidum lacticum, acidum malicum (*aliquando exigue*), 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, acetonom, ethyl acetate nec hexadecanum. Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosom. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 30 °C at non 37 °C. Habitatio in floribus et in insectis. Typus stirps CBS 6009^T (h⁺). Isotypus stirps UWO(PS)97-118¹ (h⁻). In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum, sub no. CBS 8451¹ [= UWO(PS)97-118¹] isotypus stirps deposita est.

Description of *Starmerella bombicola* Rosa & Lachance sp. nov.

Because the number of physiological tests performed with our strains exceeds that given in the original description of *Torulopsis bombicola* (Spencer *et al.*, 1970), an extensive description of the teleomorph is given.

In yeast extract (0.5%)/glucose (2%) broth after 3 d at 25 °C, the cells are ovoid to ellipsoidal (1–2 × 2–4 µm). Budding is multilateral. A sediment and a

ring are formed after a month. On malt agar after 2 weeks at 17 °C, the colonies are small, convex and white, with an entire edge. In Dalmau plates after 2 weeks on cornmeal agar, pseudomycelia or true mycelia are not formed. After 1 d on GY agar, mixed cells of compatible mating types fuse in pairs. After 3 d, conjugated asci form one spheroidal ascospore with a convoluted wall and a membranous basal ledge. The ascospores are released terminally and have a strong tendency to agglutinate. Glucose and sucrose are fermented. Fermentation of raffinose is variable. Sucrose (sometimes weak), galactose (delayed and variable), raffinose (variable), L-sorbose (sometimes weak or slow), ethanol (weak or slow), glycerol (often slow), mannitol, glucitol (often slow), succinic acid (variable), citric acid (variable), gluconic acid (variable) and glucono- δ -lactone (often slow or weak) are assimilated. Inulin, melibiose, lactose, trehalose, maltose, melezitose, methyl α -D-glucoside, starch, cellobiose, salicin, L-rhamnose, xylose, L-arabinose, D-arabinose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, ribitol, xylitol, galactitol, *myo*-inositol, lactic acid, malic acid (sometimes weak), 2-ketogluconate, glucosamine, N-acetylglucosamine, acetone, ethyl acetate and hexadecane are not assimilated. Ethylamine, lysine and cadaverine are assimilated as nitrogen sources, but not nitrate and nitrite. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 30 °C is positive; at 37 °C it is negative. Gelatin liquefaction and casein hydrolysis are negative. Lipolytic activity on Tween 80 agar is negative. Acid formation on chalk agar is negative. Growth in YM agar with 5% NaCl is positive or weak; with 10% NaCl growth is negative. Growth in 50% glucose/yeast extract agar is slow. Starch-like compounds are not produced. Growth in the presence of 10 mg cycloheximide l⁻¹ is positive; with 100 µg ml⁻¹ it is negative. Growth in the presence of 8 µg digitonin ml⁻¹ at 25 °C is negative. Diazonium Blue B reaction is negative. G + C content: 49.8 mol% (Stenderup *et al.*, 1972). Ubiquinone system: Q-9 (Lee *et al.*, 1993). The habitat is flowers and insects. The type strain is CBS 6009^T, the type strain of *Candida bombicola* (Spencer *et al.*, 1970). Its mating type is designated arbitrarily as h⁺. The isotype is strain UWO(PS)97-118¹ (h⁻; CBS 8451¹) isolated from hedge bindweed (*Calystegia sepium*).

Variation

Some of the physiological properties determined for our isolates are at variance with those given in the literature. Raffinose assimilation was negative. The original description of *T. bombicola* (Spencer *et al.*, 1970) reported it as positive, Barnett *et al.* (1990) reported it as delayed and Meyer *et al.* (1984) reported it as variable. Acid production was reported as positive in the original description, while it was negative in Barnett *et al.* (1990) and weak in our study. Barnett *et al.* (1990) reported a variable response on ribose and a delayed response on 100 mg cycloheximide l⁻¹, both of

which are at variance with other sources, which report these as negative.

As noted earlier, the Syracuse strain (97-211.1) differed from others in the intensity of many growth responses. Fermentation and the assimilation of galactose, sorbose, glucitol and succinic acid were more intense, whereas the utilization of sucrose, citric acid, malic acid, gluconic acid and glucono- δ -lactone, as well as NaCl tolerance were less intense. Such intrinsic differences within the species may account for the variability observed when contrasting various reports.

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