# Cryptococcus socialis sp. nov. and Cryptococcus consortionis sp. nov., Antarctic Basidioblastomycetes

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New yeasts from the Ross Desert (dry valley area) of Antarctica include *Cryptococcus socialis* sp. nov. and *Cryptococcus consortionis* sp. nov. *Cryptococcus socialis* MYSW A801-3aY1 (= ATCC 56685) requires no vitamins, assimilates L-arabinose, cellobiose, D-glucuronate, maltose, melezitose, raffinose, soluble starch, sucrose, and trehalose, and may be distinguished from all other basidioblastomycetes by the combination of amylose production, cellobiose assimilates L-arabinose, Cryptococcus consortionis MYSW A801-3aY2 (= ATCC 56686) requires thiamine, assimilates L-arabinose, D-glucuronate, 2-ketogluconate, salicin, succinate, sucrose, trehalose, and D-xylose, and may be distinguished from all other basidioblastomycetes by the combination, succinate, sucrose, trehalose, and D-xylose, and may be distinguished from all other basidioblastomycetes by the combination of amylose production and failure to utilize nitrate, cellobiose, D-galactose, *myo*-inositol, and mannitol. Its guanine-plus-cytosine content is 56 mol%.

During an examination of the microbiota of soils from the Ross Desert (dry valley area) of Antarctica, two unidentifiable yeasts were isolated; these yeasts are here proposed as new species. Both are representatives of a small group of Antarctic yeast biotypes that fail to utilize nitrate N and therefore are restricted to sites (rare in deserts) in which other N sources are available.

## MATERIALS AND METHODS

Cultures were maintained at 4°C on 1.8% agar slants of YY-2 medium (Y-2 mineral base of Vishniac and Baharaeen [21] supplemented with 0.5% glucose, 2.0 mM NH<sub>4</sub>Cl, 2.0 mM NaH-glutamate [pH 6.0], 4.0 mM potassium phosphate buffer [pH 6.0], 0.05% yeast extract, and a vitamin mixture [13]). Cycloheximide resistance (at 10°C), NaCl tolerance (at 10°C), and temperature tolerance were examined on YY-2 medium. Colonial morphology was also observed on the following commercially available media (Difco Laboratories): cornmeal agar, malt extract agar, Sabouraud dextrose agar, and Wickerham agar (yeast carbon base without nitrogen, supplemented as suggested by Phaff et al. [13]). The exponentially growing cultures used for cell measurement and electron microscopy by the method of Baharaeen and Vishniac (2, 3) were prepared in liquid YY-2 medium at 10°C with aeration provided by continuous shaking. Carbon and nitrogen utilization profiles were determined at 10°C by multipoint inoculation of 2.0% agar plates containing Y-2 base plus glucose or another substrate (0.2%) and NH<sub>4</sub>Cl or another nitrogen source (2.0 mM), with appropriate negative controls. Amylose production required the use of 0.5 to 1.0%glucose and 10 mM NH<sub>4</sub>Cl. Fermentative ability was tested in heavily inoculated broth containing a mixture of 0.5%glucose and 0.5% sucrose. The production of extracellular urease was examined on Difco urea broth to which 0.2% glucose and 1.8% agar had been added. Production of extracellular proteinase and of melanin from dihydroxyphenylalanine (DOPA) was examined by previously described methods (21). Reaction to diazonium blue B (DBB) was determined by the method of Hagler and Ahearn (9). Guanine plus cytosine (G+C) contents of nuclear deoxyribonucleic acids (DNAs) were determined by the buoyant density method (1).

The following compounds were tested as sole sources of carbon and energy: acetate (0.1%, pH 6.0 and 7.0),  $\gamma$ -aminobutyrate (pH 6.1), p-arabinitol, L-arabinitol, p-arabi-

nose, L-arabinose, arbutin, L-aspartate (pH 5.0), butyrate (pH 5.0), cellobiose, citrate (pH 6.0), erythritol, ethanol, D-fructose, D-fucose, fumarate (pH 5.1), galactitol, D-galactose, D-glucitol, D-gluconate (pH 5.8), D-glucose, D-glucosamine (pH 6.0), D-glucuronate (pH 5.5), L-glutamate (pH 6.0), glycerol, hexadecane, myo-inositol, inulin, 2-ketogluconate (hemicalcium salt), 5-ketogluconate (pH 6.8), DL-lactate (pH 6.0), lactose, L-malate (pH 5.1), maltose, D-mannitol, D-mannose, melezitose, melibiose, methanol,  $\alpha$ -methyl-D-glucopyranoside, L-proline (pH 6.0), raffinose, L-rhamnose, ribitol, D-ribose, salicin, soluble starch, L-sorbose, succinate (pH 5.5), sucrose, trehalose, xylitol, and D-xylose. The following compounds were tested as sole sources of nitrogen: NH<sub>4</sub>Cl, NaNO<sub>2</sub>, KNO<sub>3</sub>, D-alanine, L-alanine,  $\gamma$ -aminobutyrate, L-arginine, D-aspartate, L-aspartate, cadaverine (pH 6.1), creatinine (pH 6.0), D-glutamate, L-glutamate, D-lysine, L-lysine, D-proline, L-proline, D-tyrosine, and L-tyrosine. The compounds which were not utilized as C or N sources are not mentioned below.

#### RESULTS

*Cryptococcus socialis* sp. nov. Vishniac (soc.i.al'is. L. adj. *socialis* of companionship, referring to occurrence only at Antarctic sites with other microbiota).

Latin diagnosis of *Cryptococcus socialis* sp. nov. Coloniae in agaro colore cremeo, marginibus integris, politae et paululum mucosae. In mediis liquidiis pellicula nulla, annulus exigue, et sedimentum lente formantur. Cellulae crescentes ovoideae,  $5.43 \pm 0.68 \times 4.05 \pm 0.44 \mu m$ , cum capsulis exiguis, e situ cicatricis natalis iterumque iterumque generatae. Parietes typici basidioblastomycetium. Nec pseudomycelium nec mycelium formatur. Non generant sed per modo blastico asexuali, calore 4°C usque ad 23°C (lente) sed non 27°C. DBB respondet. G+C, 56 mol%.

Nec glucosum nec sucrosum fermentat. Assimilat acidum aceticum, L-arabinosum, acidum L-asparticum, cellobiosum, D-fructosum, D-glucosum, acidum D-glucuronicum, acidum L-glutamicum (lente, variabile), maltosum, D-mannosum, melezitosum, raffinosum, amylosum (sine agarum candidum faciens), sucrosum, trehalosumque. Crescit cum ammonio, L-alanino, acido  $\gamma$ -aminobutyrico, acido L-arginino, acido L-aspartico, L-prolinoque. Amylosum formatum est. Crescit sine vitamines. Cum cycloheximido 0.05 µg ml<sup>-1</sup> non crescit (lente cum 0.01 µg ml<sup>-1</sup>). Cum natrio chlorido 7% non



FIG. 1. C. socialis MYSW A801-3aY1. Scanning electron micrograph. Bar =  $1 \mu m$ .

crescit (lente cum 3, 5%). Media DOPA non atrescentes. Nec lactis proteinum nec urea finditur.

Typus MYSW A801-3aY1 (= ATCC 56685), e solo, Terratio Linnaeo, ca. 1,550 m.

C. socialis sp. nov. Vishniac is typified by strain MYSW A801-3aY1 (= ATCC 56685), which was isolated from soil from Linnaeus Terrace, South Victoria Land, Antarctica, at ca. 1,550 m and by this description and (Fig. 1 under Note 1, Article 9, of the *International Code of Botanical Nomenclature* (17) (the distinguishing features of the species are not preserved in herbarium specimens).

Colonies growing on agar are cream colored, entire, and glistening but not conspicuously mucoid. Growth in liquid media is limited unless agitation and aeration are provided; in static tubes, a scanty annulus (no pellicle) and slowly produced sediment provide the only evidence of growth. Exponentially growing cells are ovoid and  $5.43 \pm 0.68$  by  $4.05 \pm 0.44 \mu$ m, with a scanty capsule. Budding is monopolar and repetitive through the birth scar site. Mycelium and pseudomycelium are not produced on Dalmau plates; exponentially growing cultures sometimes contain a very low proportion of atypical cells, including rudimentary (three-celled) pseudomycelia. Sexual reproduction has not been observed. Growth occurs at temperatures from 4 to 23°C (slowly), but not at 27°C. The DBB reaction is positive; the G+C content is 56 mol%.

Fermentation is lacking. Acetate, L-arabinose, L-aspartate, cellobiose, D-fructose, D-glucose, D-glucuronate, L-glutamate (weakly and variably), maltose, D-mannose, melezitose, raffinose, soluble starch (without clearing around the colony), sucrose, and trehalose serve as sole sources of carbon and energy. Utilizable nitrogen sources include ammonium chloride, L-alanine,  $\gamma$ -aminobutyrate, L-arginine, L-aspartate, L-glutamate, and L-proline. Amylose is produced. Vitamins are not required. Growth is suppressed by 0.05 µg of cycloheximide per ml and slowed by 0.01 µg of cycloheximide per ml. Growth is slowed by 3 and 5% NaCl and completely suppressed by 7% NaCl. Melanin production is not evident on DOPA. Extracellular proteinase and urease are not produced. *Cryptococcus consortionis* sp. nov. Vishniac (con. sort.i.on'is. L. n. f. gen. *consortionis* of a community or association, referring to occurrence only at Antarctic sites with other microbiota).

Latin diagnosis of *Cryptococcus consortionis* sp. nov. Coloniae in agaro colore cremeo, marginibus integris, politae et paululum mucosae. In mediis liquidiis pellicula nulla, annulus exigue, et sedimentum lente formantur. Cellulae crescentes ovoideae,  $5.45 \pm 0.56 \times 4.34 \pm 0.34 \mu m$ , cum capsulis exiguis, e situ cicatricis natalis iterumque iterumque generatae. Parietes typici basidioblastomycetium. Nec pseudomycelium nec mycelium formatur. Non generant sed per modo blastico asexuali, calore 4°C usque ad 23°C (lente) sed non 27°C. DBB respondet. G+C, 56 mol%.

Nec glucosum nec sucrosum fermentat. Assimilat acidum aceticum, acidum  $\gamma$ -aminobutyricum, L-arabinosum, arbutinum (lente), acidum asparticum (lente, variabile), D-fructosum, acidum fumaricum (lente), D-glucosum, acidum Dglucuronicum, acidum glutamicum (lente, variabile), acidum 2-ketogluconicum, acidum malicum, D-mannosum, L-prolinum, salicinum, acidum succinum, sucrosum, trehalosum, xylosumque. Crescit cum ammonio, L-alanino, acido  $\gamma$ aminobutyrico, acido L-aspartico, acido D-glutamico (lente), acido L-glutamico, L-prolinoque. Amylosum formatum est. Thiaminum necessitat. Cum cycloheximido 0.05  $\mu$ g ml<sup>-1</sup> non crescit (lente cum 0.01  $\mu$ g ml<sup>-1</sup>). Cum natrio chlorido 7% non crescit (lente cum 3, 5%). Media DOPA non atrescentes. Nec lactis proteinum nec urea finditur.

Typus MYSW A801-3aY92 (= ATCC 56686), e solo, Terratio Linnaeo, ca. 1,550 m.

Cryptococcus consortionis sp. nov. Vishniac is typified by strain MYSW A801-3aY92 (= ATCC 56686), which was isolated from soil from Linnaeus Terrace, South Victoria Land, Antarctica, at ca. 1,550 m and by this description and Fig. 2.

Colonies growing on agar are cream colored, entire, and glistening but not conspicuously mucoid. Growth in liquid media is limited unless agitation and aeration are provided; in static tubes, a scanty annulus (no pellicle) and slowly produced sediment provide the only evidence of growth. Exponentially growing cells are ovoid and  $5.45 \pm 0.56$  by  $4.34 \pm 0.34 \mu m$ , with a scanty capsule. Budding is monopo-



FIG. 2. C. consortionis MYSW A801-3aY92. Scanning electron micrograph. Bar =  $2 \mu m$ .

lar and repetitive through the birth scar site. Mycelium and pseudomycelium are not produced on Dalmau plates; exponentially growing cultures sometimes contain a very low proportion of atypical cells, including rudimentary (three-celled) pseudomycelia. Sexual reproduction has not been observed. Growth occurs at temperatures from 4 to  $23^{\circ}$ C (slowly), but not at  $27^{\circ}$ C. The DBB reaction is positive; the G+C content is 56 mol%.

Fermentation is lacking. Acetate,  $\gamma$ -aminobutyrate, L-arabinose, arbutin (slowly), aspartate (weakly and variably), D-fructose, fumarate (slowly), D-glucose, D-glucuronate, L-glutamate (weakly and variably), 2-ketogluconate, malate, D-mannose, L-proline, salicin, succinate, sucrose, trehalose, and D-xylose serve as sole sources of carbon and energy. Utilizable nitrogen sources include ammonium chloride, L-alanine,  $\gamma$ -aminobutyrate, L-aspartate, D-glutamate (weakly), L-glutamate, and L-proline. Thiamine is required for growth. Amylose is produced. Growth is suppressed by 0.05  $\mu$ g of cycloheximide per ml and slowed by 0.01  $\mu$ g of cycloheximide per ml. Growth is slowed by 3 and 5% NaCl and completely suppressed by 7% NaCl. Melanin is not produced on DOPA. Extracellular proteinase and urease are not produced.

## DISCUSSION

C. socialis and C. consortionis are considered basidioblastomycetes (11) by virtue of possessing a layered cell wall (evident in the fraying collars of Fig. 1 and 2) and a positive DBB response. These species are assignable to the genus *Cryptococcus* as defined by the modification of Golubev et al. (8) of the definition of Phaff and Fell (12) to include yeasts which assimilate D-glucuronate, although not necessarily *myo*-inositol. These species also answer to the description of *Cryptococcus* suggested by Baharaeen and Vishniac (4), since they are cream-colored anamorphic yeasts with a smooth, layered, cell wall and typically bud enteroblastically and repetitively at the birth scar site during uninterrupted exponential growth, through a collar left by the abscission of the primary (holoblastic) and succeeding buds.

Each species was compared with all basidiomycetous yeasts which are identifiable as such by sexual reproduction, DBB reaction, or wall structure (5, 16, 21). Each is presumptively identifiable by a short list of tests to which its reactions (in combination) are unique. *C. socialis* is the only basidioblastomycete which produces amylose, assimilates cellobiose, and fails to utilize nitrate, D-galactose, *myo*-inositol, and mannitol. *C. consortionis* is the only basidioblastomycete which produces amylose but fails to utilize nitrate, cellobiose, D-galactose, *myo*-inositol, and mannitol. Such distinctions are insufficient to justify the description of these isolates as new species.

Species of anamorphic yeasts can be distinguished either by G+C content or DNA-DNA homology values similar to those correlated with the failure of teleomorphic species to produce fertile interspecific hybrids or by phenotypic differences greater than those found within well-investigated species. The proposed species qualify by neither criterion. The two species proposed here do not differ significantly in G+C content from most of the species of the Antarctic *Cryptococcus vishniacii* complex or, given the variation from laboratory to laboratory, from many other *Cryptococcus* spp. DNA-DNA hybridizations have not been performed; for reasons given below, it appears that shotgun experiments would be required. Although many species of basidioblastomycetes have been described as possessing highly variable physiological profiles (5), the profiles of

biotypes known to be interfertile or to possess high levels of DNA-DNA homology are rarely recorded. A strain of Cryptococcus neoformans has been reported to mate with the a type of Filobasidiella neoformans despite nine substrate assimilation differences (15). Differences in the assimilation of five conventional substrates separate C. vishniacii var. wolfii from C. vishniacii var. vladimiri, although these varieties, with more than 60% relative binding in DNA-DNA hybridization experiments (1), are conspecific by most standards. It has been clear for some time that fermentation and assimilation patterns of yeasts often do not reflect similarities in total genome (14). Pichia cactophila and Pichia pseudocactophila (10) and Cryptococcus wrightensis and C. vishniacii (1, 21), with about 35% relative binding in DNA-DNA hybridization experiments, do not differ as species in conventional utilization characters. If these are not valid species, they are certainly far advanced in the process of speciation.

C. socialis differs from C. consortionis in the assimilation of y-aminobutyrate, arbutin, fumarate, 2-ketogluconate, malate, maltose, melezitose, L-proline, raffinose, salicin, soluble starch, succinate, and D-xylose, in the utilization of L-arginine N, and in thiamine requirement. These differences appear to be sufficient to exclude conspecificity. However, each of these species is more similar to previously described yeasts. Similarity to Rhodotorula aurantiaca, a highly variable species (5), is probably spurious; it is unlikely that yeasts differing so greatly in morphology, color, and amylose production are conspecific. C. socialis differs in only six characters (utilization of nitrite and nitrate N, assimilation of  $\gamma$ -aminobutyrate, 2-ketogluconate, salicin, and xylose) from Cryptococcus baldrensis (21). C. consortionis differs in nine characters (thiamine synthesis, utilization of nitrite and nitrate N, assimilation of  $\gamma$ -aminobutyrate, L-arabinose, maltose, melizitose, L-proline, and raffinose) from Cryptococcus hempflingii (21). When only the conventional physiological characters are considered, both species fail to be excludable on phenotypic grounds not only from Antarctic species of the C. vishniacii complex (21), but also from a number of species of basidioblastomycetes classified in diverse genera (5).

Ecological differences provide another basis for proposing new species. It is customary in making phenotypic comparisons to give equal weight to each character. Differences in habitat and community composition impose different constraints upon evolution. The effect of such constraints on yeast speciation may give great importance to individual physiological characters or to characteristics which are not generally included in phenotypic comparisons. The best example of ecological determinants in yeast speciation is seen in the effect of host cactus chemistry on the evolution of cactophilic yeasts (18, 19).

The Ross Desert of Antarctica is the most extreme cold arid habitat on earth. The multiple stresses of this environment have resulted in depauperate soil communities containing an average of ca. 1 microcolony of microbes known to be indigenous per g of soil (Vishniac, Antarct. J. U.S. in press). In these soils, the ability to utilize abiogenic nitrate N (6) is a character of great importance, since other nitrogen sources (those provided by the activities and dissolutions of soil biota in most soils) are generally not available (6, 7). The recorded indigenous yeasts (22) of Ross Desert soils utilize nitrate N. Nitrate-utilizing yeasts resembling the indigenous *C. vishniacii* complex (which includes *C. baldrensis* and *C. hempflingii*) have been isolated in my laboratory from soil samples collected at 13 Ross Desert sites, and yeasts failing to utilize nitrate from soil samples have been collected at only 3 sites. The two proposed species are justified by this ecologically important difference from other yeasts occurring in the same macrohabitat and by habitat-related psychrophily, morphological differences, or 12 or more differences in physiological profile from other species of *Cryptococcus* failing to utilize nitrate N.

The microhabitat of the proposed species differs in population density and diversity from the microhabitats typical of Ross Desert soils. The soil sample (sample A801-3a, the 0- to 1-cm layer of soil directly under a lichenous rock ledge on Linnaeus Terrace) contained 59 CFU of C. consortionis (as biotype 20) per g, as well as yeasts (not enumerable by elution methods) belonging to two other biotypes (20). This soil sample was unfortunately lost before a complete microbiological census could be taken. The soils of the other two sites containing social yeasts (i.e., yeasts dependent upon biogenic N sources) contained a relatively large and diverse microbiota (up to 100 CFU of yeasts per g,  $10^3$  to  $10^4$ CFU of bacteria per g, and in one case algae) and were also situated in the vicinity of rocks containing cryptoendolithic communities. Clearly, the proposed species (and social yeasts of as-yet-undescribed biotypes) did have access to biogenic N sources. The sites containing asocial yeasts may have population densities as low as 1 microcolony of yeasts per 5 g of soil, less than  $10^2$  CFU of bacteria per g, and no detectable algal population and were not necessarily in the same valley as rocks containing cryptoendolithic lichen communities.

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