Cryptococcus fildesensis sp. nov., a psychrophilic basidiomycetous yeast isolated from Antarctic moss

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Two yeast strains isolated from the moss Chorisodontium aciphyllum from the Fildes Region, King George Island, maritime Antarctica, were classified as members of the genus Cryptococcus based on sequence analyses of the D1/D2 domains of the large subunit rRNA gene and the internal transcribed spacer (ITS) regions. The rRNA gene sequence analyses indicated that the two strains represented a novel species of the genus Cryptococcus, for which the name Cryptococcus fildesensis sp. nov. is proposed (type strain: CPCC 300017T = DSM 26442T = CBS 12705T). The MycoBank number of the novel species is MB 805542.

The genus Cryptococcus Vuillemin (1901) comprises a great variety of asexually reproducing species which differ in morphology (e.g. cell shape, colony form and colour, formation of hyphae or pseudohyphae), physiology (e.g. nutritional capabilities) and habitat ( Fonseca et al., 2011). The genus is also polyphyletic as species occur in four orders within the Tremellomycetes (Agaricomycotina, Basidiomycota): Tremellales, Trichosporonales, Filobasidiales and Cystofilobasidiales ( Fell et al., 2000; Scorza et al., 2002). Therefore, the taxonomy of these yeasts should be revised in future.

Antarctica is geographically isolated from other continents and one of the harshest ecosystems on earth. Several studies have discussed the yeasts isolated from samples in Antarctica and indicated that basidiomycetous yeasts (particularly strains of species of the genus Cryptococcus) predominate in Antarctic habitats. Hitherto, 11 species from the genus Cryptococcus have been found in Antarctica and most of them are psychrophilic or psychrotolerant, including Cryptococcus albidus (Goto et al., 1969), Cryptococcus antarcticus (Vishniac & Kurtzman 1992), Cryptococcus adeliensis (Scorza et al., 2000), Cryptococcus albidosimilis (Vishniac & Kurtzman 1992; Connell et al., 2008), Cryptococcus carnescens (Connell et al., 2008; Loque et al., 2010), Cryptococcus gastricus (Carrasco et al., 2012), Cryptococcus gilvescens (Carrasco et al., 2012), Cryptococcus friedmannii (Vishniac, 1985), Cryptococcus laurentii (Vishniac & Hempfling, 1979; Godinho et al., 2013), Cryptococcus saitoi (Connell et al., 2008), Cryptococcus victoriae (Carrasco et al., 2012; Godinho et al., 2013) and Cryptococcus vishniacii (Vishniac & Bahareen 1979; Vishniac & Hempfling, 1979; Arenz et al., 2006; Connell et al., 2008). Species of the genus Cryptococcus have also been found in mountainous glacial habitats, e.g. Cryptococcus carnescens, Cryptococcus folicola, Cryptococcus frias, Cryptococcus fonsecaei, Cryptococcus psychrotolerans, Cryptococcus aff. tephrensis, Cryptococcus tro-nadorensis, Cryptococcus victoriae (de Garcia et al., 2012), Cryptococcus gilvescens and Cryptococcus terricolus (Turchetti et al., 2008).

The flora of Antarctica consists largely of bryophytes (approximately 111 species of mosses and 27 species of liverworts) (Bednarek-Ochyra et al. 2000; Ochyra et al. 2008) and includes only two native flowering plant species (Convey et al., 2011). Given that bryophytes are the dominant plant component of the Antarctic terrestrial ecosystem, we investigated the fungal diversity associated with three bryophyte species in the Fildes Region, i.e. the liverwort Barbilophozia hatcheri (Evans) Loeske and the mosses Chorisodontium aciphyllum (Hook. f.) Broth and Sanionia uncinata (Hedw.) Loeske. A total of 29 yeast isolates were isolated from six samples and were recognized by sequence analyses of the D1/D2 domains of the large subunit rRNA gene and the internal transcribed spacer (ITS) regions. The majority of these yeasts belonged to known species including Rhodotorula psychrophilica, Mrakia psychrophilia and Mrakia robertii. However, two of them, each isolated from a different sample, were found to belong to a novel species of the genus Cryptococcus,
within the order Filobasidiales. The name Cryptococcus fildesensis sp. nov. is proposed.

The study area was located in the south-western part of King George Island (62° 08′–62° 14′ S, 59° 02′–58° 51′ W). In the area, bryophytes prevail in wetter niches on the ground, whereas lichens predominate in dry and rocky places. The native flowering plants are only a minor component of the vegetation (Olech, 2002). A total of 61 species of mosses and 11 species of liverworts have been reported from the island (Ochyra, 1998; Ochyra & Vana 1989). From 1982 to 2002, the mean air temperature of the area varied from −6.4 °C in July to 2.3 °C in February. Mean annual precipitation is 366.7 mm rainfall equivalent, falling as snow and rain (Setzer et al., 2004). Sampling of bryophytes was carried out during China’s 28th Antarctic expedition in December 2011. Strain CPCC 300016 was isolated from sample number 451 of the moss Chorisodontium aciphyllum (collected at 62° 12′ 44.77″ S, 58° 56′ 27.47″ W, altitude 34 m), whereas strain CPCC 300017 was isolated from the moss sample number 390 of Chorisodontium aciphyllum (collected at 62° 13′ 39.29″ S, 58° 56′ 53.51″ W). The samples were cut into segments of 0.1–0.3 cm without surface-sterilization. Between 20 and 30 tissue segments were then evenly placed in 9 cm-diameter Petri dishes containing potato dextrose agar (PDA, 1.5 %), tetracycline (50 mg l⁻¹) and streptomycin sulfate (50 mg l⁻¹). The plates were incubated at 4 °C, and all colonies were streaked for purification. Purified yeast strains were suspended in YM broth supplemented with 10 % glycerol (v/v) and maintained at −80 °C.

Morphological characteristics were observed by light microscopy (Axio scope A1; Zeiss) and scanning electron microscopy (Quanta 200; FEI). Physiological and biochemical tests were performed by standard methods described by Kurtzman et al. (2011). For mating tests, two strains of 2-day-old cultures were crossed on cornmeal agar and incubated at low temperature (12 °C) and at room temperature (18 °C). The crosses were examined after 1 month.

Nuclear DNA was extracted using a modified CTAB method (Cubero et al., 1999). The ITS (ITS1–5.8 S–ITS2) region of the rRNA gene was amplified with the primers ITS1F and ITS4 as described by White et al. (1990). Amplification of the ITS region was performed using the following conditions: 95 °C for 3 min, followed by 37 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s; and a final extension at 72 °C for 10 min. The D1/D2 domain was amplified with the primers NL1 and NL4 as described by Kurtzman & Robnett (1991). Amplification of the D1/D2 domain was performed as follows: 94 °C for 6 min, followed by 40 cycles of 94 °C for 60 s, 50 °C for 60 s and 72 °C for 60 s; and a final extension at 72 °C for 5 min. PCR products were purified and sequenced with the same primers by Sangon Biotech (Beijing, China). The sequences generated in this study were compared with those of other yeasts in the GenBank database (using the BLAST program) and the MyCoID database (using a pairwise sequence alignment program). Sequence alignments were done using the MUSCLE algorithm implemented in MEGA 5.05 software. Phylogenetic and molecular evolutionary analyses were also conducted with MEGA 5.05 (Tamura et al., 2011) using the neighbour-joining method with the maximum composite likelihood distance measure. Confidence values were estimated from bootstrap analysis of 1000 replicates. Pairwise deletion was adopted for the treatment of gaps and missing data.

The D1/D2 sequence indicated that the two novel strains are representatives of a novel species in the order Filobasidiales (Fig. 1). Within the order Filobasidiales, Cryptococcus fildesensis sp. nov., occupied a isolated position with Cryptococcus sp. CBS 12610 (7 nt mismatches), Cryptococcus sp. CBS 12605 (12 nt mismatches), which had been isolated from forest soil in Germany, and uncultured fungus clone BOTU020, isolated from forest leaf litter in the USA (11 nt mismatches) forming a subclade of nearest relatives, supported by a high bootstrap value. Sequence data and phylogenetic analysis of the ITS region also confirmed the positioning of Cryptococcus fildesensis sp. nov. in the order Filobasidiales and supported the recognition of Cryptococcus fildesensis sp. nov. as a novel species (a phylogenetic tree based on the ITS sequences is available as Fig. S1 in the online Supplementary Material). Additionally, the results of mating tests were negative. Therefore, it is proposed that those two strains represent a novel species of the genus Cryptococcus with the name Cryptococcus fildesensis sp. nov.

Hitherto, 11 species of the genus Cryptococcus have been found in Antarctica. Among them, eight species are psychrophilic or psychrotolerant, including Cryptococcus antarcticus, Cryptococcus carnescens, Cryptococcus friedmannii, Cryptococcus gastricus, Cryptococcus gilvescens, Cryptococcus saitoi, Cryptococcus victoriae and Cryptococcus vishniaci (Vishniac & Baharaeen 1979; Vishniac & Hempfling, 1979; Vishniac, 1985; Vishniac & Kurtzman, 1992; Connell et al., 2008; Carrasco et al., 2012). Cryptococcus albidus, Cryptococcus adelensis, Cryptococcus albidosimilis and Cryptococcus laurentii are mesophilic as their maximum temperatures for growth are ≥30 °C (Vishniac & Hempfling, 1979; Vishniac & Kurtzman, 1992; Scorzetti et al., 2000). In this study, the two strains of Cryptococcus fildesensis were isolated from Antarctic moss at 4 °C and could grow well at 12 °C and 18 °C, but not at 22 °C and 25 °C, indicating that this novel species is psychrophilic.

Description of Cryptococcus fildesensis T. Zhang & L.-Y. Yu sp. nov.

Cryptococcus fildesensis (fil.des.en’sis. N.L. masc. adj. fildesen-sis referring to the Fildes Region, King George Island, maritime Antarctica, where the type strain was isolated).

In YM broth, after 7 days at 12 °C, the cells are globose to elongate, 1.8–4.6 × 2.8–7.7 μm. Polar budding is observed
**Fig. 1.** Phylogenetic placement of *Cryptococcus fildesensis* sp. nov. within the order Filobasidiales. Neighbour-joining analysis of the D1/D2 domain of the large subunit rRNA gene sequences. Numbers on the branches are bootstrap values (1000 replicates; values <50% are not shown). Species of the genus *Holtermanniella* in the order Holtermanniales were used as the outgroup in the analysis. Numbers in parentheses are GenBank accession numbers. Bar, 0.01 substitutions per nucleotide position.
and cells occur singly or in pairs (Fig. 2). After 1 month at 12 °C, sediment is present. After 1 month at 12 °C, the streak culture is butyrous and cream-coloured. The surface is smooth and the margin entire. Pseudohyphae are not observed in cultures grown on cornmeal agar. No fermentation is observed. D-Arabinose, cellobiose, D-galactose, D-glucose, lactose (weakly), maltose, D-mannose, melezitose, melibiose (weakly), sucrose, trehalose, D-xylose, L-arabinose, L-rhamnose (weakly), L-sorbose (weakly), N-acetyl-D-glucosamine, methyl z-D-glucoside, D-glucuronic acid, succinic acid, salicin (weakly), D-mannitol, ethanol, glycerol (weakly), inositol and D-sorbitol are assimilated. Citric acid, raffinose, D-ribose, D-galactitol, DL-lactic acid, erythritol, methanol, ribitol, hexadecane, inulin and soluble starch are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, cadaverine and L-lysine are assimilated; creatinine, creatine, ethylamine and imidazole are not assimilated. No extracellular starch-like compounds are produced. Reaction with Diazonium Blue B and urease production are positive. Vitamins are not required for growth. No growth is observed in the presence of 0.1 % (w/v) cycloheximide, 10 % sodium chloride/5 % glucose or 50 % glucose. Weak growth is observed in the presence of 0.01 % (w/v) cycloheximide. Growth is observed at temperatures of 12 °C and 18 °C, but not at 22 °C and 25 °C.

The type strain, CPCC 300017T, was isolated from the moss Chorisodontium aciphyllum in Fildes Region, King George Island, maritime Antarctica in July 2012. This strain has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, as DSM 26442T and in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, as CBS 12705T. Strain CPCC 300016 is an additional strain of the species and has been deposited in the above two collections, as DSM 26441 and CBS 12704, respectively. The MycoBank number of the novel species is MB 805542.

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