Cryptococcus anemochoreius sp. nov., a novel anamorphic basidiomycetous yeast isolated from the atmosphere in central South Africa

Carolina H. Pohl, Johan L. F. Kock, Pieter W. J. van Wyk and Jacobus Albertyn

1Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, PO Box 339, Bloemfontein 9301, South Africa
2Centre for Confocal and Electron Microscopy, University of the Free State, PO Box 339, Bloemfontein 9301, South Africa

A novel yeast strain, CBS 10258T, was isolated from the atmosphere in central South Africa. Sequence analysis of the D1/D2 domain and internal transcribed spacer region of the novel strain indicates that it represents a novel species within the Cryptococcus laurentii complex. Phylogenetic analyses based on the D1/D2 domain revealed that the novel strain occupies a relatively isolated position within this complex with Papiliotrema bandonii, Cryptococcus perniciosus, Cryptococcus nemorosus and Cryptococcus sp. CBS 8363 being the closest relatives. However, the novel strain could be distinguished from related species by standard physiological tests including the inability to assimilate rhamnose, methyl-\(\alpha\)-D-glucoside, salicin, lactose, erythritol, ribitol, xylitol, citrate and ethanol. In addition, no extracellular starch production was observed and the isolate was able to grow in the absence of additional vitamins. On the basis of these results, we suggest that the new strain represents a novel species for which the name Cryptococcus anemochoreius sp. nov. is proposed (type strain CBS 10258T (≡ NRRL Y-27920T)).

Members of the anamorphic basidiomycetous genus Cryptococcus Vuillemin are characterized by their ability to assimilate D-glucuronate, their lack of fermentative ability, the presence of xylose in cell hydrolysates, positive urease and Diazonium blue B reactions as well as the presence of coenzymes Q-9 and Q-10 (Fell & Statzell-Tallman, 1998). Most species produce extracellular starch and those that do not, utilize inositol. It is well known that the genus Cryptococcus is polyphyletic, occurring in the Tremellales, Trichosporonales, Filobasidiales and Cystofilobasidiales (Fell et al., 2002). In addition, members of this genus have been isolated from a diverse range of habitats worldwide and several reports exist for the isolation of Cryptococcus species, such as Cryptococcus aenetus, Cryptococcus albicus, Cryptococcus flavus, Cryptococcus laurentii, Cryptococcus luteolus and Cryptococcus magnus, from the atmosphere (Fell & Statzell-Tallman, 1998). It is also known that the pathogenic Cryptococcus species, Cryptococcus neoformans, often occurs in the air as small, respirable particles (Ruiz & Bulmer, 1981; Sukroongreung et al., 1999).

Due to the prevalence of HIV in the mining workforce of South Africa, a study of the diversity of culturable airborne fungi (which may pose health risks to immunocompromised workers) was conducted in an active gold mine. During this study, comparative samples of the atmosphere outside were taken in order to establish a possible source of the fungal species found indoors. Of the isolates obtained from the atmosphere outside the mine, 25-9% were yeasts. The majority of these isolates belong to known species in the genera Candida, Rhodotorula, Filobasidium and Cryptococcus. However, a novel anamorphic basidiomycetous isolate was obtained and we propose the name Cryptococcus anemochoreius sp. nov. for this isolate on the basis of morphological, physiological and molecular characteristics.

Since this species was isolated from the atmosphere, it is difficult to speculate on the ecology of C. anemochoreius. However, trees belonging to the genera Pinus, Rhus, Schinus and Eucalyptus grow in the vicinity of the sampling site and since several reports exist regarding the association of Cryptococcus species with trees, especially Eucalyptus (Ellis & Pfeiffer, 1990; Sorrell & Ellis, 1997; Fortes et al., 2001; Randhawa et al., 2001; Granados & Castaneda, 2005), it is
tempting to speculate that *C. anemochoreius* may be associated with one or more of these trees.

**Yeast isolation and characterization**

Air samples were taken in June 2004 in the Free State province in central South Africa using a SAS Super 90 single stage impactor. The sampling time was 30 seconds at 30 l min⁻¹. In order to facilitate the isolation of yeasts, rose Bengal agar (5·0 g l⁻¹ peptone, 10·0 g l⁻¹ glucose, 1·0 g l⁻¹ potassium phosphate, 0·5 g l⁻¹ magnesium sulphate, 0·05 g l⁻¹ rose Bengal, 0·1 g l⁻¹ chloramphenicol, 15·0 g l⁻¹ agar) was used. Plates were incubated at 25 °C and yeast colonies were purified on 2 % yeast–malt extract (YM) agar. Morphological and physiological tests were performed in duplicate according to standard techniques (Yarrow, 1998). In addition, scanning electron microscopy was performed on the isolate using a JEOL 6400 WIN-SEM instrument (Van Wyk & Wingfield, 1994).

**rDNA sequence analyses**

The 26S rDNA D1/D2 and internal transcribed spacer regions were PCR amplified and sequenced using primer pair NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-GTTCCGTTTTCAGAGCCG-3') (Kurtzman & Robnett, 1998) and primer pair ITS4 (5'-TCCGTTTATGGATATGC-3') and ITS5 (5'-GGAAGTAAAAAGCTGAAACAGG-3') (White *et al.*, 1990), respectively. The resulting sequence was compared with sequences obtained from GenBank. Sequences for phylogenetic analysis were aligned using CLUSTAL_X 1.83 (Thompson *et al.*, 1997).
Phylogenetic and molecular evolutionary analyses were conducted with MEGA version 3.1 (Kumar et al., 2004) using the neighbour-joining method with the Kimura two-parameter distance measure. Confidence values were estimated from bootstrap analysis of 1000 replicates. The three species of Filobasidium were used as outgroup.

Species delineation and identification

The D1/D2 sequence was compared with related sequences and phylogenetic analyses (Fig. 1) indicated that Cryptococcus anemochoreius represents a novel species in the Cryptococcus laurentii phylogenetic group 1 as described by Sugita et al. (2000) and Takashima et al. (2003). Within the C. laurentii phylogenetic group 1, C. anemochoreius occupies a relatively isolated position with Papiliotrema bandonii (20 nucleotide differences), Cryptococcus pittiaricus (25 nucleotide differences), Cryptococcus nemorosus (21 nucleotide differences) and Cryptococcus sp. CBS 8363 (17 nucleotide differences) forming a clade of nearest relatives. The relatively low bootstrap value between C. anemochoreius and the P. bandonii clade (of 68 %) may increase with the isolation of more strains related to C. anemochoreius. Sequence data and phylogenetic analysis of the internal transcribed spacer region confirmed the positioning of C. anemochoreius in the C. laurentii phylogenetic group 1 and supported the recognition of C. anemochoreius as a novel isolate (a phylogenetic tree based on the internal transcribed spacer sequences is available as Supplementary Fig. S1 in IJSEM Online). Physiologically Cryptococcus anemochoreius can be distinguished from these related taxa as indicated in Table 1. Based on these differences, we conclude that the new isolate represents a novel species, Cryptococcus anemochoreius sp. nov.

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Table 1. Salient characteristics of Cryptococcus anemochoreius sp. nov. and related species

Phylogenetic analyses

Strains: 1, Cryptococcus anemochoreius sp. nov. CBS 10258T; 2, Cryptococcus nemorosus VKM Y-2906T (data from Golubev et al., 2003); 3, Cryptococcus perniciosus VKM Y-2905T (Golubev et al., 2003); 4, Cryptococcus laurentii (Fell & Statzell-Tallman, 1998); 5, Papiliotrema bandonii PYCC 5473 (Sampaio et al., 2002). +, Positive; −, negative; D/W, delayed or weak; ND, not determined.


![Fig. 2. Scanning electron micrograph of cells of Cryptococcus anemochoreius indicating polar budding of globose to elongate cells. Bar, 1 μm.](image-url)
Description of Cryptococcus anemochoreius

Pohl, J. L. F. Kock, P. W. J. van Wyk & Albertyn sp. nov.

Cryptococcus anemochoreius (a.ne.mo’cho.rei.us. Gr. n. anemos wind; Gr. adj. choreios of, or belonging to, a dance; N.L. masc. adj. anemochoreius referring to the windblown nature of the isolate).

In yeast-malt extract (YM) broth after 3 days of growth at 25 °C, cells are globose to elongate (2-8-6-4 μm × 2-3-3-7 μm). Polar budding is observed and cells occur as parent bud pairs (Fig. 2). On YM agar after 7 days at 25 °C, colonies are smooth with an entire margin and salmon coloured. In Dalmau plate cultures on YM agar, no pseudohyphae or true hyphae can be observed. No fermentation is observed. Able to assimilate D-glucose, D-galactose, L-sorbose, D-glucosamine, D-ribose (weakly), D-xylene, L-arabinose, D-arabinose, sucrose, maltose, trehalose, cellobiose, arbutin, melibiose, raffinose, melezitose, inulin, starch, D-glucitol, D-mannitol, galactitol, myo-inositol, D-gluconate, D-glucuronate, D-galacturonate, DL-lactate (weakly) and succinate (weakly), but not L-rhamnose, methyl α-D-glucoside, salicin, lactose, glycerol, erythritol, ribitol, xylitol, L-arabinitol, citrate, propane-1,2-diol, butane-2,3-diol, malic acid or butyric acid. No growth is observed on methanol and ethanol as carbon sources. L-Lysine, cadaverine and ethylenemine are utilized as nitrogen sources, but not nitrate, nitrite, creatinine, creatine, glucosamine or imidazole. Urea is hydrolysed, but no extracellular starch is produced. Vitamins are not necessary for growth, however, no growth is observed in the presence of cycloheximide (0-01 %), 1 % acetic acid or 50 % glucose. No growth is observed at 37 °C.

The type strain, CBS 10258T (=NRRL Y-27920T), is deposited in the yeast culture collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, with a copy at the yeast culture collection at the Agricultural Research Services United States Department of Agriculture, Peoria, Illinois.

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References


