



The basidiomycetous yeast *Rhodotorula yarrowii* comb. nov

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Abstract

Sequence analysis of the D1/D2 domains of the large subunit rDNA of *Cryptococcus yarrowii* (CBS 7417) indicates that this species does not belong to the hymenomycetous fungi, but instead is of urediniomycetous affinity. Therefore, the name change *Rhodotorula yarrowii* comb. nov. is proposed. The cell wall of the species contains xylose, a character considered by most authors to indicate fungi of hymenomycetous affinity. However, our results show that xylose may occur in minor amounts in the cell walls of urediniomycetous fungi. A high mannose content of the cell walls may be a more reliable character for urediniomycetous yeasts.

Cryptococcus yarrowii is an anamorphic basidiomycetous yeast described by Fonseca and van Uden (1991). The presence of budding cells, lack of fermentation, assimilation of m-inositol and D-glucuronic acid, presence of xylose in whole-cell hydrolysates, staining with Diazonium Blue B salt, and a positive urease reaction indicated placement of this species in the genus *Cryptococcus*. However, the inability of *C. yarrowii* to produce extracellular starch-like compounds is unusual for a member of *Cryptococcus* (see Table 79, Fell & Statzell-Tallman 1998).

The genus *Cryptococcus* is a polyphyletic member of the Tremellomycetidae of the Hymenomycetes *sensu* Swann & Taylor (1993,1995) and Fell et al. (1995). Sequence analysis of the D1/D2 large subunit ribosomal (LSU) DNA placed *C. yarrowii* outside this phylogenetic group. The species clusters in a clade among the Urediniomycetes that represents the Sporidiobolaceae of Boekhout et al. (1998) and the Microbotryomycetidae of Swann et al. (1999) (Figure 2). This clade consists of fungi belonging to several orders: *Colacogloea peniophorae* and *Kriegeria eriophori* in the Platygloeaales, *Heterogastridium pycnidioideum* in the Heterogastridiales (Bandoni 1995) and *Microbotryum violaceum* and *Ustilentyloma fluit-*

ans in the Microbotryales (Bauer et al. 1997). The tree lacks prominent structure in terms of statistical support, which may be the result of phylogenetic diversity and/or the limited number of species that have been investigated. Despite this lack of support the data suggest that the ballistoconidial yeast *Sporobolomyces griseoflavus* is the closest relative of *C. yarrowii*.

There are six genera of yeasts in this clade: the teliospore forming genera *Leucosporidium* and *Mastigobasidium* (Golubev 1999) and species of the anamorphic genera *Rhodotorula*, *Sporobolomyces*, *Bensingtonia* and *Reniforma*. The presence of teliospores is a characteristic shared with the teliospore forming plant parasites *Microbotryum*, *Sphacelotheca* and *Kriegeria*. Most species in this clade utilize D-glucuronate and contain co-enzyme Q9 or Q10. However, *Reniforma strues* contains coenzyme Q7 (H.J. Roelijmans, unpubl. observ.) and does not utilize D-glucuronate. Simultaneous utilization of D-glucuronate and inositol as occurs in *C. yarrowii* is a rare feature among the urediniomycetous yeasts, but occurs in *Sporobolomyces inositolophilus*, a member of the same clade (Fell et al. 2000).

Cell wall composition is an important character for inferring taxonomic and phylogenetic relationships

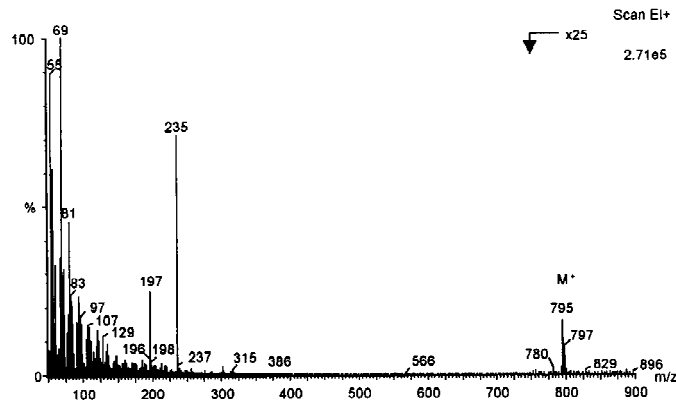


Figure 1. Mass spectrum of CBS 7417 showing the M⁺ peak of coenzyme Q9.

among basidiomycetous yeasts. Dominance of mannose, and the presence of fucose and rhamnose suggest an urediniomycetous affinity, whereas xylose indicates a hymenomycetous affinity (Weijman & Rodrigues de Miranda 1983; Sugiyama et al. 1985; Suzuki & Nakase 1988; Weijman et al. 1988; Prillinger et al. 1991a, b, 1993; Swann & Taylor, 1995). Because of the conflicting phylogenetic position of *C. yarrowii* that resulted from analyses of molecular sequences and cell wall composition, we examined the cell wall carbohydrates of the type strain of *C. yarrowii* (CBS 7417). Two analytical methods were employed: 1, gas chromatography of acid hydrolyzates of whole cells with derivatization using capillary columns and 2, Gas-liquid chromatography of purified cell walls (Prillinger et al. 1993; Lopandic et al. 1996; Roeljmans et al. 1998). The following sugars were found in CBS 7417: glucose (20%), mannose (58%), rhamnose (13%), and trace amounts of galactose (2%), xylose (5%), and fucose (2%). Fucose was only demonstrated to be present using purified cell walls. These results on the cell wall composition were in agreement with the earlier results of Fonseca and van Uden (1991), who, because of the presence of xylose, inferred that the fungus had a hymenomycetous affinity, and was best placed in the genus *Cryptococcus*. However, examination of the literature demonstrates that xylose has been reported from other urediniomycetous yeasts, specifically the type strains of *Sporodiobolus salmonicolor* and *Rhodotorula muscorum* and strain YK 131 of *R. minuta* (Sugiyama et al. 1985). These observations suggest that the presence of xylose is not limited to the hymenomycetous yeasts, and may be present in trace amounts in some urediniomycetous yeasts.

Our examination demonstrated that the major coenzyme is Q9 (Figure 1) and the minor co-enzyme is Q8. Coenzyme Q was extracted according to Yamada et al. (1989). The mass spectrum of 0.3 μ l of the acetone extract was recorded at 70 eV with the probe programmed from 50–400 °C. Peaks with Mz 197 and 235 showed typical fragments of ubiquinones and the peak 795 (M⁺) from coenzyme Q9.

Based on our molecular data we conclude that *Cryptococcus yarrowii* should be transferred to the Urediniomycetes. Using the D1/D2 sequences and phenotypic data, *Rhodotorula* appears the most appropriate genus, as the alternatives are the ballistoconidia-forming yeasts *Sporobolomyces* and *Bensingtonia*, and the genus *Reniforma*, which is unique among the basidiomycetous yeasts due to the formation of reniform shaped yeast cells.

Rhodotorula yarrowii (A. Fonseca & van Uden) Boekhout, Fell, Fonseca, Prillinger & Roeljmans comb. nov.

Basionym: *Cryptococcus yarrowii* Fonseca & van Uden, Antonie van Leeuwenhoek 59: 177, 1991.

Type strain: CBS 7417

The taxonomic characteristics follow Fonseca & van Uden (1991) with the exception that the original description listed the presence of co-enzyme Q10. A second isolate identical or very close to this species, PYCC 5629 (= CBS 8749), isolated from a Strawberry tree fruit (*Arbutus unedo*), Arrábida Natural Park (Setúbal, Portugal), differed in 4 nucleotide substitutions in the D1/D2 region (A. Fonseca, unpubl. observ.), possessed Q9 as the major coenzyme and Q10 as the minor coenzyme, and contained glucose,

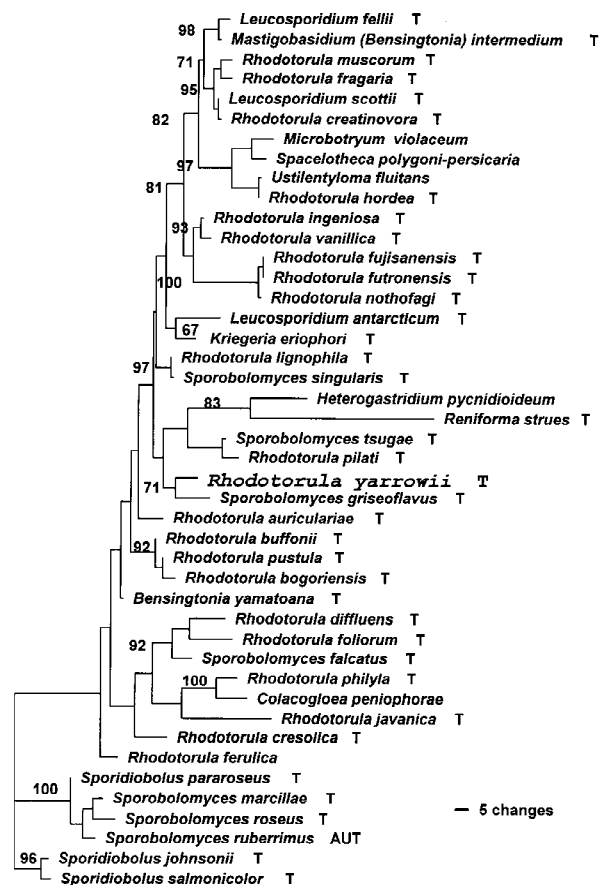


Figure 2. Phylogenetic tree based on partial large subunit (LSU) ribosomal DNA (D1/D2 domains) sequences of a selection of basidiomycetous yeasts. One out of six equally parsimonious trees generated using PAUP 4.0* with random step-wise addition and tree bisection reconnection (Swofford 1998). The tree length is 687 steps with 614 characters, of which 373 are constant, 177 are parsimony informative, 64 are parsimony uninformative, CI = 0.4945, HI = 0.5051, RI = 0.7014, RC = 0.3471. Bootstrap values using the full heuristic search option with 1000 replicates are indicated on the branches. Strain and Genebank numbers see Fell et al. (2000). *Rhodotorula yarrowii* is indicated in larger font.

mannose, xylose, fucose and arabinose in whole cell hydrolysates (H.J. Roeljmans, unpubl. observ.).

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