
MOLECULAR RELATEDNESS BETWEEN THE BASIDIOMYCETOUS YEASTS
Sporidiobolus ruinenii and Sporobolomyces coprophilus

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The anamorphic yeast genera Rhodotorula and Sporobolomyces and their respective teleomorphs, Rhodosporidium and Sporidiobolus, are characterized, sensu Kreger-van Rij (1984), by the presence of visible red pigments. The prominent distinction between the genera is the formation of ballistospores by Sporobolomyces and Sporidiobolus. In cases where the ability to produce ballistospores is lost, classification of strains would be incorrect. For example, original descriptions of Sporobolomyces albo-rubescens Derx (1930), Sporobolomyces gracilis Derx (1930) and Sporobolomyces coprophilus Sugiyama & Goto (1967) depicted ballistospores, but subsequent examinations were unable to substantiate their presence (Phaff and Ahearn, 1970). Consequently, without previous knowledge of ballistospore formation, all three species would be classified in Rhodotorula.

During a study of various red yeasts, Sporobolomyces coprophilus became of interest because of its phenotypic similarity to Sporidiobolus ruinenii Holzschu et al. (1981) and certain other taxa. As described by Sugiyama and Goto (1967), Sporobolomyces coprophilus produced ballistospores, albeit infrequently. Phaff and Ahearn (1970) did not report ballistospores and found that the type strain was "identical in all respects with Rhodotorula graminis di Menna." We did not find ballistospores in the type strain, but we did observe homokaryotic production of teliospores. Based on the presence of teliospores, Sporobolomyces coprophilus would be phenotypically aligned with either Rhodosporidium paludigenum Fell & Tallman (ballistospores absent) or Sporidiobolus ruinenii (ballistospores present). Barnett et al. (1983) designated Sporobolomyces coprophilus as an anamorphic state of Sporidiobolus ruinenii, but they did not present their rationale or discuss the presence of teliospores.

In an effort to resolve the relationship of Sporobolomyces coprophilus with these other red yeasts, comparisons of nuclear DNA relatedness were made. Procedures for DNA extraction and purification were previously described (Fell et al., 1988). G + C contents were determined by buoyant density, and extent of nuclear DNA complementarity was measured spectrophotometrically (Kurtzman et al., 1980).

The G + C contents (Table I) of Sporidiobolus ruinenii (64.9%), Sporobolomyces coprophilus (64.5%) and Rhodosporidium paludigenum (65.3%) were similar but differed from Rhodotorula glutinis (Fresenius) Harrison (69.0%) and Rhodotorula graminis (68.3%). Because the G + C content is not a species-specific descriptor but serves only an exclusionary role (Price et al., 1978), the extent of DNA hybridization between taxa was also measured. Reassociation between strains of Sporidiobolus ruinenii was 100% (Table I), whereas reassociation between strains of Sporidiobolus ruinenii and Sporobolomyces coprophilus was 63%. There was less than 10% DNA
relatedness between Sporobolomyces coprophilus and strains of Rhodosporidium paludigenum, Rhodotorula graminis and Rhodotorula glutinis. Rhodotorula glutinis and Rhodotorula graminis showed 30% DNA relatedness, suggesting a previously unsuspected close relationship that has been verified from measurements of ribosomal RNA sequence divergence (Fell and Kurtzman, 1990).

The DNA comparisons (Table I) demonstrate that Sporobolomyces coprophilus is closely related to Sporidiobolus ruinenii but not to other phenotypically similar red yeasts. The 63% DNA relatedness between these taxa is lower than usually found between strains of the same species but it is higher than observed between reproductively isolated species. For example, Price et al. (1978) examined several genera of ascomycetous yeasts and considered that strains with 80–100% complementarity were conspecific. Kurtzman (1987) presented evidence that some ascomycetous species have strains with 25–60% DNA relatedness, although this may not be a common occurrence. Aulakh et al. (1981) studied the basidiomycetes Filobasidiella neoformans Kwon-Chung and F. bacillispora Kwon-Chung and reported that reassocation of strains within species was 87–93% and reassocation between species was 55–63%. Kwon-Chung et al. (1982) examined mating between the two Filobasidiella species and demonstrated the occurrence of meiosis, concluding that the taxa represent a single divergent species. Because of ecological, biochemical, epidemiological, and DNA hybridization differences, they established separate varieties of F. neoformans. The data presented by Kwon-Chung et al. suggest that a similar relationship may exist between Sporobolomyces coprophilus and Sporidiobolus ruinenii.

Phenotypic differences offer little help in deciding whether Sporobolomyces coprophilus and Sporidiobolus ruinenii represent divergent varieties of a single species or closely related separate species. Sporobolomyces coprophilus is similar in cell size, colony color, and biochemical characteristics to Sporidiobolus ruinenii (Holzchu et al., 1981; Fell and Tallman, 1984). One difference on growth tests is that Sporidiobolus ruinenii assimilates L-rhamnose slowly, whereas Sporobolomyces coprophilus does not grow on this carbon source. Neither species is prevalent in nature: Sporobolomyces coprophilus was isolated from goat dung in Pakistan, Sporidiobolus ruinenii from the phyllosphere of tropical foliage of Malpighia coccigera L. in the Botanical Garden at Bogor, Indonesia. The original isolates of both species are the only available strains; therefore, variability between and within species is unknown. These yeasts do not have nucleotide differences in the highly variable 25S-635 region of ribosomal RNA (Fell and Kurtzman, 1990) suggesting that they are conspecific strains.

Differences have been observed in mode of teliospore formation, but little precedent exists for their interpretation. Both strains produce teliospores in the absence of mating, but by different mechanisms. In both cases teliospore formation is initiated by uninucleate cells.
Sporidiobolus ruinenii develops binucleate hyphae with clamp connections. In this type of teliospore formation (homothallic fruiting) the uninucleate cell is diploid, reduction division takes place in the hyphae, and karyogamy occurs in the teliospore. The resulting sporidia are diploid. Sporobolomyces coprophilus sporulates from unclamped hyphae indicating homokaryotic fruiting, a condition in which the hyphae and teliospores remain uninucleate. Ploidy is unknown and does not appear to change throughout the course of teliospore and sporidial formation (Fell, 1984). The occurrence of both homokaryotic and homothallic teliospore formation in the same species has not been reported. Other combinations of teleomorph formation have been described within a species. For example, homokaryotic and heterothallic strains of Rhodosporidium sphaerocarpum Newell & Fell are considered to represent a single species. All strains have identical G + C values (62.5–62.7%) and the same ubiquinone (Q10). In contrast, some homothallic and heterothallic strains of Rhodosporidium toruloides Banno are reported to be distinct species because they differ in G + C contents (65% and 59–60%) and types of ubiquinones (Q8 and Q10) (Hamamoto et al., 1987).

Some morphological differences occur between Sporobolomyces coprophilus and Sporidiobolus ruinenii. Teliospores of Sporidiobolus ruinenii are spheroidal (4–20 μm), whereas teliospores of Sporobolomyces coprophilus are spheroidal to ovoidal (8.7–10.7 × 9.4–11.4 μm). Formation of metabasidia also appears to differ. Both species produce phragmometabasidia. The majority of the Sporidiobolus ruinenii metabasidia are produced on stalks, although unstalked metabasidia do occur. Stalked metabasidia have not been observed in Sporobolomyces coprophilus.

In summary, we have reported a Sporidiobolus teleomorph for Sporobolomyces coprophilus and have shown from comparisons of DNA complementarity and from nucleotide sequence analysis that Sporobolomyces coprophilus is closely related to Sporidiobolus ruinenii. As a consequence, Sporobolomyces coprophilus needs to be identified as a member of Sporidiobolus. Strains showing intermediate levels of DNA relatedness are not particularly common. The decision to describe Sporobolomyces coprophilus and Sporidiobolus ruinenii as either taxonomic varieties or as closely related separate species depends on whether or not the strains are intersterile. Lacking the prospect of obtaining this information, we propose, on the basis of previous studies (Kurtzman, 1987; Kwon-Chung et al., 1982), that Sporobolomyces coprophilus be considered a variety of Sporidiobolus ruinenii.


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on its DNA relatedness to other species of the genus *Sporidiobolus*. Curr. Microbiol. 5: 73–76.


