Short Communication

PSEUDOZYM A BANDONI EMEND. BOEKHOUT, A GENUS FOR YEAST-LIKE ANAMORPHS OF USTILAGINALES

TEUN BOEKHOUT

Centraalbureau voor Schimmelcultures, Yeast Division, 2528 BC Delft, The Netherlands

(Received March 28, 1995; Accepted June 1, 1995)

A number of anamorphic basidiomycetous yeasts, erroneously described in diverse genera, such as Candida Berkhout, Pseudozyma Bandoni, Sporobolomyces Kluvyer & van Niel, Sterigmatomyces Fell, Stephanoascus M. Th. Smith et al. and Trichosporon Behrend, share a similar set of morphological and physiological characteristics. The species C. fusiformata Buhagiar (6), C. tsukubaensis Onishi (20), P. prolifica Bandoni (1), Sporobolomyces antarcticus Goto, Sugiyama & Iizuka (10), Stephanoascus flocculosus Traquair, Shaw & Jarvis (21), S. rugulosus Traquair, Shaw & Jarvis (21), Sterigmatomyces aphidis Henninger & Windisch (11), and T. oryzae Ito, Iizuka & Sato (14) all have flat, rapidly expanding colonies on commonly used media such as 1% yeast extract-0.1% peptone-4% glucose agar (YPGA), and usually are covered with a thin aerial mycelium made up of ramifying, acropetal chains of fusiform blastoconidia originating from short denticles, sterigma-like structures or attenuating hyphae. The colonies usually become zonated near the margin, which in most species is fringed. Microscopically they all show septate hyphae with the cytoplasm retracted in some cells and with retraction septa. Initial growth can be with budding yeast cells. Budding is polar, enteroblastic and with sympodial proliferation. Sterigma-like outgrowths, occurring near the septa and along the hyphae, give rise to cylindrical, ellipsoidal, fusiform or lanceolate (chains of) blastoconidia. Intercalary or terminal chlamydospores may be present. Ballistoconidia are absent. Urease reaction is positive, myo-inositol and D-glucuronate are assimilated, but extracellular starch-like compounds are not formed. Staining with Diazonium Blue B salt is positive. Xylose is absent from whole-cell hydrolyzates, and the major ubiquinone is Q-10.

It has been suggested that part of these fungi represent anamorphs of Ustilaginales (4), as the above characteristics occur in pure cultures of Ustilaginales, e.g.
Ustilago maydis (DC.) Corda. Hygroscopic aerial conidia have been described for several species of Ustilaginales, such as Ustilago hypodytes (Schlecht.) Fr. and Sorosporium saponariae Rudolphi (13).

While we try to avoid introducing separate anamorph binomials for holomorphic species, the species listed above seem to lack a perfect state or it is presently unknown. As species of smuts can be identified only on teleomorphic characteristics, such as host range, morphology of sori and teliospores (19, 22), it is difficult to compare the anamorphic species listed above with known species of smuts. Therefore comparative morphological, physiological, biochemical and molecular investigations of anamorphs of smuts will be useful. However, because several hundreds of species of smuts are involved, this task is not easily performed. A second possibility is to compare ribosomal DNA sequences in order to establish their phylogenetic position. Partial nucleotide sequences of the 26S ribosomal DNA of these anamorphic species clearly place them in the same group together with U. maydis (Fig. 1) (5, 8). For all these reasons, their status as anamorphs of Ustilaginales (s.s.) is well established. On the contrary, Microbotryum violaceum

![Fig. 1. Strict consensus of 5 most parsimonious trees based on partial large subunit rRNA nucleotide sequences of some tilletiaceous and ustilaginaceous fungi using PAUP, version 3.1. Neurospora crassa is used as an outgroup. The ustilaginaceous anamorphs discussed taxonomically all belong to cluster 3 (reproduced from Ref. 5).]
Anamorphs of Ustilaginales

(Pers.: Pers.) G. Deml & Oberwinkler was found to belong to a different group based on partial 26S rDNA sequences, thus supporting Deml’s conclusion on the divergence of smuts occurring on mono- and dicotyledons (7). This dichotomy is supported by 5S rRNA sequences (2, 3) and recent work on the 18S rDNA (E.C. Swann, unpubl. results).

When it was proposed to restrict Candida Berkhout to anamorphic ascomycetous yeasts with multilateral budding, the species C. antarctica and C. fusiformata could not unambiguously be placed in either Rhodotorula or Cryptococcus (25) and consequently ended up as “ghost” species in the taxonomic system of basidiomycetous yeasts.

Two species recently described in Stephanoascus, viz. S. flocculosus and S. rugulosus (21), fit the above characteristics well. The detailed description provided of conidiogenesis and colony morphology fully agrees with the characteristics given above. Moreover, these strains of Stephanoascus are DBB- and urease-positive, utilize myo-inositol and α-glucuronate and do not produce extracellular starch-like compounds. All of the above, together with partial 26S rDNA sequences place these species in the Ustilaginales s.s. (Fig. 1) (5). This conflicts with the finding of asci with 2–4 galeate ascospores (Figs. 11–14, 23–27 in Ref. 21). However, this observation was not confirmed by the present author. Reinvestigation of acid fuchsin-stained preparations revealed the presence of one to three positively stained structures in the chlamydospores (J.A. Traquair, pers. comm.). These structures may represent nuclei, but are not ascospores. Old chlamydospores contained numerous refractile, but non-stained and presumably lipidic contents. Therefore, we are convinced that there is enough morphological, physiological and molecular evidence that S. flocculosus and S. rugulosus are not ascomycetes, but belong to the basidiomycetes and, more specifically, the Ustilaginales s.s.

When isolating fungi from litter of Scirpus microcarpus Presl, Bandoni (1) observed abundant Tricladium-like conidia, for which he created a monotypic genus Pseudozyma with P. prolifica Bandoni as the only species. Upon germination of the Tricladium-like conidia, dimorphic colonies appeared. The “mycelial” colonies were reported to grow slowly, being dark grey to black, with septate hyphae 1.5–5.0(–8.0) μm in diameter. Arthro- and scolecoconidia were observed on water agar. The “conidial” colonies were yeast-like, with scarce hyphae forming blastoconidia. The blastoconidia were described as hyaline, simple, 0–1-septate, narrowly obclavate, 8–20 × 1.5–2.0(–5.0) μm, straight or curved, with the base truncate then conical, developing into a primary axis, initially unbranched, 1–3(–4)-septate, 26–45(–100) μm long, becoming mostly 1–3(–4)-branched. The branches often 1–3-branched, finally seceding from the primary axis, and ultimately all cells separating. In addition, arthro- and scoleconidia and chlamydospores were described by this author. P. prolifica, CBS 319.87, exhibits the same morphological, physiological and molecular characteristics as the smut anamorphs (Fig. 2). Partial 26S rDNA sequences strongly suggest that this species is a close relative of U. maydis (DC.) Corda (Fig. 1) (5), differing only in two of the ca. 600 nucleotides
investigated. Our results indicate that *P. prolifica* is an anamorphic smut fungus. We therefore propose to emend the generic diagnosis in order to accommodate all these anamorphs of Ustilaginales (s.s.).

Two species, viz. *S. antarcticus* and *C. tsukubaënsis*, were recombined in the genus *Vanrija* R. T. Moore (18). We do not favor this solution as this genus is poorly defined as follows “Candida et Torulopsis simile, sed basidiomycetum” (17). Moreover, the type species, *Vanrija (Cryptococcus, Torula) humicola* (Daszewska) R. T. Moore, does not belong to the same group as the above discussed smut anamorphs based on partial 26S rRNA sequences (8, 9). The molecular investigations cited above strongly indicate that *Vanrija* as currently defined (17, 18), is polyphyletic.

Because of the observation of trace amounts of xylose in whole-cell hydrolyzates of *C. tsukubaënsis* (24), this species was transferred to *Cryptococcus* (25). However, the presence of xylose could not be confirmed in a repeat experiment (H. J. Roeijmans, unpubl. results), which does not support recombination in *Cryptococcus tsukubaënsis* (Onishi) Rodr. de Miranda & Weijman. Moreover, a number of physiological and morphological characteristics of this species do not fit the current definition of the genus *Cryptococcus*, as *Candida tsukubaënsis* does not form extracellular starch-like compounds, and has septate hyphae with sterigma-like outgrowths on which fusiform blastoconidia are formed.
Similar anamorphic fungi are regularly sent to the CBS for identification. They seem to occur widely on plants, and in soil and air, but have been isolated from medical sources as well. Understanding of their biodiversity and ecological role is hampered by the lack of taxonomic knowledge. This paper aims to set up a taxonomic framework for these yeast-like smut anamorphs. *U. maydis* is included as an example of *Ustilaginales*.

*Hyalodendron* Diddens is another basidiomycetous genus with one-celled conidia in acropetal chains (12). However, this genus differs by the presence of xylose in whole-cell hydrolyzates (23), the presence of a dolipore with a parenthesome-like structure made up of vesicles of endoplasmic reticulum (16), and the absence of sterigma-like structures on which fusiform blastoconidia originate.

Strains listed as species of *Fusidium* Link, which morphologically agree to some extent with the above discussed smut anamorphs because of the presence of acropetal chains of narrowly fusiform hyaline conidia, belong to the Ascomycetes and may be differentiated by the lack of staining with Diazonium Blue B, the absence of sterigma-like structures, holoblastic proliferation of conidia and/or molecular approaches. *Fusidium* is not the adequate genus for these fungi either as the type species was found to be a species of *Cylindrocarpon* Wollenw. (nom. cons.).

**Pseudozyma** Bandoni emend. Boekhout

Anamorphs of Ustilaginales. Colonies usually dimorphic, with the central part usually pasty, white, cream, pinkish, orange, brownish or even blackish, and usually with the margin flat with radially growing hyphae. Initial growth yeast-like, with polar budding, usually on short denticles and with sympodial proliferation. Septate hyphae hyaline, with the cytoplasm retracted in cells, separated by lysed cells, and with retraction septa, sometimes becoming pigmented on age. Sterigma-like outgrowths occurring near the septa and along the hyphae, giving rise to fusiform, ellipsoidal to cylindrical blastoconidia, frequently in short acropetal, usually branch-ed chains. Ballistoconidia absent, but chlamydospores sometimes present. Aerial mycelium made up of branched or unbranched, acropetal chains of fusiform ramoconidia, usually giving the colony a somewhat hairy, velutinous, velvety or pruinose appearance. Upon ageing, the colonies may become zonated. The margin may be entire, but more frequently it is eroded. *myo*-Inositol and D-glucuronate are assimilated; extracellular starch-like compounds are not produced; DBB- and urease reactions are positive. Type species *Pseudozyma prolifica* Bandoni (CBS 319.87).

Species accepted:
1. **Pseudozyma fusiformata** (Buhagiar) Boekhout, comb. nov.
2. **Pseudozyma tsukubaensis** (Onishi) Boekhout, comb. nov.


   
   

   
   
   Synonym: *Sporothrix flocculosa* Traquair, L. A. Shaw & Jarvis (described as its anamorph).

   
   
   Synonym: *Sporothrix rugulosa* Traquair, L. A. Shaw & Jarvis (described as its anamorph).

   

Key to the species:

1 a Lactose assimilated 2
   b Lactose not assimilated 7

2 a Glucitol assimilated 3
   b Glucitol not assimilated 6

3 a Citrate, melibiose and salicin assimilated 4
   b Citrate, melibiose and salicin not assimilated  

4 a Ethanol assimilated; growth in vitamin-free medium weak  

   b Ethanol not assimilated; growth in vitamin-free medium absent 5

5 a Saccharate assimilated  
   b Saccharate not assimilated  

   *Pseudozyma prolifica*  
   
   *Pseudozyma rugulosa*  
   
   *Pseudozyma antarctica*  
   
   *Pseudozyma aphidis*
6 a Rhamnose, galactitol, salicin and D-gluconate not assimilated; citrate assimilated
   Pseudozyma tsukubaensis

   b Rhamnose, galactitol, salicin and D-gluconate assimilated; citrate not assimilated
   Ustilago maydis

7 a Ethanol assimilated; starch and galactose not assimilated
   Pseudozyma fusiformata

   b Ethanol not assimilated; starch assimilated and galactose slowly assimilated
   Pseudozyma flocculosa

REFERENCES


