The Anamorphic Yeast Genus *Myxozyma* gen. nov.

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Abstract. The yeast species published as *Torulopsis melibiosum* and *Candida mucilagina*, together with five unidentified strains, were found to constitute a morphologically and culturally distinct anamorphic taxon, characterized by affinitive properties which relate it to the ascomycetous yeasts. As this well defined taxon cannot be identified with any of the extant anamorphic yeast genera, *T. melibiosum* and *C. mucilagina* have been assigned to the new genus *Myxozyma*, based on the type species *Myxozyma melibiosi*.

Introduction

Because the imperfect or anamorphic yeasts lack taxonomically definitive sexual stages expressed as either the ascus or the basidium, the early systematics of these taxa (Harrison, 1928; Lodder, 1934; Diddens & Lodder, 1942, Lodder & Kreger-van Rij, 1952) were primarily based on morphological and physiological properties which served previously to describe and differentiate perfect or teleomorphic ascogenous yeast species. As such descriptive properties do not provide reliable means to assess possible affinities, which these anamorphs may have with either the ascomycetous or the basidiomycetous teleomorphs, the genera *Candida, Cryptococcus, Torulopsis* and *Trichosporon* became progressively heterogeneous and cumbersome — a feature which became particularly obvious when several representatives of these anamorphic genera were found to constitute haploid mating types of undescribed, heterothallic ascomycetous or basidiomycetous teleomorphs (Smith et al. 1976; Rodrigues de Miranda, 1978; Kwon-Chung, 1975).

It should, however, not be concluded that all anamorphic strains are haploid. Nor should it be assumed that an alternative, less restrictive approach to the classification of the anamorphic yeasts is not possible.

Striving toward a more natural and less restrictive system for the

classification of the anamorphic yeast taxa, von ARX et al. (1977) proposed a reclassification on the basis of affinitive characteristics, such as the ultrastructure of the cell wall and features of conidiation, which without direct reference to possible perfect states, nevertheless relate such anamorphs to either ascomycetous or basidiomycetous teleomorphs. Proceeding along these lines WEIJMAN (1979) remodelled the arthroconidial genera *Geotrichum* and *Trichosporon*. On the basis of conidiation, ultrastructure of the cell wall and carbohydrate composition, *Geotrichum* was restricted to species which in respect of these characteristics related them to the ascogenous taxa, and *Trichosporon* to species with obvious basidiomycetous affinities. Along similar lines von ARX & WEIJMAN (1979) subsequently laid the foundation for a more natural classification of the one-hundred-and-fifty species currently accepted in *Candida* (YARROW & MEYER, 1978) by transferring several species with salient basidiomycetous characteristics to *Rhodotorula* and the reinstated genus *Apiotrichum*.

Although the exclusion of basidiomycetous anamorphs from *Candida* bestows greater homogeneity upon the genus, it nevertheless still includes a taxonomically distinct group of non-fermentative species which differ from the type and all other species of the genus, by the formation of encapsulated cells and viscous growth due to the production of extracellular polysaccharides which stain blue to greenish-blue with diluted Lugol's iodine solution.

The first of these anamorphic species was isolated from insect frass and published as *Torulopsis melibiosum* (SHIFRINE & PHAFF, 1956). PHAFF & FELL (1970) subsequently reclassified it as *Cryptococcus melibiosum*. STORCK et al. (1969) reported the molar % guanine+cytosine of the nDNA of the species to be 61. RODRIGUES de MIRANDA (1978) subsequently showed that the type strain was, however, characterized by a cell wall of the ascomycetous type. The second species was isolated from cactus rots and published as *Candida mucilagina* (PHAFF et al., 1980). Attention was drawn to the fact that in respect of its morphological, cultural and physiological characteristics as well as the composition of its capsular material this species resembled species of the genus *Lipomyces*. Its DNA base composition (mol % G+C 43.2—44.0) was also found to be close to that of several *Lipomyces* species. The systematic mating of strains listed below produced neither zygotes nor ascospores. Apart from these two described species, the group also represents five morphologically and culturally similar strains which differ from *T. melibiosum* and *C. mucilagina* in respect of their utilization of certain carbon sources.

In order to assess the uniformity and possible affinities of this group, the type strains of *T. melibiosum*, *C. mucilagina* as well as the unclassified strains were examined in greater detail.

Materials and Methods

Cultures: The cultures were obtained from the Yeast Collections of the Centraalbureau voor Schimmelcultures (CBS) Delft, The Netherlands, and the Council for Scientific and Industrial Research (CSIR) Pretoria, South Africa.

Torulopsis melibiosum SHIFRINE & PHAFF

CBS 2102 (type): isolated from *Dendroctonus monticola* (Northern California)

Candida mucilagina PHAFF, STARMER, MIRANDA & MILLER

CBS 7071 (type): isolated from rotting tissue of *Stenocereus gummosis* (Mexico).

Unclassified strains

CBS 7037: isolated from soil (Pretoria district, Transvaal)
CSIR 907: isolated from soil (Bronkhorstspruit, Transvaal)
CBS 7038: isolated from arboricolous lichen (Nylstroom district, Transvaal)
CBS 7058, CSIR 769: isolated from decaying cladodes of *Opuntia ficus-indica* (Groblersdal district, Transvaal)

Lipomyces starkeyi

CBS 1807 (type): isolated from soil (S.U.A.)

Methods.

Morphological, cultural and physiological properties:

The seven strains were examined for their morphological, cultural and physiological properties according to the standard methods adopted for yeast identification (van der WALT, 1970). In addition, the ability to utilize imidazol and imidazol-4-carboxylic acid as sole sources of nitrogen was determined according to the method of LARUE & SPENCER (1967). To ensure maximum aeration, the liquid carbon and nitrogen assimilation tests were carried out at 25° C by the roller-tube method on a Tissue Culture Rollordrum (New Brunswick Scientific Co.) rotating at a speed of 40 r. p. h.

Sexual characteristics and ploidy:

Ascospore formation by strains CBS 7037, CSIR 907, CBS 7038, CBS 7058 and CSIR 769 was examined on Gorodkowa agar, McClary's acetate agar, YM agar, V8 agar, 2% malt extract agar, potato-glucose agar and Starkey's ethanol agar (van der WALT, 1970). To detect possible mating-types the five strains were also mass-mated with the type strains of *T. melibiosum* and *C. mucilagina* according to the

method of WICKERHAM & BURTON (1954), on Gorodkowa, YM, 2% malt extract and Starkey's ethanol agars.

The ploidy of *T. melibiosum* (CBS 2102) was determined by X-ray inactivation according to the method of BEAM et al. (1954) using a 48h-old culture grown on YM agar. The X-ray source (Siemens Dermipan) was operated at 60 KV and 10.5 mA, the dose rate being 340 roentgens (R) sec^{-1} with the material 100 mm from the tube target.

Carbohydrate composition:

For their carbohydrate analyses, the strains CBS 2102, CBS 7071, 7037, 7038, 7058 and *Lipomyces starkeyi* CBS 1807 were grown in 2% glucose —1% peptone —0.5% yeast extract broth in infusion bottles for 10 days at 25° C under continuous shaking and prepared for analysis according to the method of WEIJMAN (1978). The "whole cell approach" was applied to detect cellular monosaccharides with the aid of gas-liquid chromatography (GLC) as described by LECHEVALIER & LECHEVALIER (1970) and WEIJMAN (1976). For GLC analyses of sugars and amino sugars, freeze-dried, intact cells were hydrolysed in 1N and 5N hydrochloric acid for 12h at 100° C. Hydrolysis products were analyzed as their trimethylsilyl ethers after SWEELEY et al. (1963) on Chromosorb W (HP) 80—100 mesh, coated with 3% OV—1 (Pierce Chem. Co.).

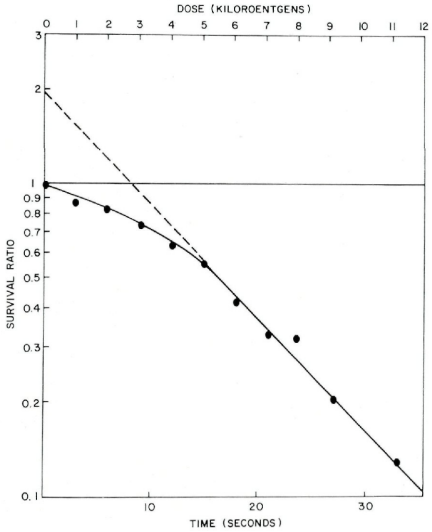
Ultrastructural characteristics:

The transmission electron microscopy (TEM) of ultrathin sections of 24h-old cultures of strains CBS 7071, CBS 7037, CBS 7038 and CBS 7058 fixed with glutaraldehyde and osmium tetroxide and stained with uranyl acetate and Reynolds' lead citrate, is based on the method described by van der WALT et al. (1974).

Results and Discussion

Morphologically and culturally the seven strains listed are indistinguishable and all characterized by multilateral budding, the formation of spheroidal to ovoid cells which are frequently encapsulated in young cultures, the absence of either pseudohyphae or true hyphae, and hyaline, flowing or viscous growth which on solid media tends to become mucoid or glutinous with age. In respect of these properties the strains resemble *Lipomyces*, as was already commented on by PHAFF et al. (1980).

Physiologically the seven strains are likewise indistinguishable on the basis of the absence of fermentation, the production on Aschner's medium of polysaccharides which stain blue to bluish green with diluted Lugol's iodine and the inability to utilize nitrate or nitrite, or to grow in the absence of an external vitamin source. All the strains



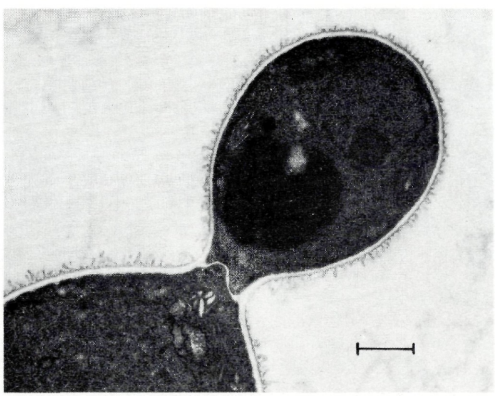


Fig. 1. (above). *Myxozyma melibiosi* CBS 2102. X-ray survival curve of a 48 h old culture grown on YM agar. Note that the linear portion of this curve when extrapolated to zero-dosage, does not intercept at the origin. — (below). *Myxozyma mucilagina* CBS 7071. Electron micrograph of an ultra-thin section of a budding cell showing characteristic holoblastic conidiogenesis in which all layers of the mothercell wall are involved in the formation of the bud wall. The bar represents 0.5 μm

nevertheless utilized imidazol and imidazol-4-carboxylic acid as sole source of nitrogen — a property common to *Lipomyces lipofer* and *Lipomyces starkeyi* (LARUE & SPENCER, 1967).

Ascus formation was not detected in any of seven strains on the seven sporulation media used. Similarly, no evidence indicative of a mating-reaction was found in any of the mass-mated, mixed cultures of the seven strains. As such the seven strains appear to lack ascigerous states. The survival curve of CBS 2102 is given in Fig. 1. a. from which an LD_{50} of 5.5 KR is deduced for this strain. Since the linear portion of the survival curve when extrapolated to zero dosage, does not intercept at the origin, the possibility of CBS 2102 being haploid is ruled out. In fact, the intercept of the extrapolated, linear portion of the survival curve suggests a probable ploidy level of two. If CBS 2102 is accepted as typical of the seven strains, this presumed ploidy level could account for the failure to detect haploid mating-types among these strains.

All four strains examined by TEM were found to be characterized by holoblastic budding and cell walls of the ascomycetous type, consisting of a rather thin dark outer layer and a broader, lighter inner layer as is shown in Figure 1 b.

The monosaccharide composition patterns of strains CBS 2102, CBS 7038, CBS 7071, CBS 7937, CBS 7058 and that of *Lipomyces starkeyi* CBS 1807 are shown in Table 1. As will be observed, the six strains show close agreement in their carbohydrate composition. In general these patterns correspond to those observed in the Saccharomycetales and anamorphs which are characterized by cell walls of the ascomycetous type with mannan and glucan as dominant components of the cell wall (von ARX & WEIJMAN, 1979). Noteworthy of the patterns shown in Table 1, is the absence of xylose which features prominently in the carbohydrate composition of the telemorphic basidiomycetous genera *Filobasidium* and *Filobasidiella* and the

Table 1. Monosaccharide patterns of hydrolyzed, intact cells [1])

Strain	Carbohydrate composition	Final pH of culture medium
CBS 2102	mannose glucose mannitol glua gal	4.0
CBS 7071	mannose glucose mannitol glua gal	4.0
CBS 7037	mannose glucose mannitol glua gal	4.0
CBS 7038	glucose mannose mannitol glua gal	4.0
CBS 7058	glucose mannose mannitol glua gal	4.0
CBS 1807	glucose mannose mannitol gal glua	5.0

[1]) Sugars listed in order of abundance
glua = glucuronic acid
gal = galactose

anamorphic genera *Apiotrichum*, *Bullera*, *Dioszegia*, *Phaffia* and *Trichosporon* which are characterized by cell walls of the basidiomycetous type (von Arx & Weijman, 1979).

Conclusions

Accepting the application of affinitive characteristics such as aspects of conidiation, ultrastructure of the cell wall and carbohydrate composition, as guide lines for generic differentiation among the imperfect yeasts, the seven strains studied emerge as a distinct and well-defined taxon which is readily differentiated from *Candida* on the basis of the copious production of extracellular polysaccharides.

As the affinitive properties of the seven strains are essentially ascomycetous, they are also excluded from the basidiomycetous genus *Cryptococcus*.

Novak & Zsolt (1961) proposed placing *T. melibiosum* in the genus *Paratorulopsis* and introduced the combination *Paratorulopsis melibiosi* (Shifrine & Phaff) Novak & Zsolt. This proposal cannot be accepted because *Paratorulopsis*, by definition, precludes all species forming extracellular, iodophilic, polysaccharides — property well expressed in all the strains when examined on Aschner's medium. Moreover, the name *Paratorulopsis* and the cited combination are not validly published as a result of the contravention of Articles 37 and 33, respectively of the International Code of Botanical Nomenclature (Stafleu et al., 1978).

Consequently, the seven strains are assigned to the new genus:

Myxozyma van der Walt, Weijman & von Arx, gen. nov. (Deuteromycotina).

Cellulae hyalinae propagantes per gemmationem holoblastica multilateralem, saepe capsula circumdatae. Ultrastructura paritietis sicut in fermentis ascomycetoideis. Coloniae hyalinae, profluentes vel viscosae, mucosescentes vel glutinosescentes in substratis solidis. Materia amyloidea iodophila formatur. Fermentatio abest.

Etymology: *Myxozyma* (f) slime yeast; from the Gr. nouns μύξα slime, and ζύμη yeast.

Type species: *Myxozyma melibiosi* (Shifrine & Phaff) van der Walt, Weijman & von Arx comb. nov. (basionym: *Torulopsis melibiosi* (as '*meliobosum*') Shifrine & Phaff, Mycologia 48: 49. 1956).

Cells hyaline, reproducing by multilateral holoblastic budding, frequently encapsulated. Ultrastructure of the cell walls as in the ascomycetous yeasts. Growth on solid substrates hyaline, flowing or viscous, becoming mucoid to glutinous. Iodophilic amyloid material is produced. Fermentation absent.

Second species: *Myxozyma mucilagina* (Phaff, Starmer, Miranda & Miller) van der Walt, Weijman & von Arx comb. nov.

(basionym: *Candida mucilagina* PHAFF, STARMER, MIRANDA & MILLER, Int. J. syst. Bact. 30: 596. 1980).

As species delimitation based exclusively on physiological differences is inadequate and as PHAFF et al. (1980) have reported that *Myxozyma mucilagina* shows considerable variation in its utilization of carbon sources, the classification of the five unclassified strains is left in abeyance. Because of the absence of ascigerous states, the classification of these strains must ultimately hinge on nDNA reassociation data.

On the basis of their morphological and physiological properties, representatives of *Myxozyma* could be regarded as anamorphs of the Lipomycetoideae. It should, however, be noted that, whereas *Lipomyces lipofer* and *L. starkeyi* possess the Coenzyme Q-9 system (YAMADA et al., 1977), *Myxozyma mucilagina* is characterized by the Coenzyme Q-8 system (PHAFF et al., 1980). Whereas *Lipomyces* appears to be primarily associated with soils, the genus *Myxozyma* has a wider distribution, being recoverable also from insect sources, lichens and decaying vegetable matter.

Acknowledgement

The authors express their gratitude to Dr. Elżbieta JOHANNSEN for her assistance in determining the ploidy of strain CBS 2102 and to Mr. N. V. D. W. LIEBENBERG for his help at the electronmicroscope.

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