Phaffia, a New Yeast Genus in the Deuteromycotina (Blastomycetes)

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A description is given of a new yeast genus, Phaffia, represented by P. rhodozyma sp. nov., to accommodate nine yeast strains isolated in Japan and one in Alaska, all from exudates of deciduous trees. The type strain of P. rhodozyma is UCD (FS&T) 67-210 (="ATCC 24202 = CBS 5905"). Phaffia, named in recognition of the contributions of Herman Jan Phaff to yeast taxonomy and ecology, is a carotenoid-producing, fermentative yeast of the Deuteromycotina (Blastomycetes), whose properties indicate a basidiomycetous origin. A comparison is made between Phaffia and other yeast genera to which it might be related.

In surveys of yeasts in tree exudates (slime fluxes) in Japan and in the Pacific Northwest of North America (16), we isolated 10 similar yeast strains that belong to a new species which represents a new genus. This yeast produces carotenoid pigments, reproduces vegetatively by budding, and lacks, as far as we have been able to determine, a sexual life cycle. It could not be accommodated in the genus Rhodotorula Harrison because species in that genus are non-fermentative, whereas the isolates from tree exudates ferment several sugars. In an earlier publication (16), this yeast was tentatively named Rhodozyma montanae. However, a Latin diagnosis, as required by the rules of the International Code of Botanical Nomenclature, was not given, and this binomial is therefore a nomen nudum. The intent of this paper is to give a complete description, to discuss the taxonomic relationships of the new organism, and to provide it with a validly published name.

MATERIALS AND METHODS

Samples of exudates were collected in new plastic vials or bags. Usually within 6 to 18 h after collection, a loopful of the slimy exudate was streaked directly on 5% malt agar acidified with hydrochloric acid to pH 3.7. If the sample was in a dehydrated condition, it was removed from the tree with a specially made chisel sterilized in alcohol. It was then rehydrated with a small amount of sterile water for a suitable length of time before streaking. The inoculated plates were stored at room temperature ranging from about 15 to 25°C. On most of the plates, relatively few fungi appeared, but many of the samples yielded significant numbers of bacterial colonies in spite of the low pH of malt agar. The plates were inspected with a dissecting microscope after 3 to 6 days of growth, and a visual estimate was made of the proportion of the various colony types. One colony of each type of yeast was picked for purification, but bacterial colonies were not isolated. Purification of the isolates was accomplished by two successive plateings on 5% malt agar. The 10 strains and their isolation sources are listed in Table 1.

In the identification of the isolates, the standard methods currently employed in yeast taxonomy (28) were used. Deoxyribonucleic acid (DNA) extraction and purification were done by a combination of the procedures of Marmur (12) and Bernardi et al. (4). The moles percent guanine plus cytosine (G + C) of the DNA was calculated from buoyant density values in cesium chloride (21, 26), based on three separate determinations. Micrococcus lysodeikticus DNA, with a buoyant density of 1.7311 g/ml, was used as a reference. The buoyant density of the M. lysodeikticus DNA was derived from comparison with Escherichia coli K-12 DNA, whose buoyant density was taken to be 1.7100. Mating attempts were made by the procedures of Wickerham and Burton (32) and van der Walt (29, 31). The scanning and transmission electron micrographs were taken by the method of Talens et al. (27).

RESULTS

In recognition of the outstanding and continuing contributions by Herman Jan Phaff to the fields of yeast taxonomy and ecology, we propose the name Phaffia for this new genus. It is represented by a single species, Phaffia rhodozyma sp. nov., and is characterized below.

The vegetative cell wall is multilayered and forms a bud in a manner characteristic of heterobasidiomycetous yeasts (8) (Fig. 1). Chlamydoспорes germinated by budding (Fig. 2), but a definitive promycelium and sporidial formation did not occur. Attempts to mate the various strains in the hope of observing subsequent dikaryotic mycelium and teliospore formation were unsuccessful. Consequently, the new
yeast genus is placed in the *Deuteromycotina* in the form order *Blastomycetes*.

**Latin diagnosis**

*Phaffia* Miller, Yoneyama, et Soneda (*Blastomycetes*), gen. nov.

Cellulae vegetativae ellipsoideae, singulae, binae, interdum catenatae. Pigmenta carotinoidea (praecipue astaxanthina, \( \beta \)-carotina quoque) formantur, cellularum ponderibus sandaracoidum aut salmonis rubrum colorem dans. Mycelium absens, sed pseudomycelium rudis formari potest. Chlamydospores are formed. Velum supra media liquida formatur.

Fermentatio praesens.

Materia amyloidea formantur (pH sejuncte).

Typus: *Phaffia rhodozyma* Miller, Yoneyama, et Soneda.

**Genus characteristics.** Vegetative cells are ellipsoidal and occur singly, in pairs, and occasionally in short chains. Carotenoid pigments are synthesized (mainly astaxanthin and a minor proportion of \( \beta \)-carotene) which give cells en masse an orange to salmon-red color. True mycelium is absent, but a rudimentary pseudomycelium may be present. Chlamydospores are formed. A pellicle is formed on liquid media.

Fermentative ability present.

Starchlike compounds are formed (synthesis is pH independent).

Type of the genus: *Phaffia rhodozyma* Miller, Yoneyama, et Soneda.

**Latin diagnosis.**

*Phaffia rhodozyma* sp. nov.

In extracto malti, post tres dies, cellulae ellipsoideae (3.8–7.5) \( \times \) (5.5–10.5) \( \mu \)m, singulae, binae, interdum catenatae. Frequente ibidem gemmant. Chlamydosporeae rotundae cum granibus fulgentibus post plures dies formari possunt. Annulus et velum tenue cum sedimentum parvum format. Post unum mensum, annulus sandaracoidus crasusque, velum tenues aut insulae et medium sedimentum praesenta sunt.

In agaro extractis malti, post unum mensum, cultura lineamentosa sandaracoida vel salmonis rubra, superficii apiaetae vel paene levis, nitida vel semi-hebetis, mollis et sectionis laxe convexae vel convexae est; ora paene illibata vel libulata.

In agaro farinae mai's (Dalmau), portio aeria interdum oram inequalem habet; sub laminella vitri, interdum cristae cellularae pseudomyce- lii rudis, ex paucis cellulis formatae, praesentes sunt. Mycelium non formatur.

Glucosum medie, maltosum, saccharum et raffinosum exigere fermentantur; galactosum et melibiosum non fermentantur.

Glucosum, maltosum, saccharum, cellobiosum, trehalosum, raffinosum, melezitosum, amyllum (lente aut –), di-xylulosum (lente), L-arabinosum, ethanolum, D-mannitolum (lente), \( \alpha \)-methyl-glucosidum (lente aut –), salicinum exigere, glucono-\( \delta \)-lactonum, calcium-2-ketogluconatum, kalium-\( \delta \)-ketogluconatum (lente), acidum lacticum (lente) et acidum succinicum assimilantur, at non galactosum, L-sorbosum, lactosum, melibiosum, inulinum, D-arabino- sum, D-ribosum, L-rhamnosum, methan- olum, glycerolum, erythritolum, ribitolum, galactitolum, D-glucitolum, acidum citricum et *meso*-inositolum.

Kalium nitricum, ethylaminum non assimilarantur.
FIG. 1. Transmission electron micrograph of Phaffia rhodozyma UCD (FS&T) 67-210 revealing the multilayered nature of the cell wall. Remnants of the outer wall layers form a collar as the new bud emerges while the inner layer is continuous. Also note the capsular material (electron-transparent area) surrounding the cell. ×41,000.

FIG. 2. Scanning electron micrograph of Phaffia rhodozyma UCD (FS&T) 67-210 showing a budding chlamydospore and a budding vegetative cell. The chlamydospore is easily distinguished by its spheroidal shape and large size. Granular appearance of the cell surfaces is due to the presence of capsular material. ×6,500.

Ad crescentiam biotinum necessarium est.
Mol% G+C: 48.3 ± 0.18.
Typus: stirps UCD (FS&T) 67-210, ex Fagus crenatae isolata. In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum sub No. 5905 deposita est.

Species characteristics. (i) Growth in malt extract. After 3 days, the cells are ellipsoidal 3.8 to 7.5 by 5.5 to 10.5 μm, occurring singly, in pairs, or occasionally in short chains. Budding occurs frequently at the same site. Spheroidal chlamydospores with refractile granules may be formed after several days. A thin ring and
pellicle are formed, and there is little sediment. After 1 month, there are a thick, orange ring, a pellicle are formed, and there is little sediment.

(ii) Growth on malt extract agar. After 1 month, the streak culture is orange- to salmon-red, punctilicate to nearly smooth, glossy to semidull, soft and broadly convex to convex in cross section; the border is lobulate to nearly entire.

(iii) Dalmau plate culture on corn meal agar. In the aerobic portion, the border is occasionally irregular; under the cover slip, there is an occasional tuft of cells or rudimentary pseudomycelium consisting of limited numbers of cells in a chain. No true mycelium is formed.

(iv) Others. Fermentation: D-Glucose, moderate (a full tube of gas is formed in 5 to 11 days); maltose, weak; sucrose, weak; raffinose, weak. D-Galactose and melibiose are not fermented.

Assimilation of carbon compounds: D-Glucose, +; D-galactose, −; L-sorbose, −; maltose, +; sucrose, +; cellobiose, +; trehalose, +; lactose, −; melibiose, −; raffinose, +; melezitose, +; inulin, −; soluble starch, + (latent) or −; D-xylene, + (latent); L-arabinose, +; D-arabinose, −; D-ribose, −; L-rhamnose, −; ethanol, + (latent) or −; methanol, −; glycerol, −; erythritol, −; ribitol, −; galactitol, −; D-mannitol, + (latent); D-glucitol, −; alpha-methyl-D-glucoside, + (latent or −); salicin, + (weak); glucono-δ-lactone, +; calcium-2-ketogluconate, +; potassium-5-ketogluconate, + (latent); DL-lactate, + (latent); succinate, +; citrate, −; and meso-inositol, −.

Assimilation of nitrogen compounds: Potassium nitrate, −; ethylamine, −.

Growth in vitamin-free medium: Absent. Biotin is required for growth.

Growth on 50% (wt/wt) glucose-yeast extract agar: Absent.

Growth on 10% sodium chloride-yeast extract agar: Absent.

Temperature range of growth: 0 to 27 C.

Acid formation on chalk agar: Weak.

Synthesis of starchlike compounds: Positive (pH independent).

Gelatin liquefaction: Weak.

Casein hydrolysis: Absent.

Hydrolysis of urea: Positive.

Lipolytic activity: Absent.

Growth in the presence of 0.1 μg of cycloheximide per ml: Absent.

Synthesis of carotenoid pigments: Positive (approximately 85% is astaxanthin).

Mol% G+C: 48.3 ± 0.18.

(v) Type. The type strain UCD (FS&T) 67-210, isolated from Fagus crenata at Uchimi-

yama (Kyoto Prefecture) at an elevation of 1,200 m, has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft, The Netherlands as CBS 5905 and in the American Type Culture Collection, Rockville Md., as ATCC 24202.

(vi) Habitat. Ten strains have been isolated. All came from exudates (slime fluxes) of deciduous trees (Table 1).

DISCUSSION

Attempts to disclose a sexual life cycle in Phaffia rhodozyma were unsuccessful. The various isolates were mixed in all possible combinations on media proven successful for sporulation or mating of other species, and heat treatments of cells showed no resistant stages and no mated generations leading to dikaryotic mycelium. The chlamydospores show a morphological similarity with those of Aessosporon. Upon germination of the chlamydospore, vegetative cells are produced by budding. However, these cells could not be construed in any definitive manner as promycelia with sporidia similar to those described by van der Walt (30) for Aessosporon. Thus, no basis exists for regarding these chlamydospores as gonotoconits or their buds as the haploid generation. Nuclear stains were inconclusive as to a diploidization at any stage of growth. Transmission electron micrographs revealed but a single nucleus during all phases of growth.

Criteria that may be used for imperfect yeasts as indicators of an ascomycetous or basidiomycetous origin are the ability to form ballistospores (9), the ability to synthesize carotenoid pigments (not known to occur in ascogenous yeasts), the base composition of DNA acid expressed as moles percent G + C (14, 24, 25), the cell wall structure and the mode of bud formation (8), and the nature of the polysaccharides that make up the capsules of encapsulated yeasts (17). In connection with the latter criterion, the acidic heteropolysaccharides of Cryptococcus and of some species of Trichosporon relate these yeasts to the Tremellaceae of the Ustilaginaceae (Basidiomycotina). The linear β-(1→3)- and β-(1→4)-linked mannan of Rhodosporidium is unknown among asccomycetous yeasts.

Criteria for determining the phylogenetic origin of Phaffia rhodozyma were as follows. The G+C value of 48.3 ± 0.18 mol% was not helpful since it falls in the "grey zone," being at the upper limit of asccomycetous yeasts (approximate range, 30 to 50 mol% G+C) and the lower limit of basidiomycetous yeasts (approximate range, 50 to 70 mol%) (13). The cell wall polysaccharides contain a high proportion of α-1,3-
glucan (pseudonigeran), which is found in basidiomycetous yeasts as well as in the nonbudding, ascomycetous genera Schizosaccharomyces and Endomyces (2; Melvin T. Meyer, Ph.D. thesis, Univ. of California, Davis, 1975). The ability of _P. rhodozyma _to hydrolyze urea is more commonly found among basidiomycetous than among ascomycetous yeasts (1, 22). _P. rhodozyma _contains carotenoid pigments, which are not found in ascogenous yeasts. The most conclusive evidence of the phylogenetic origin of _Phaffia _is the disclosure of a multilayered cell wall near the area of repeated bud formation. This budding mechanism is similar to that of heterobasidiomycetous yeasts (8). Bud scars shown by transmission and scanning electron microscopy are also characteristic of this origin.

In recent years, several asporogenous yeast genera of basidiomycetous affinity have been shown to have sexual cycles. Banno (3) was the first to discover _Rhodotorula glutinis _strains that mated. The zygotes produced a dikaryotic mycelium with clamp connections and gave rise to teliospores. The germination of these spores to a septate promycelium and budding spores (sporidia) confirmed the basidiomycetous nature of these yeasts. He established the genus _Rhodosporidium (Ustilaginales) _to accommodate the perfect stage. Banno's attempts to find a sexual cycle in _Sporobolomyces _were unsuccessful. Kluyver and van Niel (7) suggested a basidiomycetous origin for _Sporobolomyces _since its spores were released by the same drop-secretion mechanism that is responsible for the discharge of basidiospores. Sainclivier (18–20) postulated that the ballistospores of _Sporobolomyces _were sexual basidiospores. However, van der Walt (30) was successful in inducing a single strain of _Sporobolomyces salmonicolor _to form teliospores which germinated, usually by a nonseptate promycelium bearing two to four sporidia. He described _Aessosporon (Tilletiaceae) _to accommodate these sexual forms. Thus, while suggested over 50 years ago, the basidiomycetous origins of several yeast genera have been confirmed only recently by the demonstration of their sexual life cycles. Thus far, none of the yeasts that produce carotenoid pigments are fermentative. Only some species of _Leucosporidium _and of _Filobasidium (Ustilaginales), _genera containing only nonpigmented species, are fermentative.

Upon comparison of _Phaffia _with other yeast genera, there are a number of differences that necessitate establishment of a new genus. The ability to ferment sugars is not shared by any other genus of carotenoid-producing yeasts, and this property sharply differentiates _Phaffia _from those yeasts. Equally distinctive is the production of astaxanthin, which represents ca. 85% of the carotenoid pigments produced by _Phaffia _A. G. Andrewes, H. J. Phaff, and M. P. Starr, Phytochemistry, in press) and is not known to be produced by yeasts of other genera. Conversely, torulene and torularhodin, which are known to be the main carotenoid pigments of _Rhodotorula _and of other red-pigmented, carotenoid-producing yeasts (23), were not found in _Phaffia_. Astaxanthin is commonly associated with marine crustacea (i.e., shrimp, crab, lobster, etc.), although it is known to be produced by some plants and algae and has also been reported in a few basidiomycetous fungi (J. L. Fiasson, Ph.D. thesis, Univ. of Lyon, Lyon, France, 1968).

Specific criteria differentiating _Phaffia _from other genera are: the inability to form ballistospores (positive for _Sporobolomyces, Sporidiobolus, _and _Aessosporon _); the absence of an observed sexual cycle (positive for _Rhodosporidium, Aessosporon, _and _Sporidiobolus _); and fermentative ability (negative for _Cryptococcus _and _Rhodotorula _). A comparison was made also with _Rhodomyces dendrorheus, _a yeastlike organism superficially described by Ludwig (10, 11). Other than color and source of isolation (red flux of _Betula _sp.), we could determine no similarity. Apparently Ludwig never isolated this organism in pure culture. Saccardo (17) placed _Rhodomyces _in synonymy with _Monilia _.

In summary, the properties that make _Phaffia _unique among the genera with which it can be compared are the production of carotenoid pigments, of which astaxanthin is the principal component, and the ability to ferment sugars, a property not shared by any other carotenoid-producing yeast.

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REPRINT REQUESTS

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LITERATURE CITED


