Pichia scutulata, a New Species from Tree Exudates

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A novel representative of the yeast genus *Pichia* has been recovered 11 times during 1968, 1971, and 1972. We regard this organism as belonging to a new species, *Pichia scutulata*, with two varieties: *P. scutulata* var. *scutulata*, the type variety; and *P. scutulata* var. *exigua*. Strains of both varieties were found in tree exudates but were geographically separated. *P. scutulata* var. *scutulata* was isolated from slime exudates and flux-wetted soil of *Myoporum* trees on the island of Hawaii (six strains), whereas *P. scutulata* var. *exigua* was found in fluxes or insect borings of various trees in the state of Washington, and in the province of British Columbia, Canada (five strains). *P. scutulata* var. *exigua* differs from *P. scutulata* var. *scutulata* by its slower fermentation rate, weak ability to utilize glycerol, and higher maximum temperature for growth. The type strain of *P. scutulata* var. *scutulata* is UCD-FST 71-102 (= ATCC 32651 = CBS 6644), and that of *P. scutulata* var. *exigua* is UCD-FST 68-979B1 (= ATCC 24185 = CBS 6836).

During an extensive survey of yeasts associated with trees, Phaff et al. (7) isolated six strains of yeast, which they discussed either in relation to *Pichia terricola* as a variety or as a possible new species. Five strains came from tree exudates or insect borings (frass) in trees from the North American Pacific Northwest, and one strain came from the flux of a *Myoporum* tree on the island of Hawaii. Subsequent isolations from Hawaii yielded additional strains of this yeast. All strains grew well in a vitamin-free medium and formed four spheroidal spores, which were not dehiscent from the ascus when mature. However, the strains differed in the vigor of glucose fermentation: those from Hawaii had a rather strong fermentation, whereas the strains from the Pacific Northwest were latent and slow in fermenting glucose.

In the Kreger-van Rij key to the species of *Pichia* (2), the properties of the new isolates led to a group of three yeasts: *P. terricola*, *P. kudriavzevii*, and *P. membranaefaciens*. These three species are differentiated by their vigor of glucose fermentation and vitamin requirements. *P. terricola* is vitamin dependent, *P. kudriavzevii* is independent, and *P. membranaefaciens* is variable in this respect. On the basis of glucose fermentation rate, the Pacific Northwest strains showed some resemblance to either *P. terricola* or *P. membranaefaciens*, whereas the Hawaiian strains fermented glucose at about the same rate as *P. kudriavzevii*.

Conclusive proof that the strains from tree exudates represent a new species was obtained from the base compositions of their deoxyribonucleic acids (DNA), which were significantly

lower than those of the other three species. The new species is named *Pichia scutulata* after the frequent occurrence of the four spheroidal spores in a diamond-shaped, planar arrangement (L. adj. *scutulatus* diamond-shaped). Since the Hawaiian and the Pacific Northwest strains differed in a number of phenotypic properties, two varieties will be described, *P. scutulata* var. *scutulata* for the Hawaiian strains and *P. scutulata* var. *exigua* for the Pacific Northwest strains. The latter variety is named after the latent and slow fermentation of glucose (L. adj. *exiguus* of low intensity or weak).

MATERIALS AND METHODS

Yeast strains. Samples were collected from trees in British Columbia and the state of Washington in 1968 (7) and from trees on the island of Hawaii in 1971 and 1972. The tree exudates or insect borings were usually streaked directly (one loopful per plate) or, if desiccated, after about 1 g of material was soaked in 3 ml of sterile water. Streaking was done (on the day of collection) on acidified malt-extract agar (5% Fleischmann Diamalt plus 0.7 ml of 1 N HCl added per 100 ml of medium after autoclaving, pH 3.7 to 3.8). The plates were placed at 20 to 25°C until colonies developed (3 to 6 days), at which time counts of distinct morphological colony types were made. Each type was purified by restreaking on 5% malt-extract agar. All isolates reported in this paper were obtained from different trees or soils (Table 1).

Methods. The characteristics of the isolates were determined by standard methods currently employed in yeast taxonomy (12). DNA extraction and purification were done by a combination of the procedures of Marmur (4) and Bernardi et al. (1). The guanine plus cytosine (G+C) content of the DNA

TABLE 1. Isolation data of *Pichia scutulata*

Strain no.	Source	Locality	Percent of population ^a	Isolation date
<i>Pichia scutulata</i> var. <i>scutulata</i>				
71-102	Slime flux of <i>Myoporum sandwicense</i>	Island of Hawaii	— ^b	1971
71-103	Slime flux of <i>M. sandwicense</i>	Island of Hawaii	—	1971
71-145	Slime flux of <i>M. sandwicense</i>	Island of Hawaii	100	1971
71-180	Wet soil under <i>Myoporum</i> flux	Island of Hawaii	—	1971
72-135	Slime flux of <i>M. sandwicense</i>	Island of Hawaii	4	1972
72-147	Slime flux of <i>M. sandwicense</i>	Island of Hawaii	100	1972
<i>Pichia scutulata</i> var. <i>exigua</i>				
68-914B	Slime flux of <i>Populus trichocarpa</i>	Near Lillooet, British Columbia	10	1968
68-917C	Slime flux of <i>Acer</i> sp.	Emory Creek, British Columbia	10	1968
68-930	Slime flux of <i>Salix</i> sp.	Near Agassiz, British Columbia	100	1968
68-931A	Slime flux of <i>Salix</i> sp.	Near Agassiz, British Columbia	100	1968
68-979B1	Insect boring in <i>Picea sitchensis</i>	Beach section, Olympic National Park, Washington	30	1968

^a The percentage of the yeast from each source represents the estimated frequency of its colonies on the isolation plate.

^b Not determined.

was calculated from buoyant density values in cesium chloride (8, 10) and was based on four separate determinations. *Micrococcus lysodeikticus* DNA, with a buoyant density of 1.7311 g/cm³, was used as a reference. The buoyant density of the *M. lysodeikticus* DNA was derived from comparison with *Escherichia coli* K-12 DNA, the buoyant density of which was taken to be 1.7100 g/cm³. Scanning electron microscopy was by the method of Talens et al. (11).

RESULTS

Latin diagnosis

Pichia scutulata sp. n.

Pichia scutulata var. *scutulata* var. n.

In extracto multi cellulae ovoideae, interdum longovoideae, (2.6–6.6) × (3.9–11.8) μm, singulae, binae, aut in catenis brevis, sedimentum, annulus et pellicula tenuis aut crispulata, non-nitida formantur.

Cultura in agarō multi post unum mensem (18°C) crenea, mollis, nitida et glabra aut farinacea; prope plana; margine glabro et undulato.

In agarō farinae Zea maïs post dies 10 pseudomycelium nullum; interdum paucae cellulae oblongae formantur.

Species heterothallica, diploidea. Asci inconjugati fiunt, habentes 4 sporos spherioideos; asci non rumpuntur.

Fermentatio glucosi solius.

Glusum, ethanolum, glycerolum, DL-acidum lacticum et acidum succinicum assimilantur at non galactosum, L-sorbosum, maltosum, saccharum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitosum, inulinum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rham-

nosum, methanolum, iso-erythritolum, ribitolum, galactitolum, α-methyl-D-glucosidum, salicinum, glucono-δ-lactonum, 2-ketogluconatum, 5-ketogluconatum, D-glucosaminum, acidum citricum nec inositolum.

Kalium nitricum et kalium nitrosus non assimilantur.

Ad crescentiam vitaminarum additae non necessariae sunt.

Crescere non potest in 33°C.

G+C acidi deoxyribonucleati = 32.7 ± 0.14 mol%.

Typus: stirps UCD (FST) 71-102 ex *Myoporum sandwicense* Hawaiiensis isolata est.

In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum sub. no. 6644 deposita est.

Pichia scutulata var. *exigua* var. n.

Varietas a varietate *scutulata* differt: fermentatio glucosi lente aut exigue; glycerolum assimilatur exigue; crescere potest in 33°C at non potest in 36°C.

Typus: stirps UCD (FST) 68-979 B1 ex *Picea sitchensis* Washingtonensis isolata est.

In collectione zymotica Centraalbureau voor Schimmelcultures Delphi Batavorum sub. no. 6836 deposita est.

Standard description of *Pichia scutulata* var. *scutulata*

Growth in malt extract: After 3 days at 25°C, the cells are spheroid to ovoid and occasionally elongate (2.6 to 6.6) by (3.9 to 11.8) μm, single, and in pairs or short chains. An ascending, smooth, thin pellicle, or a slightly wrinkled pellicle, and a ring are present. After 1 month

there is a sediment, a thin pellicle, and a ring.

Growth on malt agar: After 1 month at 18°C, streak cultures are whitish to cream colored. The surface is semiglossy and smooth but, in some strains, it is finely granular to rugose. The texture is soft. The cross section is low convex to umbonate or nearly flat. The border is entire with fine lobes to slightly serrate.

Slide culture on corn meal agar: After 7 to 10 days at 25°C, a rudimentary pseudomycelium may develop consisting of a few branched chains of ovoid to cylindrical cells. Some strains lack the formation of pseudomycelium.

Formation of ascospores: Vegetative cells are heterothallic diploid. Ascospores are formed on most solid media. The spores are spheroidal, and there are usually four per ascus, often arranged in diamond-shaped tetrads. Two of the spores are of mating type α and two are of mating type α . The slightly warty spore surface is evident only in a scanning electron microscope (Fig. 1). The spores contain a small lipid globule. The asci do not release the spores upon maturity.

Fermentation: Only glucose is fermented. A full tube of gas is produced in 3 to 4 days.

Assimilation of carbon compounds: Glucose, ethanol, glycerol, DL-lactic acid, and succinic acid are assimilated. The following compounds are not assimilated: D-galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, iso-erythritol, ribitol, galactitol, D-mannitol, D-glucitol, α -methyl-D-glucoside, salicin, glucono- δ -lactone, Ca-2-ketogluconate, K-5-ketogluconate, D-glucosamine, citric acid, and meso-inositol.

Assimilation of nitrogen compounds: Potassium nitrate, -; potassium nitrite, -; ethylamine, +; ammonium sulfate, +.

Growth in vitamin-free medium: Positive.

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

Growth in 10% NaCl plus 5% glucose in yeast nitrogen base: Positive.

Maximum temperature for growth: Positive

at 30°C; negative at 33°C.

Acid formation on chalk agar: Weak or absent.

Hydrolysis of urea: Variable (weak to strong).

Synthesis of starchlike compounds: Negative.

Gelatin liquefaction: Negative.

Casein hydrolysis: Negative.

Lipolytic activity: Negative.

Growth in the presence of 0.1 mg of cycloheximide per ml: Negative.

G+C content of the nuclear DNA: 32.7 ± 0.14 mol%.

Habitat: Six strains were recovered during 1971 and 1972 on the island of Hawaii from exudates of *Myoporum sandwicense* trees and from soil wetted by dripping fluxes (Table 1).

Type: The type strain UCD-FST 71-102, isolated from a frothy slime flux of *M. sandwicense* on the island of Hawaii, has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft, The Netherlands, as CBS 6644 and in the American Type Culture Collection, Rockville, Md., as ATCC 32651.

Supplementary description of *Pichia scutulata* var. *exigua*

Fermentation of glucose: Latent and slow. Usually not more than 50 to 70% of the inverted vials become filled with gas after 7 to 10 days.

Assimilation of glycerol: Very weak rather than positive.

Maximum temperature for growth: Positive at 33°C; negative at 36°C.

Acid formation on chalk agar: Positive.

Hydrolysis of urea: Strongly positive.

Growth in 10% NaCl plus 5% glucose in yeast nitrogen base: Negative.

G+C content of the nuclear DNA: 32.5 ± 0.23 mol%.

Habitat: Five strains were recovered from slime fluxes or insect borings in various trees of the Pacific Northwest (Table 1).

Type: The type strain of *P. scutulata* var. *exigua*, UCD-FST 68-979B1 (Fig. 1), isolated

FIG. 1. Ascospores and asci of *Pichia scutulata* var. *scutulata* (a, c, d, and e) and of *P. scutulata* var. *exigua* (b and f) observed by scanning electron microscopy (a through d) and by phase-contrast microscopy (e and f). (a through c) Asci that have been enzymatically digested to remove the ascus wall; (d) intact ascus. The spore surface of *P. scutulata* var. *scutulata* appears to have distinct warts, whereas that of *P. scutulata* var. *exigua* has a more corrugated surface. The planar tetrad configuration appears to result from incomplete separation of the spores after the process of spore wall formation (a and e). Characteristic lipid globules are distinct in the spores of *P. scutulata* var. *scutulata* (e). The fact that the lipid inclusions are less distinct in *P. scutulata* var. *exigua* (f) may be related to spore maturity. Note the distended appearance of the ascus wall (d and e) and the swollen appearance of the ascospores, which result in the diamond shape. (a through d) Bar represents 1 μ m ($\times 10,000$); (e and f) bar represents 2 μ m ($\times 5,000$).

FIG. 1
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from insect borings in *Picea sitchensis*, Olympic National Park, Wash., has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft, The Netherlands as CBS 6836 and in the American Type Culture Collection, Rockville, Md., as ATCC 24185.

DISCUSSION

Although *Pichia scutulata* bears some resemblance to *P. terricola*, *P. kudriavzevii*, and *P. membranaefaciens*, the values obtained for the DNA base composition of the four species (Table 2) are distinctly different, and *P. scutulata* therefore represents an independent species. Our G+C values, determined by buoyant density equilibrium centrifugation in CsCl, are generally higher than values given in the literature, based on the melting technique (5, 6) but, no matter which data are used, the differences between the four species are clear-cut. Since the assimilatory properties of the four species are virtually the same, the most convenient phenotypic properties used to differentiate *P. scutulata* from the others are as follows. *P. scutulata* differs from *P. terricola* by its growth in vitamin-free medium and by its four-spored asci (those of *P. terricola* nearly always contain two spores and only rarely four). It differs from *P. kudriavzevii* by its

much lower maximum temperature for growth (lower than 36°C, versus 43 to 44°C), its four-spored asci, and by its inability to assimilate D-glucosamine. The phenotypic properties available to differentiate *P. scutulata* from *P. membranaefaciens* are the latter's very weak, or lack of, glucose fermentation and its inability to hydrolyze urea. It should also be noted that the spores of *P. scutulata* are not released from the ascus but that they are easily liberated by nearly all strains of *P. membranaefaciens*. Moreover, most *P. membranaefaciens* strains form hat-shaped rather than spheroidal spores. Mating types of *P. scutulata* do not mate with the mating types of either *P. terricola* or *P. kudriavzevii* (C. P. Kurtzman, personal communication). The wartlike spore wall ornamentation of *P. scutulata* (Fig. 1) is quite similar to that of *P. terricola* and of *P. kudriavzevii* (3). Although Kreger-van Rij (2) reported that *P. terricola* strains grew at 37°C, we found this property variable for the strains included in this study (Table 2). *P. scutulata* cannot, therefore, be differentiated from *P. terricola* by maximum temperature of growth.

The Hawaiian strains differed in a number of relatively minor properties from the Pacific Northwest strains. This observation induced us to split *P. scutulata* into two varieties: *P. scutulata* var. *scutulata*, the type variety, for the

TABLE 2. Salient characteristics of several species of *Pichia*

Species and variety	Strain no.	G+C content (mol%)	Growth in vitamin-free medium	Growth on glycerol	Growth on glucosamine	Maximum temp for growth	No. of spores per ascus	Ascus dehiscence	Fermentation of glucose
<i>Pichia scutulata</i> var. <i>scutulata</i>	71-102 ^a	32.7 ± 0.14	+	+	—	>30; <33	4	No	Strong
	71-145	32.6 ± 0.36	+	+	—	>30; <33	4	No	Strong
	72-147	33.0 ± 0.18	+	+	—	>30; <33	4	No	Strong
<i>P. scutulata</i> var. <i>exigua</i>	68-917C	32.4 ± 0.23	+	— or weak	—	>33; <36	4	No	Latent, slow
	68-979B1 ^a	32.5 ± 0.23	+	— or weak	—	>33; <36	4	No	Latent, slow
	68-931A	32.6 ± 0.13	+	— or weak	—	>33; <36	4	No	Latent, slow
<i>P. terricola</i> van der Walt	CBS 2617 ^a	47.4 ± 0.24 ^b	—	+	—	>33; <36	2	No	Slow
<i>P. terricola</i>	68-145 ^c	37.3 ± 0.13	—	+	—	37	2	No	Slow
	56-107 ^d	37.2 ± 0.34	—	+	—	>33; <36	2	No	Slow
<i>P. kudriavzevii</i> Boidin, Pignal, and Besson	CBS 5147 ^a	40.1 ± 0.36 ^b	+	+	+	43-44	2	No	Strong
<i>P. membranaefaciens</i> Hansen	CBS 107 ^a	44.3 ± 0.44	+ or —	+ or —	—	37 or lower	4	Yes	Very weak or negative

^a Type strain.

^b Data supplied by C. W. Price (manuscript in preparation).

^c This strain was isolated in British Honduras, C.A., from soil.

^d This strain (= CBS 5376) was isolated in our laboratory from spoiled figs.

Hawaiian strains; and *P. scutulata* var. *exigua* for the Pacific Northwest strains (Table 2). Although representative strains of the two varieties have essentially the same DNA base composition, the possibility that they represent different species cannot be excluded. DNA-DNA hybridization experiments should settle this point.

We considered the possibility that *P. scutulata* var. *exigua* could be identical to species whose names are now included among the numerous synonyms of *P. membranaefaciens* (2). Two strains were obtained from the CBS culture collection: *Pichia punctispora* (Mélard) Dekker CBS 190, on the basis of its spore wall ornamentation (9); and *Pichia membranaefaciens* Hansen var. *calliphorae* (Kloecker) Dekker CBS 189, since it forms four spheroidal to irregularly shaped spores per ascus (9). However, the DNA base composition of CBS 190 was found to be 43.42 ± 0.54 mol% G+C; that of CBS 189 was found to be $45.60 \pm 0.37\%$ G+C. These values are in agreement with the value found for the type strain of *P. membranaefaciens* (Table 2).

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REPRINT REQUESTS

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