Candida populi, a New Species of Yeast Occurring in Exudates of *Populus* and *Betula* Species

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During a survey of yeasts occurring in exudates of various tree species in the Pacific Northwest of North America, 24 strains of an imperfect yeast were isolated in a wide geographic area, mainly from species of the genus *Populus*. The isolates were studied by traditional as well as molecular methods, and the results revealed a new species of the genus *Candida*. The new species is named *Candida populi*, because its principal habitat is in exudates of *Populus* species. *C. populi* resembles *Candida molischiana* but differs from this species in habitat, guanine-plus-cytosine content of the nuclear deoxyribonucleic acid, maximum growth temperature, and ability to assimilate several carbon compounds. The type strain of *C. populi* is strain UCD-FST 68-675B (= CBS 7351 = ATCC 64933).

In 1968 Phaff et al. (2) isolated 24 strains of a novel yeast species from sap exudates of various broad-leaved, deciduous trees in the Pacific Northwest of North America. A total of 12 strains were isolated from aspen (*Populus tremuloides* Michaux), 10 strains were isolated from cottonwoods (*Populus trichocarpa* Torrey and Gray), and 2 strains were isolated from a species of birch. These isolates were referred to as *Torulopsis* sp., and they were found in a large area spanning from northern California to central Alaska (Table 1). Sporulation was not observed in individual strains or after mixing in various combinations. All of the isolates exhibited a more or less mucous appearance on agar media, assimilated nitrate as a sole nitrogen source, and demonstrated a fairly uniform pattern of assimilation of carbon compounds. The new species, although mucous, does not fit the definition of the genus *Myxozyma* (8) because of its fermentative ability and lack of production of amyloid iodophilic material. Therefore, we place it in the genus *Candida* sensu Yarrow and Meyer (10) and propose to name it *Candida populi* after the most common genus of host trees.

MATERIALS AND METHODS

Samples of tree exudates were collected in sterile plastic bags during June and July of 1968 in various locations of Alaska, the Yukon Territory, British Columbia, Washington, Oregon, and northern California (2). The precise locations of the trees with exudates are given in Table 1. The isolation of pure yeast cultures by streaking onto acidified malt agar (pH 3.7) has been described in detail by Phaff et al. (2). Taxonomic responses were determined by methods currently used in yeast identification (7), but additional carbon compounds used in assimilation tests were also employed. These included D-glucosamine, N-acetyl-D-glucosamine, hexadecane, methanol, acetone, 2-propanol, and ethyl acetate. The last three compounds were tested by incorporating them at concentrations of 1% in yeast nitrogen base agar because in liquid yeast nitrogen base media they proved to be inhibitory, even in those cases where good growth was observed on solid medium.

Deoxyribonucleic acid (DNA) extraction and purification were carried out by using the methods described by Price et al. (4), except that purified DNA was concentrated by

electrophoresis in a concentrator (ISCO, Lincoln, Nebr.) and the reference DNA was labeled with ¹²⁵I as described by Holzschu et al. (1). The guanine-plus-cytosine (G+C) contents of the nuclear DNAs were calculated from buoyant density values in cesium chloride gradients established in a Spinco model E analytical ultracentrifuge equipped with photographic optics (5, 6) and were based on three separate determinations. DNA from *Micrococcus luteus* ICPB 2039 (International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis), with a buoyant density of 1.7310 g/ml, was used as a reference. The buoyant density of the *M. luteus* DNA was derived from a comparison with plasmid-free *Escherichia coli* K-12 DNA, the buoyant density of which was taken to be 1.7100 g/ml. Denaturation of DNA, incubation for reassociation, analysis of the renaturation kinetics and reannealing reactions, and quantitation of single- and double-stranded DNAs were carried out by using the protocol of Price et al. (4). Samples were counted with a gamma ray counter (90% efficiency; model 1185; Nuclear-Chicago Corp., Des Plaines, Ill.).

RESULTS

The fieldwork associated with this study yielded 24 glossy, somewhat mucous yeast isolates (Table 1). These organisms had fermentative ability, assimilated nitrate as a sole nitrogen source, had a fairly uniform pattern of assimilation of carbon compounds, did not form pseudo or true hyphae, and lacked the capacity for ascospore formation. They were originally considered to belong in the genus *Torulopsis* (9) and were keyed to *Torulopsis molischiana*, which has since been transferred to the genus *Candida* by Yarrow and Meyer (10) as *Candida molischiana*. Although there are some similarities in phenotypic properties between *C. molischiana* and the new isolates, the G+C content of the nuclear DNA of *C. molischiana* is nearly 12 mol% higher (Table 2), which virtually precludes conspecificity. Nevertheless, a DNA hybridization experiment was designed to compare the new isolates with *C. molischiana* and several *Hansenula* species with DNA base compositions much closer to that of the new species. *Pichia nakazawae* was used as a negative control. Our results (Table 2) indicated clearly that there is no significant DNA relatedness between the strains from tree exudates and several phenotypically similar species. We describe the strains from tree exudates as a new species below.

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TABLE 1. Strain numbers, host trees, and localities of the isolates studied

UCD-FST strain no. ^a	Host tree With exudate	Location
68-675B ^{Tb} , 68-685	<i>Populus tremuloides</i>	Delta Junction, Ala.
68-680	<i>Populus trichocarpa</i>	Delta Junction, Ala.
68-728A	<i>Populus trichocarpa</i>	Canyon Creek, Yukon Territory, Canada
68-731	<i>Populus trichocarpa</i>	Mile 982, Alcan Highway, Yukon Territory, Canada
68-791B	<i>Populus trichocarpa</i>	Mile 734, Alcan Highway, Yukon Territory, Canada
68-763	<i>Populus tremuloides</i>	Mile 620, Alcan Highway, Yukon Territory, Canada
68-775A	<i>Betula</i> sp.	Liard Hot Springs, British Columbia, Canada
68-781B	<i>Populus trichocarpa</i>	Mile 480, Alcan Highway, British Columbia, Canada
68-783B	<i>Populus tremuloides</i>	Mile 456, Alcan Highway, British Columbia, Canada
68-804	<i>Betula</i> sp.	Mile 345, Alcan Highway, British Columbia, Canada
68-811B	<i>Populus trichocarpa</i>	Mile 298, Alcan Highway, British Columbia, Canada
68-835B, 68-840B	<i>Populus trichocarpa</i>	Mile 65, Alcan Highway, British Columbia, Canada
68-845B, 68-842	<i>Populus tremuloides</i>	Mile 55, Alcan Highway, British Columbia, Canada
68-852, 68-857	<i>Populus tremuloides</i>	Dawson Creek, British Columbia, Canada
68-865 ^c , 68-868	<i>Populus tremuloides</i>	Moberly Lake, British Columbia, Canada
68-951D	<i>Populus trichocarpa</i>	Mile 6, South of Nanaimo, Vancouver Island, Canada
68-1020B	<i>Populus tremuloides</i>	Diamond Lake, Oreg.
68-1013A	<i>Populus trichocarpa</i>	Klamath Agency, Calif.

^a UCD-FST, Culture Collection of the Department of Food Science and Technology, University of California, Davis.

^b T = type strain.

^c From a pupa in the bark of *Populus tremuloides*.

Latin diagnosis of *Candida populi* sp. nov. In extracto malti post dies 3 cellulae singulae, binae, aut in catenis brevis; cellulae sphaericae vel ovoideae (2.2-4.6 × 2.9-5.2 µm). Post unum mensem annulus et sedimentum formantur. Cultura in agar malti post dies 21 (25°C) mucosa, glabra, nitida; cellulae capsulae.

In agar farinae *Zea mays* post dies 14 pseudomycelium vel mycelium verum nullum.

Glucosum, cellobiosum (exigue), et trehalosum (exigue) fermentantur. Glucosum, maltosum, cellobiosum, trehalosum, melezitiosum (lente), D-xylosum, L-arabiosum (lente), D-arabiosum (lente, exigue), D-ribosum, L-rhamnosum, ethanolum, glycerolum, ribitolum, D-mannitolum, D-glucitolum, methyl-α-D-glucosidum (lente, exigue), salicinum (lente), glucono-Δ-lactonum, acidum succinicum, acidum citricum, hexadecanum, et N-acetylglucosaminum assimulantur at non D-galactosum, L-sorboseum, saccharosum, lac-

tosum, melibiosum, raffinolum, inulinum, amyllum solubile, erythritolum, galactitolum, 2- et 5-ketogluconatum, acidum lacticum, meso-inositolum, methanolum, 2-propanolum, acetoneum, ethyl acetum, nec D-glucosaminum.

Kalium nitricum et ethylaminum assimilantur.

Ad crescentiam vitaminarum externarum necessariae sunt.

Augmentum in 30°C, raro in 37°C.

G+C acidi deoxyribonucleati, 37.4-38.9 mol% (6 stirpes).

Habitatio in exudati species populi et betuli.

Typus stirpis UCD-FS&T 68-675B (= CBS 7351) ex exudato *Populus tremuloides* isolata est.

In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum, sub. no. CBS 7351 deposita est.

Standard description of the type strain of *Candida populi*. *Candida populi* (pop'u. li. L. gen. n. *populi*, of poplar, the principal host trees of the yeast). In malt extract after 3 days at 25°C, the cells are short ovoidal to spheroidal, occur

TABLE 2. Nuclear DNA base compositions and reannealing between labeled DNA from *C. populi* UCD-FST 68-775A and DNAs from 10 yeast strains and calf thymus^a

Organism or tissue ^b	G+C content (mol%) ^c	Actual binding (%) ^d	Relative binding (%) ^e
<i>C. populi</i> UCD-FST 68-775A	38.4 ± 0.4	71.2 ± 1.2	(100)
<i>C. populi</i> UCD-FST 68-675B ^T	38.1 ± 0.2	69.8 ± 3.7	97.9
<i>C. populi</i> UCD-FST 68-731	38.2 ± 0.4	67.5 ± 2.3	94.4
<i>C. populi</i> UCD-FST 68-791B	38.9 ± 0.2	71.5 ± 1.7	100.2
<i>C. populi</i> UCD-FST 68-804	37.4 ± 0.3	68.1 ± 3.5	95.5
<i>C. populi</i> UCD-FST 68-1020B	38.3 ± 0.1	54.2 ± 1.9	74.2
<i>C. molischiana</i> NRRL Y-2237 ^T	50.3 ± 0.0	5.2 ± 0.3	0.2
<i>Hansenula holstii</i> UCD-FST 61-13 ^T	36.5 ± 0.2	6.4 ± 0.3	1.8
<i>Hansenula beckii</i> UCD-FST 74-68 ^T	36.4 ± 0.2	7.5 ± 0.7	3.6
<i>Hansenula muscicola</i> NRRL Y-7005 ^T	37.0 ± 0.3	6.3 ± 1.0	1.7
<i>Pichia nakazawae</i> NRRL Y-7903 ^T	40.4 ± 0.2	4.8 ± 0.6	0
Calf thymus		4.8 ± 0.6	0

^a 125I-labeled DNA (0.2 µg) and unlabeled DNA (200 µg) were incubated for 24 h in 0.5 ml of 280 mM phosphate buffer (pH 6.8) at 63°C.

^b NRRL, Northern Regional Research Laboratory, United States Department of Agriculture, Peoria, Ill.

^c Average ± standard deviation of three or more buoyant density determinations.

^d Average ± standard deviation for three samples, corrected for zero-time binding (1.9%).

^e Corrected for self-renaturation of labeled DNA (5.2%) (4).

singly, in pairs, or in small clusters of three or four cells, and measure 2.2 to 4.6 by 2.9 to 5.2 μm . A sediment and a thin ring develop after 1 week.

On malt agar after 2 weeks at 20°C, streak cultures are low convex to flat, glossy, smooth, greyish, and somewhat mucous.

In Dalmau plate cultures on cornmeal agar after 2 weeks pseudomycelium is absent, or a few spotty, rudimentary tufts develop into the agar.

Fermentation: a full tube of gas develops in about 5 days with glucose; cellobiose and trehalose are fermented weakly (about 50% gas in 2 weeks).

Assimilation of carbon compounds: glucose, maltose, cellobiose, trehalose, melezitose (latent), D-xylose, L-arabinose (latent), D-arabinose (latent and weak), D-ribose, L-rhamnose, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, methyl- α -D-glucoside (latent, weak), salicin (latent), glucono- Δ -lactone, succinic acid, citric acid, hexadecane, and N-acetylglucosamine are assimilated; no growth occurs on D-galactose, L-sorbose, sucrose, lactose, melibiose, raffinose, inulin, soluble starch, i-erythritol, galactitol, 2-keto-gluconate, 5-ketogluconate, lactic acid, *meso*-inositol, methanol, 2-propanol, acetone, ethyl acetate, and D-glucosamine.

Assimilation of nitrogen compounds: potassium nitrate, ammonium sulfate, and ethyl amine positive.

Diazonium Blue B reaction is negative.

Growth in a vitamin-free medium is negative.

Growth in amino acid-free medium is positive.

Growth at 30°C is positive; growth at 37°C is negative.

Acid formation on chalk agar is weak.

Urease activity is negative.

Gelatin liquefaction is negative.

Casein hydrolysis is negative.

Lipolytic activity is negative.

Production of amyloid compounds is negative.

Growth in Wickerham osmotic medium is negative.

Growth in the presence of 100 μg of cycloheximide per ml is positive.

Ubiquinone type, Q-8.

The G+C content of the nuclear DNA is 38.8 ± 0.1 mol% (mean $\pm$ standard deviation).

The habitat is in slime exudates of species of *Populus* in the Pacific Northwest of North America.

The type strain of *C. populi*, strain UCD-FST 68-675B, was isolated from an exudate of *Populus tremuloides* (trembling aspen) at Delta Junction, Ala. This strain has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, The Netherlands, as strain CBS 7351 and in the American Type Culture Collection, Rockville, Md., as strain ATCC 64933.

DISCUSSION

C. populi is not the anamorph of *Hansenula populi*, a species that is sympatric with *C. populi* but occurs only in black cottonwood (*Populus trichocarpa*) and not in *Populus tremuloides* (3). *H. populi* has a nuclear DNA composition that is almost 6 mol% G+C higher than that of *C. populi*, and moreover there are many differences in physiological properties. *C. populi*, which appears to occur in a large area of the Pacific Northwest in tree exudates of the genera *Populus* and *Betula*, was not found during an extensive survey of yeasts occurring in exudates of numerous species of trees on

various Japanese islands (2). A possible reason could be that *Populus* species do not occur in Japanese forests and *C. populi* may be dependent on some insect vector that utilizes *Populus* exudates for its nutrition or life cycle. As shown in Table 1, *C. populi* is occasionally isolated in mixed forests from birch fluxes, but is relatively rare there. Among the various isolates there were minor variations in the degree of growth on melezitose, D-arabinose, methyl- α -D-glucoside, and the organic acids. A few of the strains grew weakly to moderately at 37°C, but most were negative for this characteristic. Strain UCD-FST 68-1020B, which was used in the DNA hybridization experiment (Table 2), was somewhat atypical in that no fermentation of cellobiose and trehalose was observed; also, both the assimilation of melezitose and the assimilation of methyl- α -D-glucoside were negative, characteristics which were also observed for strains UCD-FST 68-1013A and UCD-FST 68-951D. The somewhat lower homology value for strain UCD-FST 68-1020B (Table 2) might have been due to allopatry and a resulting change in metabolic potential.

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LITERATURE CITED

- Holzschu, D. L., H. L. Presley, M. Miranda, and H. J. Phaff. 1979. Identification of *Candida lusitanae* as an opportunistic yeast in humans. *J. Clin. Microbiol.* 10:202-205.
- Phaff, H. J., M. W. Miller, M. Yoneyama, and M. Soneda. 1972. A comparative study of the yeast flora associated with trees on the Japanese Islands and on the west coast of North America, p. 759-774. In G. Terui (ed.), *Fermentation technology today*. Society of Fermentation Technology, Osaka, Japan.
- Phaff, H. J., Y. Yamada, J. Tredick, and M. Miranda. 1983. *Hansenula populi*, a new homothallic species of yeast from exudates of cottonwood trees. *Int. J. Syst. Bacteriol.* 33:375-380.
- Price, C. W., G. B. Fuson, and H. J. Phaff. 1978. Genome comparison in yeast systematics: delimitation of species within the genera *Schwanniomyces*, *Saccharomyces*, *Debaryomyces*, and *Pichia*. *Microbiol. Rev.* 42:161-193.
- Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. Mol. Biol.* 4:430-433.
- Szybalski, W. 1968. Use of cesium sulfate for equilibrium density gradient centrifugation. *Methods Enzymol.* 128:330-360.
- van der Walt, J. P. 1970. Criteria and methods used in classification, p. 34-113. In J. Lodder (ed.), *The yeasts—a taxonomic study*. North-Holland Publishing Co., Amsterdam.
- van der Walt, J. P., A. C. M. Weijman, and J. A. von Arx. 1981. The anamorphic yeast genus *Myxozyma* gen. nov. *Sydowia Ann. Mycol.* 34:191-198.
- van Uden, N., and M. Vidal-Leiria. 1970. *Torulopsis* Berlese, p. 1235-1308. In J. Lodder (ed.), *The yeasts—a taxonomic study*. North-Holland Publishing Co., Amsterdam.
- Yarrow, D., and S. A. Meyer. 1978. Proposal for amendment of the diagnosis of the genus *Candida* Berkhout nom. cons. *Int. J. Syst. Bacteriol.* 28:611-615.