Candida mucilagina, a New Species of Yeast Found in Decaying Cladodes of *Opuntia inermis* and in Necrotic Tissue of Cereoid Cacti

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A new species of *Candida* has been recovered 40 times from necrotic cactus tissue occurring in widely separated geographic areas. The organism has been named *Candida mucilagina* because of the extremely slimy, almost watery appearance of its colonies on solid media. Eleven strains were isolated in Baja California Sur, Mexico, where its principal host plant was *Stenocereus gummosus* (agria), and 29 strains came from rotting cladodes of *Opuntia inermis* in New South Wales and Queensland, Australia. The base composition of the nuclear deoxyribonucleic acid of *C. mucilagina* is 43.2 to 44.0 mol% (range of five strains). The type strain of *C. mucilagina* is UCD-FS&T 76-236C (= ATCC 42174 = CBS 7071).

During a survey of yeasts associated with necroses of Mexican columnar cacti of the North American Sonoran Desert and opuntia cacti of eastern Australia, we isolated 40 strains of a slimy, asporogenous, multilaterally budding yeast that represents a new species of the genus *Candida* sensu Yarrow and Meyer (9). On the isolation medium, the colonies of the new species could be easily recognized by their highly mucous, nearly watery appearance, which led us to name it *Candida mucilagina* sp. nov. The Mexican strains were nearly all associated with agria cacti (*Stenocereus gummosus* (Engelm.) Gibson and Horak), and those from Australia were all isolated from rots in *Opuntia inermis* de Candolle.

MATERIALS AND METHODS

Samples of necrotic cactus tissue were collected from eight areas of Baja California Sur, Mexico in the North American Sonoran Desert (Table 1, Fig. 1). These collections were part of an extensive survey of this region carried out in March 1976. Additional samples were collected in Australia in 1977 and 1978 by J. S. F. Barker, University of Sydney, New South Wales, from decaying cladodes of *O. inermis* growing in New South Wales and Queensland (Table 1). The 1977 strains (strains 12 through 17) were isolated by J. S. F. Barker and mailed to Davis for identification. The 1978 strains (strains 18 through 40) were isolated in Davis from samples of cactus rot airmailed to the University of California (Table 1).

Most of the isolations were accomplished by streaking a loopful of necrotic tissue directly on acidified yeast extract-malt extract agar (Difco YM agar + 0.7% [vol/vol] 1 N HCl = AYM, pH 3.7 to 3.8) in the field

or in the laboratory. In some cases, dilutions of the tissue were made in sterile water as described earlier (6). The plates were stored at ambient temperatures (24 to 30°C) until colonies appeared. Counts of morphologically distinct colony types were then made, and for identification, a representative of each type was brought into pure culture by two successive platings on YM agar.

Identification was done by standard methods currently used in yeast taxonomy (8). Deoxyribonucleic acid (DNA) extraction and purification were done by a combination of the procedures of Marmur (2) and Bernardi et al. (1), as described by Price et al. (3). The guanine plus cytosine (G+C) content of the DNA was calculated from buoyant density values in cesium chloride (4, 7) and was based on two or three separate determinations. *Micrococcus luteus* (syn.: "*Micrococcus lysodeikticus*" [not on the Approved Lists (5)]) strain 2039 (International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis) 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 plasmid-free *Escherichia coli* K-12 DNA, the buoyant density of which was taken to be 1.7100 g/ml.

RESULTS

Latin diagnosis of *Candida mucilagina* sp. nov. In extracto malti cellulae sphaericae, interdum ovoideae, $(3.2-7.9) \times (3.9-7.9) \mu\text{m}$, singulae, binae, interdum ternae; post dies 6 annulus et sedimentum non formantur. Post dies 21 annulus tenuis et sedimentum formantur.

Cultura in agaro malti post dies 21 (25°C) mucosa, glabra, nitida; cellulae capsulae.

In agaro farinae Zea mais post dies 14 pseudomycelium vel mycelium verum nullum.

Fermentatio glucosi nullum.

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TABLE 1. Localities and sources of isolates of *C. mucilagina*

Serial no.	UCD-FST strain no.	Locality ^a	Habitat/substrate
1	76-214B	Rancho Cuñaño, Baja Calif. Sur, Mexico (a)	<i>Stenocereus thurberi</i> (Engelmann) Buxbaum (organ pipe) ^b
2	76-266B	Punta Pescadero, Baja Calif. Sur, Mexico (b)	<i>Stenocereus gummosis</i> (Engelmann) Gibson and Horak (agria) ^b
3, 4	76-324C, 76-236C	Todos Santos, Baja Calif. Sur, Mexico (c)	<i>S. gummosis</i>
5	76-318C	Loreto, Baja Calif. Sur, Mexico (d)	<i>S. gummosis</i>
6, 7	76-329C, 76-380C	Mulegé, Baja Calif. Sur, Mexico (e)	<i>S. gummosis</i>
8	76-354C	12 km South of Mulegé, Baja Calif. Sur, Mexico	<i>S. gummosis</i>
9	76-365B	San Jose de Castro, Baja Calif. Sur, Mexico (f)	<i>S. gummosis</i>
10	76-370B	San Ignacio, Baja Calif. Sur, Mexico (g)	<i>S. gummosis</i>
11	76-388C	Bahia San Nicolás, Baja Calif. Sur, Mexico (h)	<i>S. gummosis</i>
12, 13	77-27, 77-166	Yarrowonga, New South Wales, Australia (170 km NNW of Sydney)	<i>Opuntia inermis</i> de Candolle
14, 15	77-228B, 77-247C	Yarrowonga	<i>O. inermis</i>
16, 17	77-248A, 77-249B	Yarrowonga	<i>O. inermis</i>
18, 19	78-191, 78-206B	Yarrowonga	<i>O. inermis</i>
20, 21	78-214A, 78-218	Yarrowonga	<i>O. inermis</i>
22, 23	78-231, 78-264B	Yarrowonga	<i>O. inermis</i>
24, 25	78-265B, 78-266A	Yarrowonga	<i>O. inermis</i>
26, 27	78-345, 78-360	Yarrowonga	<i>O. inermis</i>
28,	78-361A	Yarrowonga	<i>O. inermis</i>
29, 30	78-538F, 78-539E	Borallon, Queensland, Australia	<i>O. inermis</i>
31, 32	78-544A, 78-550C	Grand Chester Hill, Queensland, Australia	<i>O. inermis</i>
33	78-552C		
34, 35	78-556B, 78-557D	Hemment, Queensland, Australia	<i>O. inermis</i>
36, 37	78-559B, 78-567D		
38, 39	78-576D, 78-587A	Hemment	<i>O. inermis</i>
40	78-601D	Hemment	<i>O. inermis</i>

^a Letters in parentheses indicate the map localities depicted in Fig. 1.^b Common names of cacti are given in parentheses.

Glucosum, galactosum, L-sorboseum (interdum lente), maltosum (raro nullum), saccharum (raro nullum), cellobiosum, trehalosum (valde aut lente), lactosum (lente aut nullum), melezitium (raro nullum), D-xylosum, L-arabiosum, L-rhamnosum (interdum lente), ethanolum (interdum lente), glycerolum, ribitolium (interdum lente), D-mannitolium (variabile), D-glucitolium (valde aut lente), alpha-methyl D-glucosidum, salicinum, glucono-δ-lactonum (lente, exigue), acidum lacticum (exiguitate aut nullum), acidum succinicum (exiguitate, lente) assimilantur at non melibiosum, raffinoseum, inulinum, amyllum solubile, D-arabiosum, D-ribosum, erythritolum, methanolum, galactitolium, acidum citricum, inositolium nec hexadecanum.

Nitras kalii non assimilatur; ethyl aminum non assimilatur.

Ad crescentiam vitamina externi necessariae sunt.

Crescere potest in temperatura 37°C, interdum in 39–42°C.

G+C acidi deoxyribonucleati = 43.2–44.0 mol% (5 stirpes, vide tabulum 2).

Amyllum fit; gelatinum non liquescit.

Typus: Stirps UCD (FS&T) 76-236C ex tabidosis sacculis cacti *Stenocereus gummosis* isolata est.

In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum sub no. 7071 deposita est.

Standard description of *Candida mucilagina* sp. nov. Growth in malt extract: after 6 days at 25°C the cells are spheroidal to globose, sometimes ovoidal, 3.2 to 7.9 by 3.9 to 7.9 μm, single, in pairs, and sometimes in groups of three,

surrounded by a mucilaginous capsule (Fig. 2); a pellicle, ring, and a visible sediment are lacking. After 21 days a thin ring and a moderate amount of sediment are present.

Growth on malt agar: After 21 days at 25°C,

FIG. 1. Collection localities for isolates of *C. mucilagina* listed in Table 1.

the streak culture is watery and very slimy. The growth runs to the bottom of the tube.

Dalmau plate culture on cornmeal agar: Pseudohyphae or true hyphae are lacking.

Other characteristics: Ability to ferment glucose is absent. Assimilation of carbon compounds: D-glucose, D-galactose, L-sorbose (sometimes latent), maltose (rarely negative), sucrose (rarely negative), cellobiose, trehalose (strong or latent), lactose (latent or negative), melezitose (rarely negative), D-xylose, L-arabinose, L-rhamnose (sometimes latent), ethanol (sometimes latent), glycerol, ribitol (sometimes latent), D-mannitol (some strains negative), D-glucitol (strong or latent), α -methyl D-glucoside, salicin, glucono- δ -lactone (latent and weak), DL-lactic acid (weak or negative), and succinic acid (latent and weak) are assimilated. The following compounds are not assimilated: melibiose, raffinose, inulin, soluble starch, D-arabinose, D-ribose, erythritol, methanol, galactitol, citric acid, meso-inositol, and hexadecane.

Assimilation of nitrogen compounds: Potassium nitrate, negative; sodium nitrite, negative; ethyl amine, negative; ammonium sulfate, positive.

Growth in vitamin-free medium: Absent. Thiamine is required. Biotin is stimulatory.

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

Growth on osmotic salt agar: On 5% NaCl plus 5% glucose-yeast extract agar, moderate growth; on 7.5% NaCl plus 5% glucose-yeast extract agar, absent.

FIG. 2. (A) Budding cell of *C. mucilagina* surrounded by a capsule; (B) slimy, watery colonies on malt agar. The bar represents 5 μ m.

Maximum temperature for growth: 37 to 39°C (a few strains from Mexico grew at 42°C).

Acid formation on chalk agar: Negative.

Hydrolysis of urea: Negative.

Gelatin liquefaction: Negative.

Casein hydrolysis: Negative.

Lipolytic activity: Negative.

Formation of starch-like compounds: Positive (upon addition of Lugol's iodine, a greenish-blue color develops).

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

Coenzyme Q system: Q-8

G+C content of the nuclear DNA: 43.2 to 44.0 mol% (five strains, Table 2).

Habitat: Forty strains were recovered from rotting cactus tissue in the North American Sonoran Desert (principal host plant: *Stenocereus gummosus*) and in Eastern Australia, from *Opuntia inermis* (Table 1).

Type: The type strain of *Candida mucilagina*, UCD (FS&T) 76-236C, was isolated from rotting tissue of *Stenocereus gummosus* (agria) near Todos Santos, Baja California Sur, Mexico. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft, The Netherlands as CBS 7071 and in the American Type Culture Collection, Rockville, Md., as ATCC 42174.

Etymology: *mucilagina* L.L. adj. *mucilaginus* mucilaginous; referring to the organism's slimy appearance on solid media.

DISCUSSION

Candida mucilagina is a yeast characteristically associated with cactus rots. It is nonfermentative and produces slimy, almost watery colonies on solid media (Fig. 2). Its principal habitat in the North American Sonoran Desert

appears to be rotted tissue of *S. gummosus* (agria), and in Australia, that of *O. inermis*. It is the only species that forms exceptionally slimy colonies that we have isolated from necrotic cactus tissue. It is usually present as a minority organism in cactus-specific yeast communities.

Attempts to induce ascosporeulation by cultivating representative strains on a variety of sporulation media were unsuccessful. Similarly, systematic mixing of strains on several media to reveal heterothallism produced neither zygotes nor ascospores.

Superficially, *C. mucilagina* resembles species of the genus *Lipomyces*, whose members have their habitat in soil. Similarities include the very slimy appearance of colonies, lack of fermentation, assimilation of a relatively large assortment of carbon compounds, and the production of starchlike compounds on acidic media (e.g., YNB-glucose) that give a blue or blue-green color with Lugol solution. The nuclear DNA base composition of the new species (Table 2) is close to that of several species of *Lipomyces* (Holzschu, Tredick, and Phaff, unpublished data). In addition, the composition of the capsular slime bears some similarity to the composition of the capsular slime of *Lipomyces*. The capsular material of *C. mucilagina* consists primarily of two glycoproteins: (i) a mannan-type glycoprotein, containing 90% D-mannose and precipitable with both concanavalin A and with Fehling solution, and (ii) a glycoprotein containing D-glucose and D-mannose in approximately equimolar proportions and precipitable by concanavalin A but not by Fehling solution. Both glycoproteins contain ca. 5% uronic acids (Villa and Phaff, unpublished data).

Although *C. mucilagina* forms no pseudomycelium and could therefore be considered a new

TABLE 2. Nuclear DNA base compositions of representative strains of *C. mucilagina*

Strain no.	Locality ^a	Substrate ^a	Distinctive physiological criteria, assimilation of:	Mol% G+C of nuclear DNA $\pm$ SD ^b
3	Todos Santos, Mexico	<i>Stenocereus gummosus</i>	Maltose-, Sucrose-, Melezitose-	43.5 $\pm$ 0.1
4 ^c	Todos Santos, Mexico	<i>Stenocereus gummosus</i>	Maltose+, Sucrose+, Melezitose+	43.2 $\pm$ 0.3
8	12 km South of Mulegé, Mexico	<i>Stenocereus gummosus</i>	Maltose+, Sucrose+, Melezitose-	44.0 $\pm$ 0.3
11	Bahia San Nicolás, Mexico	<i>Stenocereus gummosus</i>	Maltose-, Sucrose-, Melezitose-	43.3 $\pm$ 0.01
12	Yarrowonga, Australia	<i>Opuntia inermis</i>	Maltose+, Sucrose+, Melezitose+	43.5 $\pm$ 0.3

^a See Table 1.

^b SD, Standard deviation; G + C, guanine plus cytosine.

^c Type strain.

species of the genus *Torulopsis* Berlese, we are following the proposal by Yarrow and Meyer (9) to include *Torulopsis* species in their amended genus *Candida* Berkhout.

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REPRINT REQUESTS

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