Candida caseinolytica sp. nov., a New Species of Yeast Occurring in Necrotic Tissue of *Opuntia* and *Stenocereus* Species in the Southwestern United States and Baja California, Mexico

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We describe *Candida caseinolytica*, a new yeast species which occurs in rotting tissues of opuntias and other cacti in the North American Sonoran Desert and a few other localities. This small-celled, slowly growing yeast does not ferment any sugar and assimilates a limited number of carbon compounds, including 2- and 5-ketogluconic acids. It exhibits strong extracellular proteolytic activity on casein at pH 6.5, but gelatin is not hydrolyzed or is only weakly hydrolyzed by a few strains. The type strain of *C. caseinolytica* is strain UCD-FST 83-438.3 (= ATCC 90546 = CBS 7781).

During extensive surveys of more than 5,000 isolates in the yeast biota found in rotting tissues of various cactus species obtained from a wide variety of geographic areas (2, 12, 15), we isolated a small number of strains of a unique, slowly growing, small-celled yeast species; these strains were isolated mainly from various *Opuntia* and *Stenocereus* species (Table 1) growing in a rather restricted region of the North American Sonoran Desert and a few other localities. The colonies of this yeast species were recognized on the isolation medium used (pH ca. 3.8) by their small size, late appearance compared with other cactus-specific species, very glossy smooth surfaces, and white color. The unusually small budding cells occurred mainly as single cells and in pairs. Subsequent physiological tests showed that these strains exhibited an exceptionally strong ability to hydrolyze casein at pH 6.5, a property that had not been observed previously in any of the other cactus-specific species described by us. As ascosporeulation was not observed, the strains fit the description of the genus *Candida* sensu Yarrow and Meyer (17). A comparison of the phenotypic properties of this organism with the properties of other *Candida* species (3, 8) and a recent up-to-date key to species of the genus (7) revealed that the cactus isolates represent a new species, for which we propose the name *Candida caseinolytica*.

MATERIALS AND METHODS

Samples of rotting cactus tissues collected during 1981, 1983, 1984, 1985, 1986, 1987, and 1991 were obtained from host plants in the locations shown in Table 1. The technique used for yeast isolation and purification has been described in detail previously by Starmer and Phaff (13).

The isolates were characterized phenotypically by methods currently used in yeast taxonomy (16), but additional carbon compounds were used in the assimilation tests. These additional carbon compounds included hexadecane, glucosamine, *N*-acetylglucosamine, methanol, 2-propanol, acetone, and ethyl acetate. 2-Propanol, acetone, and ethyl acetate were tested by adding each compound at a concentration of 1% (vol/vol, based on medium volume) to the inverted cover of a flat-bottom glass petri dish after inoculation of the test cultures with a multipoint inoculator. The dishes were then sealed with

Parafilm M (American Can Co., Greenwich, Conn.) and incubated upside down.

The medium used for the casein hydrolysis test, which is not a routine test in yeast taxonomy, was a slightly modified version of the medium used by Ahearn et al. (1). First, 1.5 g of beef extract (Difco), 2.5 g of Bacto Tryptone, 0.5 g of glucose, and 8.5 g of agar were added to 440 ml of deionized water, and the preparation was autoclaved. Then 12 g of skim milk powder (Difco) was mixed with deionized water with a mortar and pestle, the volume was brought to 60 ml, and the preparation was autoclaved at 12 lb/in² for 12 min. The two solutions were cooled to about 50°C and mixed together thoroughly before the final preparation was poured into petri dishes. The final pH was 6.5. The extent of clearing caused by casein hydrolysis was determined daily at 25°C for up to 1 week rather than 3 weeks, which was the time period used by Ahearn et al. (1).

Susceptibility to triterpene glycosides was tested at 25 and 37°C in YM agar plates containing 1% dried, powdered *Stenocereus gummosus* (agria) tissue, as described by Starmer et al. (11).

DNA was extracted, purified, and hybridized by the methods described by Price et al. (10), 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. (5). The 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 (10).

RESULTS

Latin diagnosis of *Candida caseinolytica* sp. nov. In YM (Difco) liquido post dies 5 ad 30°C, cellulæ ovoideæ vel brevi-cylindricæ, 1.3–2.5 × 2.5–5.0 µm, singulæ, binæ vel catenis brevissimæ; sedimentum; pellicula nulla. Cultura in agaro malti post dies 21 ad 25°C cana-cremea, butyrosa vel mollis, glabra, nitida, margine glabro.

In agaro farinae *Zea mays* post dies 10 pseudomycelium nullum.

Fermentatio glucosi nullum. Glucosum, galactosum, sorbosum (lente), trehalosum (lente), xylosum, ethanolum, gluconod-lactonum, gluconatum, 2-ketogluconatum, 5-ketogluconatum, acidum lacticum (lente), acidum succinicum (interdum exigue), ethyl acetat (exiguitate) assimilantur at non maltosum,

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TABLE 1. Geographic origins and host plants of *C. caseinolytica* strains

Strain ^a	G+C content (mol%)	Host plant	Location
UCD-FST 81-434.2	46.9	<i>Opuntia phaeacantha</i>	Santa Rita Mountains, Arizona
UCD-FST 81-467.2	47.4	<i>O. phaeacantha</i>	Rincon Mountains, Arizona
UCD-FST 81-471.1	46.7	<i>O. phaeacantha</i>	Rincon Mountains, Arizona
UCD-FST 81-472.3	46.8	<i>O. phaeacantha</i>	Rincon Mountains, Arizona
UCD-FST 83-416.4		<i>O. phaeacantha</i>	Tucson Mountains, Arizona
UCD-FST 83-426.4		<i>O. phaeacantha</i>	Tucson Mountains, Arizona
W84-201.5		<i>O. phaeacantha</i>	Tucson Mountains, Arizona
UCD-FST 85-833.2		<i>O. phaeacantha</i>	Tucson Mountains, Arizona
UCD-FST 85-833.2		<i>O. phaeacantha</i>	Tucson Mountains, Arizona
G91-628.3		<i>O. phaeacantha</i>	Tucson Mountains, Arizona
G91-706.5		<i>O. phaeacantha</i>	Tucson Mountains, Arizona
UCD-FST 83-438.3 ^T	46.9	<i>O. phaeacantha</i>	Santa Rita Mountains, Arizona
UCD-FST 83-444.3		<i>O. phaeacantha</i>	Along Highway 83, Arizona
UCD-FST 81-494.4	47.3	<i>Opuntia ficus-indica</i>	Tucson, Ariz.
UCD-FST 81-522.2	46.4	<i>O. ficus-indica</i>	Tucson, Ariz.
UCD-FST 81-665.2	46.5	<i>Opuntia lindheimeri</i>	Ozona, Tex. ^b
S83-44		<i>Stenocereus thurberi</i>	Organ Pipe National Monument, Ariz.
S83-56		<i>S. thurberi</i>	Organ Pipe National Monument, Ariz.
S83-64		<i>S. thurberi</i>	Organ Pipe National Monument, Ariz.
S83-102		<i>S. thurberi</i>	Organ Pipe National Monument, Ariz.
S83-114		<i>S. thurberi</i>	Organ Pipe National Monument, Ariz.
G91-702.5		<i>S. thurberi</i>	Organ Pipe National Monument, Ariz.
G91-706.5		<i>S. thurberi</i>	Organ Pipe National Monument, Ariz.
G91-714.5		<i>S. thurberi</i>	Organ Pipe National Monument, Ariz.
S86-455.4		<i>Stenocereus gummosus</i>	Punta Prieta, Baja California Norte, Mexico
S86-457.4		<i>S. gummosus</i>	Punta Prieta, Baja California Norte, Mexico
S86-470.4		<i>S. gummosus</i>	Punta Prieta, Baja California Norte, Mexico
S86-486.4		<i>S. gummosus</i>	Punta Prieta, Baja California Norte, Mexico
S86-460.6		<i>Drosophila mojavensis</i>	Punta Prieta, Baja California Norte, Mexico
G91-613		<i>S. gummosus</i>	Santa Catarina, Baja California Norte, Mexico
G91-617		<i>Myrtillocactus cochal</i>	Santa Catarina, Baja California Norte, Mexico
G91-618.4		<i>M. cochal</i>	Santa Catarina, Baja California Norte, Mexico
G91-625		<i>Opuntia</i> sp.	Santa Catarina, Baja California Norte, Mexico
G91-506.2		<i>Opuntia</i> sp.	Encinada, Baja California Norte, Mexico
G91-510.2		<i>Opuntia</i> sp.	Encinada, Baja California Norte, Mexico
S87-477.3		<i>Opuntia (Nopalea)</i> sp.	Zanatepec, Chiapas, Mexico
W87-2430.3		<i>Opuntia megacantha</i>	Maui Island, Hawaii
W91-445.1		<i>Opuntia boarensis</i>	Santa Rosa, Tucuman, Argentina
UCD-FST 91-476.1		<i>Opuntia quimila</i>	Termas, Tucuman, Argentina

^a UCD-FST, Culture Collection of the Department of Food Science and Technology, University of California, Davis. Designations that begin with G were assigned by P. F. Ganter; designations that begin with S were assigned by W. T. Starmer; and designations that begin with W were assigned by M. A. Lachance.

^b An additional eight undesigned strains were collected from *Opuntia engelmannii* by P. F. Ganter in Texas in the valley of the Rio Grande and the Chisos Mountains.

saccharosum, cellobiosum, lactosum, melibiosum, raffinose, melezitose, inulin, amylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, glycerol, i-erythritol, ribitol, galactitol, D-mannitol, D-glucitol, α-methyl-D-glucoside, salicin, citric acid, meso-inositol, methanol, acetone, 2-propanol, N-acetylglucosamine, glucosamine, and hexadecane.

Potassium nitrate, sodium nitrite are not assimilated. Ethylamine, L-lysine, and cadaverine are assimilated. Growth requires vitamins B₁, B₂, B₆, B₁₂, and biotin. Growth at 42°C, but not at 45°C. G+C acid deoxyribonucleate 46.4–47.4 mol% (8 strains). Habitat in cacti of the tribe Opuntieae and subtribe Stenocereinae.

Type: strain UCD-FST 83-438.3 ex type culture of cacti *Opuntia phaeacantha* isolated. In collection of the Central Bureau for Schimmelfungi, Deph Batavorum sub no. CBS 7781 deposited.

Description of the type strain of *Candida caseinolytica* sp. nov. *Candida caseinolytica* (ca.se.i.no.ly' ti.ca. L. fem. adj. *caseinolytica*, casein hydrolyzing, referring to the pronounced

ability of the organism to hydrolyze casein). In YM (Difco) liquid medium after 5 days at 30°C the cells are ovoidal to short cylindrical (1.3 to 2.5 by 2.5 to 5.0 μm) and occur singly, in pairs, or in very short chains. A small amount of sediment develops after about 10 days; a pellicle or ring is not present. On YM agar the cells are similar, but occur only singly or in pairs; budding occurs mainly on the shoulders of the short cylindrical cells (Fig. 1). Streak cultures on malt agar after 3 weeks at 25°C are cream colored to greyish, convex with little spreading, smooth, glistening, and butyrous to pasty, and the periphery is entire.

In Dalmau plate cultures on cornmeal agar after 10 days, no pseudomycelium or true mycelium is produced.

Ascospore formation does not occur on various sporulation media. Mixing of strains in all possible combinations produces no evidence of a conjugation tube or zygote formation.

Does not ferment glucose.

Assimilates the following carbon compounds: glucose, D-galactose, L-sorbose (latently), trehalose (latently), D-xylose, ethanol, glucono-δ-lactone, gluconate, 2-ketogluconate, 5-ke-

FIG. 1. Vegetative cells of *C. caseinolytica* grown for 3 days on YM agar medium at 25°C. Bar = 10 μ m.

togluconate, DL-lactate (latently), succinate, and ethyl acetate (weakly). Does not assimilate maltose, sucrose, cellobiose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, glycerol, i-erythritol, ribitol, galactitol, D-mannitol, D-glucitol, α -methyl-D-glucoside, salicin, citric acid, meso-inositol, methanol, acetone, 2-propanol, N-acetylglucosamine, glucosamine, and hexadecane.

KNO₃ and NaNO₂ are not utilized as sole sources of nitrogen; ethyl amine, cadaverine, and L-lysine are utilized.

Grows in amino acid-free medium.

Does not grow in vitamin-free medium.

Growth in the presence of triterpene glycosides is moderate at 25°C and good at 37°C.

Grows in the presence of 100 μ g of cycloheximide per ml.

Growth on YM agar containing 5% NaCl is moderate. Does not grow on YM agar containing 7.5% NaCl.

Does not grow in the presence of 50% glucose.

Casein hydrolysis is strongly positive at pH 6.5 to 7.0 (Fig. 2).

Does not hydrolyze gelatin.

Lipolytic activity is weak.

Does not produce urease activity.

Grows at 42 and 45°C.

The Diazonium Blue B reaction is negative.

Habitat: necrotic tissues of various cactus species in various locations (Table 1). The type strain is UCD-FST 83-438.3, which was isolated from *Opuntia phaeacantha* in the Santa Rita Mountains of Arizona; this strain has been deposited in the American Type Culture Collection as strain ATCC 90546 and in the Centraalbureau voor Schimmelcultures, Delft, The Netherlands, as strain CBS 7781.

Some strains differ from the type strain in the following assimilation characteristics: strains UCD-FST 81-444.3 and UCD-FST 91-476.1 do not assimilate D-xylose; strains UCD-FST 81-444.3 and UCD-FST 81-467.2 do not assimilate gluconate and grow weakly on glucono- δ -lactone; and strains UCD-FST 83-416.4 and UCD-FST 83-426.4 grow weakly on gluconate and glucono- δ -lactone. However, all of these strains grow well on 2- and 5-ketogluconates. Assimilation of L-

sorbose varies from latent to negative, and the ability to grow at 42 and 45°C also varies among the strains. A few strains hydrolyze gelatin weakly, and some lack lipolytic activity.

To verify the genetic homogeneity of the *C. caseinolytica*

FIG. 2. *C. caseinolytica* strains and two control cactophilic yeast species on casein test medium after 4 days at 25°C. The inocula were grown on YM agar. The following strains were used (counterclockwise from the arrow in the outer and inner circles): UCD-FST 81-472.3, *Pichia cactophila*, UCD-FST 83-426.4, UCD-FST 83-438.3^T, UCD-FST 81-434.2, UCD-FST 81-467.2, UCD-FST 81-471.1, *Candida sonorensis*, UCD-FST 81-494.4, UCD-FST 81-522.2, UCD-FST 81-665.2, and UCD-FST 85-833.2.

TABLE 2. Reannealing between labeled DNA from *C. caseinolytica* UCD-FST 83-438.3^T and unlabeled DNAs from three yeast strains and unlabeled calf thymus control DNA^a

Source of unlabeled DNA	Relative % of binding with labeled <i>C. caseinolytica</i> UCD-FST 83-438.3 ^T DNA ^b
<i>C. caseinolytica</i> strains	
UCD-FST 83-438.3 ^T	100.0
UCD-FST 81-472.3	97.1
UCD-FST 81-665.2	91.4
<i>Pichia opuntiae</i> UCD-FST 77-40 ^T (control)	0.0
Calf thymus (control)	0.5

^a See Table 1 for the origins of the three *C. caseinolytica* strains. *Pichia opuntiae* UCD-FST 77-40^T was isolated from *Opuntia stricta* in Australia (2).

^b ¹²⁵I-labeled DNA (0.2 µg) and unlabeled DNA (200 µg) were incubated at 65.7°C for 25 h in 0.5 ml of 280 mM phosphate buffer (pH 6.8). The values are the averages of the values from three determinations and were corrected for zero-time binding and for self-renaturation (10).

strains, a DNA hybridization experiment was performed with three strains isolated from three different host plants from different geographic locations. Table 2 shows that the levels of DNA complementarity among these strains were more than 90%.

DISCUSSION

Strong extracellular proteolytic activity is relatively rare in yeasts and is most often but not exclusively observed in basidiomycetous species (1, 9). Historically, gelatin hydrolysis (liquefaction) was the parameter most commonly used in species descriptions, perhaps because of the use of wort gelatin for the description of giant-colony morphology in genetic studies, where a positive reaction resulted in total or partial liquefaction of the medium. More recently, a number of authors have used casein or albumin as a substrate to detect proteolytic activity (1, 9). Several species were found to exhibit significant activity against these proteins, although there was not necessarily a positive correlation between proteolysis of casein and proteolysis of gelatin (1), as is the case for most strains of *C. caseinolytica*. Some reports lack specific information concerning whether proteolytic activity is due to acid, neutral, or alkaline proteases.

Production of extracellular proteolytic enzymes is rarely used for taxonomic purposes. van der Walt and Yarrow (16) stated that since so few yeasts exhibit significant extracellular proteolytic activity against gelatin, only in cases where rapid and complete liquefaction is observed in about 1 week might this property be useful as a confirmatory test.

C. caseinolytica is the only cactus-specific species that exhibits strong extracellular caseinolytic activity (Fig. 1), and it also differs from them and other *Candida* species in morphology and in a number of physiological properties (3, 6, 8).

The pH values of cactus tissue necroses usually are relatively low during the early stages of bacterial invasion (ca. 4.5), but the pH rapidly increases during rot development to values ranging from 7.5 to 8.5, values at which the yeast protease is active. *C. caseinolytica* in developing necroses may gain a competitive advantage by having proteolytic activity.

It is interesting that *C. caseinolytica* appears to be geographically limited; this species has never been isolated in the Caribbean area or Australia (12, 2), even though we have analyzed numerous opuntia rots obtained from these areas. Although *Opuntia* species appear to be the most common host

plants (Table 1), *C. caseinolytica* has also been isolated from members of the columnar cactus genera *Stenocereus* and *Myrtillocactus*, which are members of the subtribe *Stenocereinae* that contain high levels of triterpene glycosides, compounds that are inhibitory to a number of cactus-specific yeast species (11). *C. caseinolytica* is moderately resistant to triterpene glycosides and can therefore grow in necrotic tissues of these cacti. Fogleman and Starmer (4) reported isolating five strains from *Stenocereus thurberi* (organ-pipe cactus) in three locations sympatric with the opuntia host plants of *C. caseinolytica* in Arizona. Ganter isolated three additional strains in 1991 from *S. thurberi* in the same areas, while in 1986 and 1991 Starmer and Ganter recovered five strains from *Stenocereus gummosus* (agria) and two strains from *Myrtillocactus cochal* in Baja California Norte, Mexico. In each of the Mexican locations, *Opuntia* species also yielded *C. caseinolytica*. The remaining question is the primary habitat of the new species. We are inclined to consider *Opuntia* necroses the primary habitat, because numerous samples from organ-pipe and agria cacti in areas where opuntias are rare or nonexistent (15) did not yield this species. Moreover, the fact that one strain of *C. caseinolytica* was isolated in Texas and two strains were isolated from *Opuntia* spp. in Argentina (Table 1), where organ-pipe and agria cacti do not occur, supports this view. In locations where *Opuntia* species occur together with members of the genus *Stenocereus*, the yeast can be transmitted by various insects that feed on both types of rot (14). *Opuntia* spp. are not native to Hawaii, and their introduction from the Americas may explain the isolation of a single strain of *C. caseinolytica* on the island of Maui (Table 1).

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