Torulopsis sonorensis, a New Species of the Genus *Torulopsis*

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A novel member of the yeast genus *Torulopsis* has been recovered 35 times during 1971, 1972, 1973, and 1974 from *Drosophila mojavensis*, from soft-rot pockets from six species of cacti, and from soil wetted by soft-rot fluid. The collections were made in the Sonoran desert of southern Arizona and of northern Mexico. The new species was named *Torulopsis sonorensis* after the geography of its habitat. The type strain is UCD (FS&T) 71-148 (= ATCC 32108 = CBS 6792).

A survey was made in the Sonoran desert areas of Arizona and northern Mexico of yeasts living in soft-rot pockets in the arms of cacti utilized as host plants by various species of *Drosophila*. A new yeast species was isolated on 35 separate occasions. Nineteen isolates were from *Drosophila mojavensis* Patterson and Crow. Fourteen isolates were recovered from cacti, most frequently in organ pipe (six) and less frequently in saguaro (three), agria (two), and barrel, cochal, and cardon (one each). Two isolates were obtained from soils which were wetted by the soft-rot fluid from decaying saguaro. In most instances, the new yeast represented a minor proportion of the total yeast flora. The colonies were selected on the basis of their farinose, semiglossy appearance on the isolation plates. The new species of *Torulopsis* was named *T. sonorensis* after the Sonoran desert, where the isolates were obtained.

MATERIALS AND METHODS

Samples were collected in the vicinity of Tucson, Arizona, in all seasons of the year between 1971 and 1974. Other collections were made in the state of Sonora, Mexico, in the spring and summer of 1971 and 1974 and the summer and fall of 1973. Additional samples were taken from Northern Baja California, Mexico, in the summer of 1974. Approximately 1 g of necrotic tissue was placed in 10 ml of sterile water, returned to the laboratory (within 1 to 2 h), and serially diluted in steps of 10^{-2} . The original suspension and the two subsequent dilutions were plated (0.1 ml) on acidified yeast extract malt-extract agar (Difco YM agar + 0.7% 1 N HCl = YMA, pH 3.7 to 3.8). Dilutions and plating were carried out in the field when necessary. These plates were incubated at 27°C until colonies appeared, at which time counts of distinct morphologic types were made. A representative of each morphologic type was brought into pure culture by two successive platings for identification. If two or more different morphologic types from a particular sample were

identified as belonging to the same species, they were considered a single isolate.

Adult flies were surface sterilized in 70% ethanol (for about 1 min), rinsed in 10 ml of sterile water, and then directly streaked on YMA plates in the field. On occasion, adults were surface sterilized (see above), rinsed in sterile water, and then homogenized with 10 ml of sterile water in a sterile potter homogenizer in the field. The homogenate was returned to the laboratory within 1 to 2 h of homogenization, serially diluted, and plated on YMA. A sample of the rinse water was plated to determine whether surface contamination was carried over after the ethanol sterilization. Both methods appeared satisfactory for detecting internal yeast flora.

Identification was done by standard methods currently employed in yeast taxonomy (9). Deoxyribonucleic acid (DNA) extraction and purification were done by a combination of the procedures of Marmur (5) and Bernardi et al. (3). The moles percent of guanine plus cytosine (mol% G+C) of the DNA was calculated from buoyant density values in cesium chloride (6, 7) and was based on four 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.

RESULTS

Latin diagnosis

Torulopsis sonorensis sp.n.

In extracto malti cellulae ovoideae, (2.4-5.4) $\times$ (2.8-7.0) μ m, singulae, binae, aut in catenis brevis; sedimentum et anulus formantur. Cultura in agaro malti post unum mensem (20°C) cultura crenea, farinacea (paene glabra); butyrosa, subelevata, nitida; margine glabro et undulato.

In agaro farinae Zea mais post dies 10 pseudomycelium nullum.

Fermentatio glucosi et tardissima cellobiosi.

Glucosum, cellobiosum, D-xylosum, L-arabinosum, D-ribosum, ethanolum, methanolum, glycerolum (exiguum), ribitolum, D-mannitolum, D-glucitolum, salicinum, glucono-delta-lactonum (exiguum), acidum lacticum (exiguum), et acidum succinicum assimilantur at non D-galactosum, L-sorboseum, maltosum, saccharum, trehalosum, lactosum, melibiosum, raffinoseum, melezitoseum, inulinum, amyllum, D-arabinosum, L-rhamnosum, i-erythritolum, dulcitolum, alpha-methyl-D-glucosidum, 2-ketogluconatum, 5-ketogluconatum, acidum citricum nec inositolum.

Kalium nitricum non assimilatur.

Amyllum non formatur.

Ad crescentiam vitaminæ externæ necessariae sunt.

Crescere potest in 39 et 12 C.

Mol% G+C: 36.1 ± 0.09 .

Typus: cultura UCD (FS&T) 71-148 ex tabidosus sacculis cacti *Lemaireocereus thurberi* isolata est.

Description

Growth in malt extract. After three days, the cells are spheroidal to elongate (2.4 to 5.4) by (2.8 to 7.0) μm , and occur singly, in pairs, or in short chains and small clusters of four to six cells. Budding occurs on broad shoulders. A pellicle is absent, but a slight ring is usually present, and there is little sediment. After 1 month, there may be small film islets and little sediment. The ring is well developed.

Growth on malt extract agar. After 1 month, the streak culture is cream colored, finely farinose to almost smooth, semiglossy, soft to butyrous, and raised to slight convex; the border is entire or slightly lobed.

Dalmau plate culture on corn meal agar. No pseudomycelium is formed.

Other characteristics. Fermentation: Glucose is fermented moderately (a full tube of gas is obtained in 4 to 7 days) and cellobiose, weakly and latently.

Assimilation of carbon compounds: D-Glucose, +; 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, -; ethanol, +; methanol, +; glycerol, weak; erythritol, -; ribitol, +; galactitol, -; D-mannitol, +; D-glucitol, +; alpha-methyl-D-glucoside, -; salacin, +; glucono- δ -lactone, latent, weak; calcium-2-ketogluconate, -; potassium-5-ketogluconate, -; DL-lactate, weak;

succinate, weak; citrate, -; and meso-inositol, -.

Assimilation of nitrogen compounds: Potassium nitrate, -; ethylamine, +.

Growth in vitamin-free medium: Absent. Biotin is required and thiamine is stimulatory to growth.

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

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

Temperature range of growth: Minimum, < 12 C; maximum, 39 to 40 C.

Acid formation on chalk agar: Very weak.

Synthesis of starch-like compounds: Negative.

Gelatin liquefaction: Negative.

Casein hydrolysis: Negative.

Hydrolysis of urea: Negative.

Lipolytic activity: Negative.

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

Mol% G+C of the DNA: 36.1 ± 0.09 .

Type strain. The type strain, UCD (FS&T) 71-148, was isolated from *Lemaireocereus thurberi* (organ-pipe cactus) at Caborca, Sonora, Mexico. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft, The Netherlands, as CBS 6792 and in the American Type Culture Collection, Rockville, Md., as ATCC 32108.

Sources. Table 1 lists the substrates and localities from which *T. sonorensis* has been recovered. The percentage of the yeast from each rot pocket represents the frequency of its colonies on the isolation plate. *Torulopsis sonorensis* was found primarily in organ pipe cactus. It occurred in 46% (6/13) of the samples of organ pipe and in 19% (10/53) of the samples from all other cacti and soil.

The majority of *Drosophila mojavensis* specimens contained few yeasts, but *T. sonorensis*, when present, was usually the most frequent species. Moreover, in five specimens, *T. sonorensis* was abundant (100 to 1,000 colonies per isolation plate).

DISCUSSION

Torulopsis sonorensis has been isolated from *Drosophila mojavensis*, soft-rots in various species of cactus, and from soils wetted by soft-rot fluids over a wide geographic range of the Sonoran desert (Table 1).

Drosophila mojavensis is the only species of *Drosophila* that regularly utilizes organ pipe and agria cacti as its host plant (4). Saguaro

TABLE 1. *Sources of isolates of Torulopsis sonorensis*

Sources	Localities	% of population	Isolation dates
Saguaro			
<i>Carnegiea gigantea</i> (Engelm.) Britt. & Rose	Tucson, Ariz.	0.9	9/20/72
<i>C. gigantea</i>	Tucson, Ariz.	73.8	2/04/74
<i>C. gigantea</i>	Tucson, Ariz.	96.6	2/04/74
Soils wetted by saguaro	Tucson, Ariz.	3.0	9/20/72
Soils wetted by saguaro	Tucson, Ariz.	8.0	12/16/72
Organ pipe			
<i>Lemaireocereus thurberi</i> (Engelm.) Britt. & Rose	Caborca, Sonora	67.0	4/20/71
<i>L. thurberi</i>	Punta Chueca, Sonora	37.2	11/10/73
<i>L. thurberi</i>	Punta Chueca, Sonora	45.4	11/11/73
<i>L. thurberi</i>	Punta Chueca, Sonora	1.4	11/12/73
<i>L. thurberi</i>	Punta Chueca, Sonora	0.8	11/12/73
<i>L. thurberi</i>	Punta Chueca, Sonora	16.4	11/12/73
Pitaya agria			
<i>Machaerocereus gummosus</i> (Engelm.) Britt. & Rose	Punta Chueca, Sonora	0.1	11/10/73
<i>M. gummosus</i>	Tiburón Island, Sonora	3.2	11/11/73
Cochal			
<i>Myrtillocactus cochal</i> (Orc.) Britt. & Rose	Arroyo Seco, Baja Calif.	22.7	7/24/74
Barrel cactus			
<i>Ferocactus wislizeni</i> (Engelm.) Britt. & Rose	Tucson, Ariz.	9.1	5/07/74
Cardon			
<i>Pachycereus pringlei</i> (S. Wats.) Britt. & Rose	Libertad, Sonora	93.4	4/16/74
Drosophila			
<i>D. mojavensis</i> Patterson & Crow 2/4 adults	Punta Chueca, Sonora	— ^a	11/12/73
<i>D. mojavensis</i> 15/53 adults	El Socorro, Baja Calif.	—	7/24/73

^a —, Not determined.

and cochal are also occasional habitats for this species of *Drosophila*. All of the cacti listed in Table 1, except the barrel cactus, are members of the subtribe *Cereanae* (the giant cacti). Many of these cacti are considered by some authorities to belong in the genus *Cereus*, i.e., *Carnegiea gigantea* (Engelm.) Britt. et Rose [= *Cereus giganteus* Engelm.] (2).

Among the yeasts isolated, *T. sonorensis* was the only species recovered which ferments D-glucose. Its ability to utilize a variety of plant sugars and its tolerance to high temperatures are indicative of the species' adaptation to desert conditions and substrates. However, the log of the cell concentration of this yeast was negatively correlated (-0.613 , $N = 12$) with the temperature of the rot pocket. The average temperature of the substrates was 29°C (range 11 to 41°C). The range of cell concentration was 2×10^3 to 4.6×10^7 cells/ml in the rot pockets.

Torulopsis sonorensis shows no apparent close similarities to any yeast species described

to the present time. When the present key (8) to the species of *Torulopsis* is used, *T. sonorensis* keys to *T. bovina*-*T. pintolopesii*. These species only grow above 22°C, utilize only glucose (and ethanol and lactate weakly), require five vitamins for growth, and are typically associated with warm-blooded animals. When the key of Barnett and Pankhurst (1) is used, the new species keys to *Pichia sargentensis*, a species described in 1971 from the effluent of a small lake in New Hampshire. The single isolate representing this species, excluding its ability to produce ascospores, differs in its ability to produce pseudomycelium and to assimilate L-arabinose, D-ribose, L-rhamnose, and ribitol.

The mol% G+C of 36.1 (a second strain, 72-314, was found to have 36.00 ± 0.13 mol% G+C) is indicative of the ascomycetous origin of *T. sonorensis* although all attempts to induce sporulation, including mixing of strains, have failed. Based on a comparison of known *Torulopsis* species to the isolates described herein, a

new species of the genus *Torulopsis*, possessing properties sufficiently different to be recognized as unique within the genus, is validated.

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REPRINT REQUESTS

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