Pichia cactophila, a New Species of Yeast Found in Decaying Tissue of Cacti

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A novel representative of the yeast genus *Pichia* has been recovered 190 times during the period 1971 to 1976. We regard this organism as belonging to a new species, *Pichia cactophila*. Strains were found in the necrotic tissue of 16 species of cactus and in the crops of 3 species of *Drosophila* which utilize the cacti as host plants. Isolates were obtained from widely separate geographic localities (throughout Mexico, Southwestern United States, and Hawaii). The new species forms predominantly two-spored asci. Both homothallic and heterothallic strains were observed. The metabolic capability of *P. cactophila*, like that of *P. membranaefaciens*, is limited to oxidative utilization of only a few compounds. *P. cactophila* can be differentiated from *P. membranaefaciens* by its strong growth on D-glucosamine and by the lower guanine-plus-cytosine content (36.3 to 37.5 mol%) of its nuclear deoxyribonucleic acid. The type strain of *P. cactophila* is UCD-FST 76-243A (= ATCC 34932 = CBS 6926).

During a recent survey of yeasts associated with necroses of cacti of the North American Sonoran Desert (4, 14), we consistently isolated yeasts that were identified (5) as *Pichia membranaefaciens* Hansen. Subsequently we extended the range of our survey of the Sonoran Desert to include most of the peninsula of Baja California, Mexico. The resulting increase in the number of yeast isolates (ca. 500) added a large number of additional strains at first considered to belong to *P. membranaefaciens*. However, slight but consistent differences in the assimilation pattern of carbohydrates, correlated with host-plant specificity and determinations of the nuclear deoxyribonucleic acid (DNA) base compositions of representative strains, revealed that the isolates represented several homogeneous groups distinct from *P. membranaefaciens*. The guanine-plus-cytosine (G+C) contents of the DNAs of the different groups were found to be 8 to 16 percentage points lower than that of *P. membranaefaciens*. By the currently used identification procedures, however, these groups were not sufficiently distinct from the standard description of *P. membranaefaciens* to be considered separate species. Nevertheless, the intra-group homogeneity with respect to nuclear DNA base composition was suggestive of a new species. It was found that the new species, to be described in this paper, can be differentiated from *P. membranaefaciens* by its strong growth on D-glucosamine, which compound is not or is only weakly assimilated by the latter species.

We have named the new species *P. cactophila* (cac.to'phi.la. M. L. mas. n. *Cactus* generic name of cactus; Gr. adj. *philus* loving; M. L. adj. *cactophilus* cactus-loving) on the basis of its frequent recovery from many species of cacti found in widely separated geographic areas. The other yeasts that we have recognized as new species will be described in subsequent reports.

MATERIALS AND METHODS

Samples of necrotic cactus tissue were collected in various areas of the North American Sonoran Desert (Fig. 1). A few samples were obtained from cacti in the Oaxaca area of Southern Mexico, and a single isolate came from a cactus on the island of Hawaii (Table 1). Most of the isolations were done by streaking a loopful of necrotic tissue directly on acidified yeast extract-malt extract agar (AYM; Difco YM agar plus 0.7% [vol/vol] 1 N HCl, pH 3.7 to 3.8) in the field. In some cases dilutions of the tissue were made in sterile water as described earlier (14). The plates were stored at ambient temperatures (24 to 30°C) until colonies appeared. Counts of morphologically distinct colony types were then made, and a representative of each type was brought into pure culture by two successive platings on YM agar for identification. On the isolation medium, colonies of the new species were recognized with a fair degree of confidence, for they were flat, rather spreading, semiglossy to dull, and sometimes possessed a textured surface or a finely ciliate border.

Some isolates were obtained from adult *drosophilae* captured near cacti with necrotic tissue. The flies were surface-sterilized in 70% ethanol (for about 1 min), rinsed in 10 ml of sterile water, and then squashed and streaked directly on AYM agar plates in the field.

Identification was done by standard methods currently used in yeast taxonomy (16). DNA extraction and purification were done by a combination of the

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FIG. 1. Collection localities for isolates of *Pichia cactophila* listed in Table 1.

procedures of Marmur (7) and Bernardi et al. (1). The G+C content (moles percent) of the DNA was calculated from buoyant density values in cesium chloride (11, 15) and was based on two to three 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.

Single ascospores were isolated from asci with a micromanipulator (3) after treatment of the sporulating suspension of cells and asci with the cell wall-digesting enzyme zymolyase.

RESULTS

Latin diagnosis of the new species: *Pichia cactophila* sp. nov. In extracto multi cellulae ovoideae, $(1.8-5.7) \times (2.9-9.0) \mu\text{m}$, singulae, binae, aut in catenis brevis; sedimentum, annulus et pellicula crispulata non-nitida formantur. Cultura in agaro malti post unum mensem (20°C) crenea, glabra, semi-nitida, mollis; margine glabro, undulato, interdum vel pseudomycelium rudimentum. In agaro farinae Zea mais post dies 10, pseudomycelium nullum aut rudimentum.

Species homothallica aut heterothallica, diploidea. Asci inconjugati fiunt, habentes 2 sporos pileiformos (raro 4) in quoque asco; asci rumpuntur.

Fermentatio glucosi nulla aut exigua. Glucosum, glucosaminum, ethanolum, glycerolum, acidum lacticum (variabile), acidum succinicum, et acidum citricum (raro nullum) assimilantur at non galactosum, L-sorbosum, maltosum, saccharum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitosum, inulinum, amylum solubile, D-xylosum, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, methanolum, erythritolum, ribitolum, galactitolum, mannitolum, glucitolum, α -methyl-D-glucosidum, salicinum, glucono- δ -lactonum, 2- et 5-ke-togluconatum nec inositolum.

Kalium nitricum et kalium nitrosum non assimilantur.

Ethyl aminum assimilatur.

Ad crescentiam thiaminum et pyridoxinum necessariae sunt.

Crescere potest in $40-45^\circ\text{C}$.

Mol% G+C = 36.9, $\sigma^2 = 0.148$.

Typus: Stirps UCD (FS&T) 76-243A ex tabidosis sacculis cacti *Lemaireocereus thurberi* Rancho San Martin, Baja Californiensis isolata est.

Description of *P. cactophila*. Growth in malt extract: After 3 days the cells are ovoid to slightly elongate and measure 1.8 to 5.7×2.9 to $9.0 \mu\text{m}$. They occur singly, in pairs, and in short chains or small clusters of four to seven cells. Budding occurs on broad shoulders or multilaterally. A finely wrinkled, dull, ascending pellicle is formed, and there is a small amount of sediment.

Growth on malt extract agar: After 3 weeks the streak culture is cream colored, the surface is smooth and dull to semiglossy, the texture is soft, the cross section is flat, and the border is slightly lobed or entire, sometimes showing rudimentary development of pseudomycelium.

Dalmau plate culture on cornmeal agar: Pseudomycelium formation is absent or rudimentary.

Formation of ascospores: The cells are diploid, and most strains produce two hat-shaped spores which are liberated from the ascus at maturity. In rare cases four-spored asci were observed among two-spored asci (Fig. 2). The two-spored strain 17 is homothallic since isolated single spores developed into ascospore-forming clones. A few strains produced predominantly four-spored asci, which lyse upon maturity. Strain 118 was shown to be heterothallic by single-spore isolation from four-spored asci. Sporulation is best on YM agar after 5 to 7 days at room temperature.

Fermentation of glucose: Most strains do not produce gas. Others may produce a bubble at 9 days, which may increase to 20 to 40% of the inverted vial in 21 days.

Assimilation of carbon compounds: Glucose, glucosamine, ethanol, glycerol (latent), DL-lactic

TABLE 1. *Localities and sources of isolates of Pichia cactophila*

Serial no. of strain	UCD-FST ^a strain designation	Locality ^b	Habitat/substrate
1, 2, 3, 4, 5	76-204A, 76-205, 76-213, 76-215, 76-217A	Rancho Cuñaño, Baja Calif. Sur, Mexico (a)	<i>Machaerocereus gummosus</i>
6	76-207A	Rancho Cuñaño, Baja Calif. Sur, Mexico (a)	<i>Lophocereus schottii</i>
7, 8	76-224A, 76-225	Punta Pescadero, Baja Calif. Sur, Mexico (b)	<i>M. gummosus</i>
9, 10	76-228B, 76-244B	San Pedro, Baja Calif. Sur, Mexico (c)	<i>Pachycereus pringlei</i>
11, 12, 13, 14, 15, 16	76-230B, 76-232B, 76-233B, 76-236B, 76-238B, 76-241B	Todos Santos, Baja Calif. Sur, Mexico (d)	<i>M. gummosus</i>
17	76-243A (type)	Rancho San Martin, Baja Calif. Sur, Mexico (e)	<i>Lemaireocereus thurberi</i>
18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29	76-246, 76-248B, 76-249, 76-251, 76-252, 76-254, 76-255, 76-257A, 76-258, 76-259A, 76-260A, 76-261A	Isla San Jose, Baja Calif. Sur, Mexico (f)	<i>M. gummosus</i>
30, 31, 32	76-265A, 76-266B, 76-267B	Mission San Luis Gonzaga, Baja Calif. Sur, Mexico (g)	<i>M. gummosus</i>
33	76-268B	Mission San Luis Gonzaga, Baja Calif. Sur, Mexico (g)	<i>L. thurberi</i>
34, 35, 36	76-274A, 76-275B, 76-278B	La Paz, Baja Calif. Sur, Mexico (h)	<i>M. gummosus</i>
37	76-276A	La Paz, Baja Calif. Sur, Mexico (h)	<i>L. thurberi</i>
38, 39, 40, 41	76-284B, 76-292A, 76-293B, 76-294	El Centenario, Baja Calif. Sur, Mexico (i)	<i>M. gummosus</i>
42, 43, 44, 45, 46	76-298B, 76-299B, 76-300B, 76-301, 76-304A	Santiago, Baja Calif. Sur, Mexico (j)	<i>M. gummosus</i>
47	76-302	Santiago, Baja Calif. Sur, Mexico (j)	<i>P. pringlei</i>
48	76-303B	Santiago, Baja Calif. Sur, Mexico (j)	<i>L. thurberi</i>
49	76-309A	Palmilla, Baja Calif. Sur, Mexico (k)	<i>M. gummosus</i>
50, 51, 52	76-318B, 76-319B, 76-321A	Loreto, Baja Calif. Sur, Mexico (l)	<i>M. gummosus</i>
53	76-322A	Rancho Santa Rosa, Baja Calif. Sur, Mexico (m)	<i>L. thurberi</i>
54	76-323B	Rancho Santa Rosa, Baja Calif. Sur, Mexico (m)	<i>M. gummosus</i>
55, 56	76-324A, 76-325A	Rancho Rosarito, Baja Calif. Sur, Mexico (n)	<i>P. pringlei</i>
57, 58, 59	76-329B, 76-331A, 76-332	Mulegé, Baja Calif. Sur, Mexico (o)	<i>M. gummosus</i>
60, 61	76-333A, 76-334A	Mulegé, Baja Calif. Sur, Mexico (o)	<i>P. pringlei</i>
62	76-338B	Sierra de San Francisco, Baja Calif. Sur, Mexico (p)	<i>Opuntia</i> sp.
63, 64	76-342, 76-343B	San Borja, Baja Calif. Norte, Mexico (q)	<i>M. gummosus</i>
65	76-345	San Borja, Baja Calif. Norte, Mexico (q)	<i>L. schottii</i>
66	76-353A	Las Flores, Bahia de Los Angeles, Baja Calif. Norte, Mexico (r)	<i>M. gummosus</i>
67, 68	76-358, 76-359	Murlegé, Baja Calif. Sur, Mexico (s)	<i>M. gummosus</i>
69, 70	76-366A, 76-367B	San Jose de Castro, Baja Calif. Sur, Mexico (t)	<i>M. gummosus</i>
71, 72, 73	76-369B, 76-370C, 76-371B	San Ignacio, Baja Calif. Sur, Mexico (u)	<i>M. gummosus</i>

TABLE 1—*Continued*

Serial no. of strain	UCD-FST ^a strain designation	Locality ^b	Habitat/substrate
74, 75, 76, 77, 78, 79	76-373, 76-374A, 76-375B, 76-377A, 76-384B, 76- 385A	Mulegé, Baja Calif. Sur, Mexico (v)	<i>P. pringlei</i>
80	76-379A	Mulegé, Baja Calif. Sur, Mexico (v)	<i>L. schottii</i>
81, 82	76-380A, 76-381B	Mulegé, Baja Calif. Sur, Mexico (v)	<i>M. gummosus</i>
83	76-387A	Bahia San Nicolás, Baja Calif. Sur, Mexico (w)	<i>L. schottii</i>
84	76-388A	Bahia San Nicolás, Baja Calif. Sur, Mexico (w)	<i>M. gummosus</i>
85	76-389A	Isla del Carmen, Baja Calif. Sur, Mexico (x)	<i>L. schottii</i>
86, 87	77-455, 77-456	El Socorro, Baja Calif. Norte, Mexico (y)	<i>M. gummosus</i>
88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103	77-355A, 77-357B, 77-359A, 77-368D, 77-371C, 77- 376A, 77-378A, 77-379A, 77-377A, 77-387A, 77- 389A, 77-391A, 77-393A, 77-396A, 77-400A, 77- 404A	El Socorro, Baja Calif. Norte, Mexico (y)	<i>Drosophila moja-</i> <i>vensis</i>
104	71-157	Alamos, Sonora, Mexico (z)	<i>Rathbunia ala-</i> <i>mosenis</i>
105, 106, 107, 108, 109	71-158, 77-432, 77-438, 77- 439, 77-440	Navojoa, Sonora, Mexico (aa)	<i>R. alamosensis</i>
110, 111, 112, 113, 114, 115	77-428, 77-430, 77-431, 77- 434, 77-435, 77-436	Navojoa, Sonora, Mexico (aa)	<i>Opuntia</i> sp.
116, 117	71-159, 77-441	Navojoa, Sonora, Mexico (aa)	<i>L. thurberi</i>
118	77-427	Navojoa, Sonora, Mexico (aa)	<i>Pachycereus pec-</i> <i>ten-aboriginum</i>
119, 120, 121	76-392A, 76-397A, 76-399A	Guaymas, Sonora, Mexico (ab)	<i>L. thurberi</i>
122, 123, 124, 125, 126, 127	76-401A, 76-402A, 76-403A, 76-404A, 76-405A, 76- 406A	Guaymas, Sonora, Mexico (ab)	<i>R. alamosensis</i>
128	76-410C	Guaymas, Sonora, Mexico (ab)	<i>P. pringlei</i>
129	71-151	Punta Chueca, Sonora, Mexico (ac)	<i>M. gummosus</i>
130	76-500	Libertad, Sonora, Mexico (ad)	<i>P. pringlei</i>
131	76-502	Libertad, Sonora, Mexico (ad)	<i>L. schottii</i>
132	71-147	Caborca, Sonora, Mexico (ae)	<i>L. thurberi</i>
133, 134, 135, 136, 37, 138	71-154, 72-308, 73-100, 73- 51, 73-57, 77-459B	Tucson, Ariz. (af)	<i>Carnegiae gigan-</i> <i>tea</i>
139, 140, 141, 142	72-304, 72-324, 73-105, 77- 461C	Tucson, Ariz. (af)	<i>C. gigantea</i> (soil)
143	76-504	Tucson, Ariz. (af)	<i>Ferocactus wisli-</i> <i>zeni</i>
144, 145, 146, 147, 148, 149	77-462A, 77-463, 77-465A, 77-470A, 77-471, 77-478A	Tucson, Ariz. (af)	<i>Drosophila ni-</i> <i>grospiracula</i> fe- males
150, 151, 152, 153, 154, 155	77-472, 77-473, 77-479, 77- 484, 77-487, 77-488	Tucson, Ariz. (af)	<i>D. nigrospiracula</i> males
156, 157, 158, 159, 160	77-466A, 77-467, 77-468, 77- 496, 77-499	Tucson, Ariz. (af)	<i>Drosophila</i> spe- cies "M" fe- males
161, 162, 163, 164, 165, 166, 167, 168, 169, 170	77-464, 77-474, 77-483B, 77- 485, 77-490, 77-491, 77- 494D, 77-495, 77-498, 77- 500	Tucson, Ariz. (af)	<i>Drosophila</i> spe- cies "M" males
171	77-312B	Apatzingan, Michoacán, Mexico	<i>Backebergia mili-</i> <i>taris</i>

TABLE 1—*Continued*

Serial no. of strain	UCD-FST ^a strain designation	Locality ^b	Habitat/substrate
172	77-314D	Tlapa, Guerrero, Mexico	<i>Lemaireocereus stellatus</i>
173, 174, 175	77-315A, 77-316A, 77-320A	Mitla, Oaxaca, Mexico	<i>Lemaireocereus pruinus</i>
176, 177, 178, 179	77-317A, 77-318A, 77-323A, 77-325B	Mitla, Oaxaca, Mexico	<i>Lemaireocereus treleasei</i>
180, 181	77-324D, 77-326B	Mitla, Oaxaca, Mexico	<i>Myrtillocactus</i> sp.
182, 183, 184	77-327B, 77-328A, 77-328B	Totolapan, Oaxaca, Mexico	<i>L. pruinus</i>
185	77-329A	Totolapan, Oaxaca, Mexico	<i>Lemaireocereus dumortieri</i>
186	77-331A	Ixtepec, Oaxaca, Mexico	<i>P. pecten-aboriginum</i>
187, 188, 189	77-332A, 77-333A, 77-334A	Ixtepec, Oaxaca, Mexico	<i>L. pruinus</i>
190	76-900B	Saddle Road, Island of Hawaii	<i>Opuntia megacantha</i>

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

^b The letters in parentheses correspond to the geographic locations shown in Fig. 1.

acid (latent or weak or negative), succinic acid, and citric acid (rarely negative) are assimilated. The following compounds are not assimilated by any of the strains: 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, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, α -methyl-D-glucoside, salicin, glucono- δ -lactone, gluconic acid, 2-keto- and 5-ketogluconic acid, and meso-inositol.

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

Growth in vitamin-free medium: Negative. Thiamine and pyridoxine are essential for growth.

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

Growth on 10% NaCl plus 5% glucose in yeast autolysate agar: Variable. Some strains show no growth; others grow moderately.

Maximum temperature for growth: Nearly all strains grow well at 42°C. Some grow moderately at 45°C.

Acid formation on chalk agar: Weak to moderate or absent.

Hydrolysis of urea: Negative.

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: 36.9 mol%, $\sigma^2 = 0.148$ (based on 9 strains [Table 2]).

Habitat: One hundred ninety strains were isolated during the period 1971 to 1976 from a wide variety of cactus species and over a wide geo-

graphic area (see Fig. 1 and Tables 1 and 3).

Type: The type strain, UCD-FS&T 76-243A, isolated from necrotic tissue of *Lemaireocereus thurberi* (organ pipe cactus) near Rancho San Martin, Baja California Sur, Mexico, has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft, The Netherlands, as CBS 6926 and in the American Type Culture Collection, Rockville, Md., as ATCC 34932.

DISCUSSION

Among the new *Pichia* species that we have recognized in cactus rots, *P. cactophila* shows the widest distribution, both in terms of geography and host-plant specificity (Table 3). It was isolated from all species of cactus throughout the entire North American Sonoran Desert. Some isolates were recovered from cacti or drosophilae outside the Sonoran Desert, namely, strain 190 from *Opuntia megacantha* on the island of Hawaii and strains 171–189 from various cacti growing in southern Mexico; one strain (Table 2) had been isolated in 1950 from the crop of *Drosophila pseudoobscura* Frolova at Piñon Flat (southern California) by Shehata (A. M. El-Tabey Shehata, Ph.D. thesis, University of California, Berkeley, 1950). This last strain was initially considered as belonging to a new species of *Pichia* by Shehata, but it was later regarded as a strain of *P. membranaefaciens* (12).

The metabolic capability of *P. cactophila*, like that of *P. membranaefaciens*, is limited to the oxidative utilization of only a few compounds (see standard description of *P. cactophila*). The only phenotypic property that distinguishes the two species is the strong growth exhibited by *P. cactophila* on D-glucosamine, a compound not

FIG. 2. Intact asci of *Pichia cactophila* strain 17 (a) and the four-spored strain 118 (b) have thin walls. As the thin ascus wall lyses (c), the brim of the spore becomes more evident. In the four-spored strain, the dehiscent ascospores appear to remain physically associated (d and f) and form clumps. Each spore contains a highly refractile body (strain 94 [e]). The sporulation cycle is completed within 24 h or less. Bars represent 2 μ m in all cases.

TABLE 2. Nuclear DNA base compositions of representative strains of *P. cactophila*

Strain	Locality	Substrate	G+C content (mol% $\pm$ SD) ^a of nuclear DNA	Predominant no. of spores/ascus
17 (type)	Rancho San Martin, Baja Calif., Mexico	<i>Lemaireocereus thurberi</i>	36.3 $\pm$ 0.27	2
52	Loreto, Baja Calif., Mexico	<i>Machaerocereus gummosus</i>	36.5 $\pm$ 0.14	2
69	San Jose de Castro, Baja Calif., Mexico	<i>M. gummosus</i>	36.7 $\pm$ 0.13	2
118	Navojoa, Sonora, Mexico	<i>Pachycereus pecten-aboriginum</i>	37.1 $\pm$ 0.05	4
171	Apatzingan, Michoacán, Mexico	<i>Backebergia militaris</i>	36.9 $\pm$ 0.11	4
175	Mitla, Oaxaca, Mexico	<i>Lemaireocereus pruinosus</i>	37.3 $\pm$ 0.07	2
182	Totolapan, Oaxaca, Mexico	<i>L. pruinosus</i>	37.5 $\pm$ 0.45	4
190	Island of Hawaii	<i>Opuntia megacantha</i>	37.1 $\pm$ 0.23	2
50-44 ^b	Piñon Flat, Calif.	<i>Drosophila pseudoobscura</i> Frolowa	37.1 $\pm$ 0.36	2

^a G+C values for strains 17 and 52 were based on three separate determinations; two separate determinations were utilized for all other strains. SD, Standard deviation.

^b Isolated by El-Tabey Shehata in 1950.

TABLE 3. Sources and frequencies of isolation of strains of *Pichia cactophila*

Source	Frequency of isolation
<i>Machaerocereus gummosus</i> Engelm. [Agria] ^a	64 ^b
<i>Lemaireocereus thurberi</i> Engelm. [Organpipe]	11
<i>Lemaireocereus pruinosus</i> (Otto) Britton and Rose	9
<i>Lemaireocereus treleasei</i> Britt. and Rose	4
<i>Lemaireocereus stellatus</i> (Pfeiffer) Britt. and Rose	1
<i>Lemaireocereus dumortieri</i> (Scheidweiler) Britt. and Rose	1
<i>Pachycereus pringlei</i> (S. Wats.) Britt. and Rose [Cardon]	15
<i>Pachycereus pecten-aboriginum</i> (Engelm.) Britt. and Rose [Hecho]	2
<i>Carnegiea gigantea</i> Engelm. [Saguaro]	6
<i>C. gigantea</i> soil	4
<i>Lophocereus schottii</i> Engelm. [Senita]	6
<i>Rathbunia alamosensis</i> (Coulter) Britt. and Rose [Cina]	12
<i>Ferocactus wislizeni</i> (Engelm.) Britt. and Rose [Barrel]	1
<i>Myrtillo cactus</i> sp.	2
<i>Backebergia militaris</i> (Audot) Bravo and Sanchez-Mejorada	1
<i>Opuntia megacantha</i> Salm-Dyck	1
<i>Opuntia</i> sp.	7
<i>Drosophila nigrospiracula</i> Patterson and Wheeler	12
<i>Drosophila mojavensis</i> Patterson and Crow	16
<i>Drosophila</i> species "M"	15

^a Common plant names are in brackets.

^b Number of times *P. cactophila* was isolated from source.

routinely employed by most yeast taxonomists. This limited set of utilizable compounds suggests that *P. cactophila* probably invades the decaying cactus tissue after active fermentation by other microorganisms has begun. Species in the bacterial genus *Erwinia* are thought to initiate the necrosis and render the succulent tissue vulnerable to saprophytic yeasts (6). Presumably adult drosophilae serve as vectors for dispersal of *P. cactophila* (as well as other yeast species) since the yeast is readily recovered from the adult crops (Table 1). The yeast-laden cactus tissue is then utilized by cactophilic drosophilae for feeding and breeding. The adaptation of *P. cactophila* to desert habitats is reflected in its relatively high maximum temperature for growth (40 to 45°C).

P. cactophila is distinctly different from *P. membranaefaciens* in DNA base composition: the former shows a range of 36.3 to 37.5 mol% and the latter has a range of 42 to 44 mol% G+C (8, 9). In addition to its ability to utilize D-glucosamine, the cactus habitat of *P. cactophila* is helpful in separating the physiologically similar *P. membranaefaciens*. Although *P. cactophila* had been regarded as a strain of *P. membranaefaciens* (4, 14), the present taxonomic study indicates that *P. membranaefaciens* is not associated with necrotic cactus tissue. A parallel ecological situation exists for certain isolates of *P. membranaefaciens*. This species (also referred to as *P. silvestris*, later regarded as synonymous with *P. membranaefaciens* [5]) is known to occur in slime fluxes of temperate trees (10), and it has been isolated from tree fluxes in which immature stages of drosophilae were found (2, 4). The similarity in ecology and phys-

iology of *P. membranaefaciens* and *P. cactophila* suggests that they are closely related, but the wide differences in their DNA base compositions indicates the possibility of evolutionary convergence. This in large part depends on the proximal causes of DNA changes before and after yeast "speciation." A better understanding of the relationship of the two taxa might be established through comparative studies of yeasts found in tree fluxes of desert and tropical regions, especially in localities where trees and cacti are known to have been sympatric for some time. A comparison of molecular changes that could establish evolutionary distance (e.g., micro-complement fixation and nucleic acid base sequence comparison) would also be helpful in elucidating the evolutionary relationships of *Pichia* species inhabiting tree fluxes and cactus rots. Phylogenetic considerations remain speculative, but ecological knowledge combined with modern biochemical techniques could provide promising data for future studies in yeast phylogeny. A comparison of G+C values for strains of *P. cactophila* isolated from the Sonoran Desert (strains 14, 52, 69, and 118) with those found outside this region (strains 171, 175, 182, 190, and 50-44) shows that the two distinct geographic groups exhibit significantly different means ($t = 2.53$, $\alpha = 0.05$). The mean G+C value of strains outside of the Sonoran Desert is higher than the mean of strains within the desert by 0.51 mol%. We are continuing to explore the evolutionary significance of this difference. The large number of isolates and the widely separated geographic localities of isolation (Table 1 and Fig. 1) provide valuable material for intra-specific population studies. Very few eucaryotic microorganisms have been investigated for allozyme variation (see Spieth [13] for an analysis of *Neurospora intermedia*). Although most strains of *P. cactophila* are assumed to be homothallic, a study of conspecific populations for variability of isozymes and inferred allozyme variation is likely to be rewarding.

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