Pichia heedii, a New Species of Yeast Indigenous to Necrotic Cacti in the North American Sonoran Desert

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A novel member of the yeast genus *Pichia* was recovered 67 times during the period 1971 to 1976. We have named this new species *Pichia heedii* in honor of William B. Heed. Most strains were isolated from the soft-rot of *Lophocereus schottii* and from *Drosophila pachea*, which utilizes *L. schottii* as a host plant. All strains were found in the Sonoran Desert. The species has four-spored asci and is heterothallic. *P. heedii* has metabolic capabilities similar to those of *P. membranaefaciens*, but the base composition of its nuclear deoxyribonucleic acid is 10 to 12 mol% lower than that of *P. membranaefaciens*. *P. heedii* may be differentiated from other cactus-specific *Pichia* species by its ability to assimilate D-xylose and from *P. membranaefaciens* by its cactus habitat, small cell size, and relatively high maximum temperature of growth. The type strain of *P. heedii* is UCD-FST 76-356 (= ATCC 34936 = CBS 6930).

The niche specificity of yeast species has become an important and interesting attribute of species delimitation (14). This is also true for yeasts that we have been collecting from soft-rot pockets of cereoid cacti. In particular, we observed that isolates of *Pichia membranaefaciens* Hansen that were capable of assimilating the pentose sugar D-xylose were recovered mainly from the necrotic tissues of *Lophocereus schottii* (Engelmann) Britton and Rose (4, 11). Since the assimilation of D-xylose is variable for *P. membranaefaciens* (6), we did not, at the time these publications were prepared, consider the ecological affinity in conjunction with the oxidative utilization of D-xylose a sufficient justification for the description of a new species. However, when we subsequently studied the guanine-plus-cytosine (G+C) contents of the nuclear deoxyribonucleic acids (DNA) of the cactus isolates, they were shown to be 10 to 12 mol% lower than those of isolates of *P. membranaefaciens* (including the type strain and several xylose-positive strains) found outside our study area (8, 9). This observation, coupled with the ecological specificity of our D-xylose-utilizing strains, is convincing evidence for the establishment of a novel species of the genus *Pichia*. We have named this highly specialized yeast *Pichia heedii* in honor of William B. Heed, who has been inspirational in the study of desert-adapted yeasts. To him we are greatly indebted for his leadership and wisdom.

MATERIALS AND METHODS

Samples of necrotic cactus tissue were collected in

various areas of the North American Sonoran Desert (Table 1 and Fig. 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 (11). 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 *P. heedii* were recognized with a fair degree of confidence for they were convex, glossy to semiglossy, and usually had a textured or irregular surface and rarely a 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 (13). DNA extraction and purification were done by a combination of the procedures of Marmur (7) and Bernardi et al. (1). The G+C content of the DNA was calculated from buoyant density values in cesium chloride (10, 12) 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 (2) 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*

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

Serial no. of strain	UCD-FST ^a strain designation	Locality ^b	Habitat/substrate
1, 2	71-162, 72-124	Navojoa, Sonora, Mexico (a)	<i>Lophocereus schottii</i>
3, 4, 5, 6	76-393A, 76-395, 76-400A, 76-408B	Guaymas, Sonora, Mexico (b)	<i>L. schottii</i>
7	71-166	Empalme, Sonora, Mexico (c)	<i>L. schottii</i>
8	72-100	San Agustia, Sonora, Mexico (d)	<i>L. schottii</i>
9	72-103	El Desemboque, Sonora, Mexico (e)	<i>L. schottii</i>
10	76-501	Libertad, Sonora, Mexico (f)	<i>L. schottii</i>
11	72-108	Caborca, Sonora, Mexico (g)	<i>L. schottii</i>
12	71-171	Santa Ana, Sonora, Mexico (h)	<i>L. schottii</i>
13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27	76-506, 76-507, 76-508, 76-509, 76-510, 76-511, 76-512, 76-513, 76-514, 76-515, 76-516, 76-517, 76-518, 76-519, 76-520	Santa Ana, Sonora, Mexico (h)	<i>Drosophila pa- chea</i>
28, 29, 30, 31, 32, 33	72-114, 73-54, 73-58, 73-66, 73-101, 76-503	Tucson, Ariz. (i)	<i>Carnegiea gigan- tea</i>
34, 35, 36	72-306, 72-325, 77-461B	Tucson, Ariz. (i)	<i>C. gigantea</i> (soil) ^c
37, 38, 39, 40	77-465B, 77-470B, 77-480, 77-486	Tucson, Ariz. (i)	<i>Drosophila ni- grosipracula</i>
41, 42, 43	77-493A, 77-484A, 77-497	Tucson, Ariz. (i)	<i>Drosophila</i> sp. "M"
44	76-207B	Rancho Cuñaño, Baja Calif. Sur, Mexico (j)	<i>L. schottii</i>
45	76-269	Mission San Luis Gonzaga, Baja Calif. Sur, Mexico (k)	<i>L. schottii</i>
46, 47, 48, 49	76-280C, 76-281A, 76-282, 76-283	La Paz, Baja Calif. Sur, Mexico (l)	<i>L. schottii</i>
50, 51	76-289, 76-290	North of La Paz, Baja Calif. Sur, Mexico (m)	<i>L. schottii</i>
52	76-295	North of La Paz, Baja Calif. Sur, Mexico (m)	<i>Machaerocereus gummosus</i>
53, 54, 55	76-311, 76-312, 76-313	East of Insurgentes, Baja Calif. Sur, Mexico (n)	<i>L. schottii</i>
56, 57, 58, 59	76-326A, 76-327A, 76-328A, 76-335	Mulegé, Baja Calif. Sur, Mexico (o)	<i>L. schottii</i>
60, 61, 62	76-349, 76-350A, 76-351B	Las Flores, Bahia de Los Angeles, Baja Calif. Norte, Mexico (p)	<i>L. schottii</i>
63, 64, 65	76-356(Type), 76-360, 76-363	Bahia de Concepción, Mulegé, Baja Calif. Sur, Mexico (q)	<i>L. schottii</i>
66	76-368A	San José de Castro, Baja Calif. Sur, Mexico (r)	<i>L. schottii</i>
67	76-378A	Mulegé, Baja Calif. Sur, Mexico (o)	<i>Pachycereus prin- glei</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.^c Soil wetted by soft-rot drippings from *C. gigantea*.

heedii sp. nov. In extracto malti cellulae ovoideae, (1.8–3.5) × (3.0–7.0) μm, singulae, binae, aut in catenis brevis; anulus et pellicula tenuis, non-nitida aut insulae formantur. Cultura in agar malti post unum mensem (20°C) crenea, glabra, mollis, plana, prope nitida; margine glabro.

In agar farinae Zea mais post dies 12, pseudomycelium nullum aut rudimentum. Species heterothallica, diploidea. Asci inconjugati fiunt. Ascosporae pileiformae ad 4 in quoqueasco; asci rumpuntur.

Fermentatio glucosi nullum. Glucosum, D-xylosum, ethanolum, glycerolum (lente aut exigue), acidum lacticum (interdum nullum), acidum succinicum, et acidum citricum (interdum nullum) assimilantur at non-D-galactosum, L-sorbosum, maltosum, saccharum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitosum, inulinum, amyllum, L- et D-arabinosum, D-ribosum, L-rhamnosum, methanolum, erythritolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, salicinum, 2- et 5-ketogluconatum, glucono-δ-

FIG. 1. Geographic locations of isolates of *P. heedii* listed in Table 1.

lactonum, acidum gluconicum, glucosaminum nec inositolum.

Kalium nitricum et natrium nitrosum non assimulantur.

Amylum non formatur.

Ad crescentiam thiaminum necessarium est.

Crescere potest in 42°C (interdum 39°C).

Mol% G+C acidi deoxyribonucleati 32.3–32.7 (6 stirpes, vide tabulam 2).

Typus: Stirps UCD (FS&T) 76-356 ex tabidosus sacculis cacti *Lophocereus schottii*, Mulegé, Baja Californiensis Sur, isolata est.

Description of *P. heedii*. Growth in malt extract: After 3 days, the cells are ovoid and measure 1.8 to 3.5 by 3.0 to 7.0 μm . Cells rarely reach 10 μm in length. They occur singly, in pairs, or in short chains or small clusters. A very thin, dull, smooth, creeping pellicle or islets and a little sediment form after 3 days. After 3 weeks the film may settle, but the amount of sediment remains small.

Growth on malt agar: After 1 month the streak culture is cream-colored, smooth, semiglossy to dull, flat, and soft; the border is entire.

Dalmau plate culture on cornmeal agar: After 12 days pseudomycelium is absent or rudimentary.

Formation of ascospores: Vegetative cells are diploid and heterothallic. Ascospores are formed

after 2 to 3 weeks on malt agar (YM agar does not induce sporulation). Vegetative cells are transformed into asci which contain four hat-shaped spores, each containing a conspicuous refractile granule. Two of the spores are of mating type α and two are of α mating type. The asci lyse slowly upon maturity, releasing the spores (Fig. 2).

Fermentation: Glucose is not fermented. Occasionally a bubble may be present in 2 to 3 weeks.

Assimilation of carbon compounds: D-Glucose, D-xylose, ethanol, glycerol (latent or weak), lactic acid (some strains are negative), succinic acid, and citric acid (some strains are negative) are assimilated.

D-Galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D- and L-arabinose, D-ribose, L-rhamnose, methanol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, α -methyl-D-glucoside, salicin, 2- and 5-ketoglucuronate, glucono- δ -lactone, gluconic acid, D-glucosamine, and *meso*-inositol are not assimilated.

Assimilation of nitrogen compounds: Potassium nitrate, –; sodium nitrite, –; ethylamine, +.

Vitamin requirements: Thiamine is essential for growth.

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

Maximum sodium chloride concentration in yeast extract agar for growth: 5 to 10%.

Maximum temperature for growth: 42°C (a few strains did not grow above 39°C).

Acid formation on chalk agar: Weak to moderate.

Synthesis of starchlike compounds: Negative.

Gelatin liquefaction: Negative.

Casein hydrolysis: Negative.

Hydrolysis of urea: Negative.

Lipolytic activity: Negative.

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

G+C content of the nuclear DNA: 32.3 to 32.7 mol% (range for six strains, Table 2).

Type strain: The type strain, UCD (FS&T) 76-356, was isolated from a soft-rot pocket of senita cactus (*Lophocereus schottii* [Engelm.] Britt. and Rose) at Bahía de Concepción, Mulegé, 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 6930 and in the American Type Culture Collection, Rockville, Md., as ATCC 34936.

Sources. Table 1 lists the substrates and localities (see also Fig. 1) from which *P. heedii* has

FIG. 2. Formation of ascospores in *P. heedii*. (a and b) Zygotes formed 6 to 8 h after mixing of haploid strains of opposite mating type. (c) Mature asci formed in zygotes as shown in (b). (d) Early stage of spore differentiation in a diploid cell. (e) Mature ascus formed from a diploid cell. (f) Four hat-shaped spores in the process of release from the ascus. Bar represents 4 μ m in all cases.

TABLE 2. Nuclear DNA base compositions of selected strains of *Pichia heedii*

Strain	Citrate utilization	G+C content ^a (mol% $\pm$ SD)	Source	Locality
63 (type)	+	32.4 $\pm$ 0.20	<i>Lophocereus schottii</i>	Bahia de Concepción, Mulegé, Baja Calif. Sur, Mexico
65	+	32.6 $\pm$ 0.12	<i>L. schottii</i>	Bahia de Concepción, Mulegé, Baja Calif. Sur, Mexico
5	+	32.3 $\pm$ 0.18	<i>L. schottii</i>	Guaymas, Sonora, Mexico
33	—	32.7 $\pm$ 0.16	<i>Carnegiea gigantea</i>	Tucson, Ariz.
31	—	32.5 $\pm$ 0.10	<i>C. gigantea</i>	Tucson, Ariz.
29	—	32.5 $\pm$ 0.14	<i>C. gigantea</i>	Tucson, Ariz.

^a The base composition values for strains 63, 65, and 5 were based on three separate determinations; the remainder were based on duplicate runs. SD, Standard deviation.

been recovered. It is evident that *L. schottii* (senita) is the principal host plant for *P. heedii*.

DISCUSSION

The principal habitat of *P. heedii* is the decaying stems of *L. schottii*, known locally as "senita" (Table 3). This substrate is interesting in that it provides an example of a niche that supports only a single species of drosophila, i.e., *Drosophila pachea* Patterson and Wheeler. This selectivity is due to the presence of an alkaloid (pilocereine) in the tissues of the host plant (5) which is toxic to other desert drosophilae. In addition, unusual sterols (e.g., schottenol [3]) occurring in senita are required by *D. pachea* for development and fertility. These two factors provide *D. pachea* with exclusive use of senita rots for larval development. This monophagy of *D. pachea* may in part restrict the distribution of *P. heedii* to senita cactus since cactus yeasts rely primarily on insect vectors for dispersal.

It is not known whether the ability of *P. heedii* to utilize D-xylose is an important adaptation to its primary host. However, the ability to utilize this compound is possibly an indicator of a metabolic potential that closely ties *P. heedii* to the host tissues. *P. heedii* is isolated occasionally from other cacti found in the same subtribe (*Pachycereinae*) to which *L. schottii* belongs, e.g., saguaro (*Carnegiea gigantea* [Engelm.] Britt. and Rose) and cardon (*Pachycereus pringlei* [S. Wats.] Britt. and Rose), whereas the yeast is conspicuously absent in cacti of a closely related subtribe, *Stenocereinae*. The closest relatives of *D. pachea*, species in the *Nannoptera* group found in southern Mexico, have been reared from soft-rots of cacti in the *Stenocereinae*, but *P. heedii* was not recovered from a small sampling of these rots (43 isolates). Thus, *P. heedii* appears to be endemic to the Sonoran Desert and restricted to a narrow niche within the desert.

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

Source	Frequency of isolation
<i>Lophocereus schottii</i> (Engelm.) Britt. and Rose [senita] ^a	34 ^b
<i>Carnegiea gigantea</i> (Engelm.) Britt. and Rose [saguaro]	6
Soil wetted by soft-rot drippings from <i>C. gigantea</i>	3
<i>Pachycereus pringlei</i> (S. Wats.) Britt. and Rose [cardon]	1
<i>Machaerocereus gummosus</i> (Engelm.) Britt. and Rose [agria]	1
<i>Drosophila pachea</i> Patterson and Wheeler	15
<i>Drosophila nigrospiracula</i> Patterson and Wheeler	4
<i>Drosophila</i> species "M"	3

^a Common names of cacti are given in brackets.

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

Furthermore, we noted that all 16 isolates of *P. heedii* from *C. gigantea*, soil wetted by soft-rot drippings from this cactus, resident *Drosophila nigrospiracula* Patterson and Wheeler, and *Drosophila* species "M" were unable to utilize citric acid under our test conditions, whereas all other strains were positive in this respect. This minor separation along host-tissue differences may well indicate incipient speciation. Clones of single ascospores of citrate-negative and citrate-positive strains were found to be interfertile. Genetic analysis of this hybrid indicates that the character of citrate assimilation is under the control of a single locus. Tetrads dissected from seven hybrid asci all segregated into two citrate-positive and two citrate-negative ascospores.

The relationship of *P. heedii* to other yeasts is an intriguing problem. The possibility of evolutionary convergence is always present when phylogenetic considerations are made. However,

the genus *Pichia* does have a natural group of tree-dependent species which may constitute possible ancestral forms for cactus-dependent yeasts such as *P. heedii*. In our opinion, there is evidence to suggest that *P. heedii* is a derived and highly specialized form arising from the "tree-dependent" yeasts.

Finding the true close relatives of *P. heedii* will require extensive study of other cactus-specific *Pichia* species, exploration into the tree habitats of *Pichia* species in neotropical regions, and molecular evolutionary approaches, such as nucleic acid pairing, protein sequences, determination of immunological distances, and others.

The physiological profile exhibited by *P. heedii* is identical to that of certain strains of *P. membranaefaciens* (D-xylose assimilation variable [6]). The problem of identifying *P. heedii* is only solvable by combining the correlated characteristics of cactus habitat, much smaller cell size, assimilation of D-xylose, and relatively high maximum temperature of growth.

ACKNOWLEDGMENTS

We are greatly indebted to Nancy Krauter for her help in determining the DNA base compositions and to André Lachance for preparing the illustrations and for his assistance with the Latin diagnosis. We greatly appreciate assistance with the field collections by Alexander Russell, Jr., Jean S. Russell, William R. Johnson, and Robert L. Mangan. A gift of Zymolyase from the Research Laboratories of Kirin Breweries Ltd., Takasaki, Japan, is gratefully acknowledged.

This research was supported by Public Health Service grant GM-16307-07 from the National Institute of General Medical Sciences to H. J. Phaff and by a grant from the National Science Foundation to W. B. Heed and H. W. Kircher (DEB 74-19148A03).

REPRINT REQUESTS

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