Pichia antillensis, a New Species of Yeast Associated with Necrotic Stems of Cactus in the Lesser Antilles

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We describe *Pichia antillensis*, a new species of yeast which is closely related to *Pichia opuntiae*. *Pichia antillensis*, 20 strains of which were isolated, is heterothallic and occurs in nature in both the haploid state and the diploid state. It produces asci with four hat-shaped spores, which are rapidly released upon maturity. The guanine-plus-cytosine content of its nuclear deoxyribonucleic acid is 33.4 to 33.7 mol%. The deoxyribonucleic acids of *Pichia antillensis* and *Pichia opuntiae* var. *opuntiae* show approximately 50% homology, but the two species are not interfertile. Moreover, the two species are separated geographically and by host plant. *Pichia antillensis* occurs principally in necrotic tissue of the columnar cactus *Cephalocereus royerii* in the West Indies, and *Pichia opuntiae* var. *opuntiae* occurs in *Opuntia* rots in Australia. The type strain of *P. antillensis* is strain UCD-FST 82-651A (= ATCC 56267 = CBS 7111).

Recently, we have been studying a collection of yeasts that were obtained from decaying stems of cacti growing on islands in the Caribbean Sea. In this collection we discovered a heterothallic yeast which has a low-level physiological profile, a deoxyribonucleic acid (DNA) base composition of 33.4 to 33.7 mol% guanine plus cytosine (G+C), and, as far as now known, a restricted geographic and host plant distribution. The sexual, physiological, and macromolecular properties of this yeast are similar to those of three previously described cactus-specific yeasts, *Pichia heedii* (9), *Pichia amethionina* (13), and *Pichia opuntiae* (14), which are known from other geographic regions. Comparisons of the new isolates with strains of these three previously described species for sexual compatibility, physiological abilities, ecological specificity, and DNA-DNA sequence homology suggested that the new isolates comprise a new species in the genus *Pichia*. We named the new isolates *Pichia antillensis* because of their unique habitat in necrotic tissues of the columnar cactus *Cephalocereus royerii* on several islands of the Lesser Antilles.

MATERIALS AND METHODS

Samples of necrotic cactus tissue yielding the new yeast were collected from islands in the eastern West Indies in May 1982 (Table 1) during cruise CF-8205 of the research vessel Cape Florida in this region. The techniques used for yeast isolation and purification have been described previously (12). The yeast strains analyzed for nuclear DNA base composition and the strains used in the DNA-DNA homology sequence studies are listed in Table 2.

The usual phenotypic characterization of the isolates was carried out by methods currently used in yeast taxonomy (16), but additional carbon compounds used in assimilation tests were also employed. These included D-glucosamine hydrochloride, N-acetyl-D-glucosamine, methanol, 2-propanol, acetone, and ethyl acetate; the last three compounds could be tested only by incorporating them at concentrations of 1.0% in yeast nitrogen base agar because in liquid yeast nitrogen base media they proved to be inhibitory, even in those cases where good growth occurred on solid media.

DNA extraction and purification were carried out 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 equipped with photographic optics (11, 15) and were based on three separate determinations. DNA from *Micrococcus luteus* strain 2039 (International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis), with a buoyant density of 1.7310 g/ml, was used as a reference. The buoyant density of the *Micrococcus luteus* 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. Denaturation of DNA, incubation for reassociation, and analysis of the renaturation kinetics, reannealing reactions, and quantitation of single- and double-stranded DNAs were carried out according to the protocol of Price et al. (10). Samples were counted with a Nuclear-Chicago model 1185 gamma ray counter (90% efficiency).

Single ascospores were isolated from mature asci with a micro-manipulator (2).

RESULTS

The physiological and morphological properties of the strains listed in Table 1 resembled those of *P. opuntiae* (14), and the nuclear DNA base compositions of these strains (Table 2) were close to values for *P. opuntiae*, *P. heedii* (9), and *P. amethionina* (13), all of which have as their habitat the necrotic tissues of cacti. These similarities induced us to carry out DNA-DNA base sequence homology studies (Table 3). The results of these experiments indicate that *P. antillensis* appears to be closely related to *P. opuntiae* var. *opuntiae* and distantly related to *P. opuntiae* var. *thermotolerans*, but not significantly related to *P. heedii* or *P. amethionina*. Because no conjugation was observed in our laboratory between haploid mating types of heterothallic *P. antillensis* and *P. opuntiae*, the strains of *P. antillensis* are described here as a new species.

Latin diagnosis of *Pichia antillensis* sp. nov. In extracto malti cellulae ovoideae, 1.6–5.3 × 2.1–5.8 μm, singulae, binae, aut in catenis brevis; annulus et sedimentum tenuis formantur; pellicula nulla.

Cultura in agaro malti post dies 14 (25°C) demissa con-

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TABLE 1. Strain numbers, mating types, localities, and cactus hosts of isolates studied

UCD-FST strain no ^a	Mating type	Locality	Host plant
82-544	h-	Fox Bay, Montserrat	<i>C. royenii</i> (L.) Britt. and Rose
82-546	h+	Fox Bay, Montserrat	<i>C. royenii</i>
82-548A	h+	Fox Bay, Montserrat	<i>C. royenii</i>
82-550A	Diploid	Airport, Montserrat	<i>C. royenii</i>
82-556C	h+	Airport, Montserrat	<i>C. royenii</i>
82-558	h-	Airport, Montserrat	<i>C. royenii</i>
82-562A	h-	Airport, Montserrat	<i>C. royenii</i>
82-563A	h+	Airport, Montserrat	<i>C. royenii</i>
82-568B	h-	Airport, Montserrat	<i>C. royenii</i>
82-589B	Diploid	Beef Island, Tortola, Virgin Islands	<i>C. royenii</i>
82-592B	h+	Beef Island, Tortola, Virgin Islands	<i>C. royenii</i>
82-603A	h+	Beef Island, Tortola, Virgin Islands	<i>C. royenii</i>
82-604B	h+	Beef Island, Tortola, Virgin Islands	<i>C. royenii</i>
82-616B	Diploid	Beef Island, Tortola, Virgin Islands	<i>C. royenii</i>
82-628B	h-	Pricklypear Island, Virgin Gorda, Virgin Islands	<i>C. royenii</i>
82-631A	h+	Pricklypear Island, Virgin Gorda, Virgin Islands	<i>C. royenii</i>
82-637A	h-	Pricklypear Island, Virgin Gorda, Virgin Islands	<i>C. royenii</i>
82-647B	h+	Pricklypear Island, Virgin Gorda, Virgin Islands	<i>Melocactus intortus</i> Urban
82-651A ^{Tb}	Diploid	Pricklypear Island, Virgin Gorda, Virgin Islands	<i>C. royenii</i>
82-651D	h-	Pricklypear Island, Virgin Gorda, Virgin Islands	<i>C. royenii</i>

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

^b T = type strain.

vexa, nitida, glabra, mollis, cremea; margo glabra aut lobata.

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

Species heterothallica haploidea aut raro diploidea. Oriuntur asci ex conjugatione inter cellulas sexus contrarii; asci ex cellulae diploidae inconjuganti fiunt, habentes 4 sporas pileiformae; asci rumpuntur post dies 3-5.

Fermentatio glusosi nullum.

Glucosum, rhamnosum (lente), ethanolum, glycerolum, mannitolum (lente, exigue, vel nullum), glucono-delta-lactonum (lente et exigue), acidum lacticum, acidum succinicum, et acidum citricum (valde) assimilantur at non galactosum, L-sorbosum, maltosum, saccharum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitum, inulinum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, methanolum, erythritolum, ribitolum, galactitolum, D-glucitolum, salicinum, α -methyl-D-glucosidum, 2- et 5-ketogluconatum, D-glucosaminum, N-acetyl-glucosaminum, acetonum, 2-propanolum, ethylacetat, nec inositolum.

Kalium nitricum et natrium nitrosum non assimilantur.

Ethylaminum assimilatur.

Ad crescentiam biotinum, thiaminum, et pyridoxinum necessariae sunt.

Crescere potest in 36°C interdum in 39°C.

G+C acidi deoxyribonucleati, 33.4-33.7 mol% (6 stirpes) (Table 2).

Habitatio cacto.

Typus: stirps UCD-FST 82-651A ex tabidosus sacculis cacti *Cephalocereus royenii* isolata est.

In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum, sub no. (7111) deposita est.

Standard description of *Pichia antillensis*. In malt extract after 3 days at 25°C, the cells are globose to ovoid to short ovoid and 1.6 to 5.3 by 2.1 to 5.8 μ m and occur singly, in pairs, or in groups of three to seven cells. There is little sediment, a thin ring, and sometimes a very thin pellicle or islets after 7 days.

On malt agar after 2 weeks at 25°C, streak cultures are low convex, glossy, smooth, soft, and white. The periphery is entire to slightly lobular.

In Dalmau plate cultures on cornmeal agar after 10 days a pseudomycelium is absent; outgrowths of clusters of cells are occasionally present.

The vegetative cells are heterothallic, usually occurring in the haploid state when isolated from nature but occasionally occurring in the diploid state. Conjugation and sporulation are observed on Difco YM agar at 25°C 3 to 5 days after the appropriate mating types have been mixed together. Ascospores are usually formed in diploid cells that bud from the zygotes. The spores are derby hat shaped, and usually four are produced per ascus. Asci from diploid strains, each containing four spores, have been dissected; these asci yield two spores of one mating type and two spores of the opposite mating type. The asci dehiscence rapidly, releasing the spores soon after formation.

Ability to ferment glucose is absent. The carbon compounds D-Glucose, L-rhamnose (latent), ethanol, glycerol, D-mannitol (latent, weak, or negative), glucono- δ -lactone (latent and weak), DL-lactic acid, succinic acid, and citric acid (strong) are assimilated. The following carbon compounds are not assimilated: D-galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, lactose, melibiose, raffinose,

TABLE 2. Nuclear DNA base compositions (G+C contents) of *P. antillensis* strains and other species used in DNA-DNA reassociation studies

UCD-FST strain no	Organism	G+C content (mol%)
82-651A ^T	<i>P. antillensis</i>	33.7 $\pm$ 0.3 ^a
82-637A	<i>P. antillensis</i>	33.5 $\pm$ 0.1
82-628B	<i>P. antillensis</i>	33.7 $\pm$ 0.3
82-604B	<i>P. antillensis</i>	33.4 $\pm$ 0.2
82-589B	<i>P. antillensis</i>	33.6 $\pm$ 0.2
82-550A	<i>P. antillensis</i>	33.4 $\pm$ 0.2
76-356 ^T	<i>P. heedii</i>	32.4 $\pm$ 0.2
76-401B ^T	<i>P. amethionina</i> var. <i>amethionina</i>	33.1 $\pm$ 0.1
76-384A ^T	<i>P. amethionina</i> var. <i>pachycereana</i>	33.0 $\pm$ 0.2
79-132D	<i>P. amethionina</i> (from Australia)	32.3 $\pm$ 0.5
81-62	<i>P. amethionina</i> (from Bahamas)	34.3 $\pm$ 0.1
77-40 ^T	<i>P. opuntiae</i> var. <i>opuntiae</i>	33.4 $\pm$ 0.5
77-159	<i>P. opuntiae</i> var. <i>opuntiae</i>	33.0 $\pm$ 0.1
76-211 ^T	<i>P. opuntiae</i> var. <i>thermotolerans</i>	33.0 $\pm$ 0.3
76-41	<i>Metschnikowia bicuspidata</i> var. <i>bicuspidata</i>	47.0 $\pm$ 0.3

^a Average $\pm$ standard deviation from three or more buoyant density determinations.

TABLE 3. Reannealing between labeled DNA from *P. antillensis* UCD-FST 82-651A^T and DNAs from 14 yeast strains and calf thymus at 61.4°C^a

Organism or tissue	Expt 1			Expt 2		
	Actual binding (%) ^b	Corrected actual binding (%) ^c	Relative binding (%) ^c	Actual binding (%) ^b	Corrected actual binding (%) ^c	Relative binding (%) ^c
<i>P. antillensis</i> UCD-FST 82-651A ^T	78.6 ± 0.5 ^d	75.9	100.0	75.7 ± 0.9	75.2	100.0
<i>P. antillensis</i> UCD-FST 82-628B	77.6 ± 0.9	74.9	98.8			
<i>P. antillensis</i> UCD-FST 82-589B	78.5 ± 0.9	75.9	99.9			
<i>P. antillensis</i> UCD-FST 82-550A	77.6 ± 1.8	75.0	98.8	72.4 ± 0.8	71.8	95.5
<i>P. antillensis</i> UCD-FST 82-637A				72.7 ± 1.1	72.1	95.9
<i>P. opuntiae</i> var. <i>opuntiae</i> UCD-FST 77-40 ^T				42.7 ± 2.0	41.5	55.2
<i>P. opuntiae</i> var. <i>opuntiae</i> UCD-FST 77-159				42.1 ± 1.8	40.9	54.4
<i>P. opuntiae</i> var. <i>thermotolerans</i> UCD-FST 76-211 ^T				21.4 ± 1.7	19.8	26.4
<i>P. heedii</i> UCD-FST 76-356 ^T	5.0 ± 0.1	2.1	2.8			
<i>P. amethionina</i> var. <i>amethionina</i> UCD-FST 76-401B ^T	9.5 ± 1.2	6.6	8.7			
<i>P. amethionina</i> var. <i>pachycereana</i> UCD-FST 76-384A ^T	9.2 ± 1.5	6.4	8.4			
<i>P. amethionina</i> UCD-FST 79-132D (from Australia)	8.0 ± 0.4	5.1	6.7	8.3 ± 0.9	6.5	8.6
<i>P. amethionina</i> UCD-FST 81-62 (from Bahamas)	7.4 ± 0.3	4.5	5.9			
<i>Metschnikowia bicuspidata</i> UCD-FST 76-41	3.2 ± 0.4	0.3	0.4			
Calf thymus	4.1 ± 0.2	1.2	1.6	3.5 ± 1.0	1.6	2.1

^a ¹²⁵I-labeled DNA (0.2 µg) and unlabeled DNA (200 µg) were incubated for 25 h in 0.5 ml of 280 mM phosphate buffer.^b Average of three samples corrected for zero-time binding.^c Corrected for self-renaturation (experiment 1, 2.9 ± 0.5%; experiment 2, 2.0 ± 0.6%) (10).^d Mean ± standard deviation.

melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, methanol, erythritol, ribitol, galactitol, D-glucitol, salicin, methyl-α-D-glucoside, 2- and 5-ketogluconate, D-glucosamine, meso-inositol, ethyl acetate, acetone, 2-propanol, hexadecane, and N-acetyl-D-glucosamine.

Assimilation of nitrogen compounds: potassium nitrate, negative; sodium nitrite, negative; ethylamine, positive; lysine, positive; cadaverine, positive; ammonium sulfate, positive.

Growth in vitamin-free medium is negative. Biotin, thiamine, and pyridoxine are required for growth.

Growth in amino acid-free medium is positive.

Growth on 50% (wt/wt) glucose-yeast extract agar is negative.

Growth on glucose-yeast extract agar containing 5% NaCl is positive; growth on the same medium containing 7.5% NaCl is weak; and growth in the presence of 10% NaCl is negative.

Maximum temperature for growth, 36 to 39°C (depending on the strain).

No acid formation on chalk agar.

Hydrolysis of urea, negative.

Gelatin liquefaction, negative.

Casein hydrolysis, negative.

Lipolytic activity, negative.

Growth in the presence of 1 µg of cycloheximide per ml, positive; growth in the presence of 10 µg of cycloheximide per ml, negative.

G+C content of the nuclear DNA, 33.4 to 33.7 mol% (six strains) (Table 2).

Habitat. A total of 20 strains were recovered from necrotic tissue of *C. royerii* on the West Indian islands of Montserrat, Tortola, and Virgin Gorda; 1 strain was recovered from necrotic tissue of *Melocactus intortus* on Virgin Gorda.

Type. The type strain of *P. antillensis*, strain UCD-FST 82-651A, was isolated from a rot of *C. royerii* on the island of Virgin Gorda in the Lesser Antilles, West Indies. This strain

has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures in Delft, The Netherlands, as strain CBS 7111 and in the American Type Culture Collection, Rockville, Md., as strain ATCC 56267.

Etymology. The specific epithet of *P. antillensis* (an. til. len' sis. M.L.f. adj. *antillensis*) is geographical, referring to the occurrence of this organism on islands of the Antilles, West Indies.

DISCUSSION

Investigations into the yeast communities associated with the decaying stems of cacti have revealed a group of yeasts which share certain physiological, ecological, and macromolecular properties. These species include *P. heedii* Phaff et al. (9), two varieties of *P. amethionina* Starmer et al. (13), two varieties of *P. opuntiae* Starmer et al. (14), and *P. antillensis*. These species are heterothallic and have similar low-level physiological abilities and DNA base compositions (G+C contents) clustered in a 2.0-mol% range (32.3 to 34.3 mol %). However, they do not conjugate in the laboratory when haploid mating types of the respective species are mixed.

A previous study on the DNA-DNA base sequence relatedness of *P. heedii*, *P. amethionina*, and *P. opuntiae* clearly demonstrated the distinct genomic separation of these three species (3). This study also demonstrated a significant sequence divergence between the two varieties of *P. amethionina*, as well as the unreliability of using mannitol assimilation as a means of separating these varieties. The mean intervarietal relative binding to hydroxylapatite of the DNAs of the two varieties of *P. opuntiae* was 28.0 ± 3.1% based on five pair-wise comparisons (3); this, together with the lack of gene flow between the organisms (8, 14), suggests that they represent distinct biological species.

Table 4 lists the known differences among the four *Pichia* species, including the previously described varieties of these species. These data show that the only phenotypic differ-

TABLE 4. Salient characteristics differentiating *P. antillensis* from several phenotypically similar cactus-associated yeasts^a

Characteristic	<i>P. antillensis</i>	<i>P. opuntiae</i> var. <i>opuntiae</i>	<i>P. opuntiae</i> var. <i>thermotolerans</i>	<i>P. amethionina</i> var. <i>amethionina</i>	<i>P. amethionina</i> var. <i>pachycereana</i>	<i>P. heedii</i>
Growth at:						
33°C	+	+/-	+	+	+	+
37°C	+/-	-	+	+	+	+
39°C	+/-	-	-	+	+	+
42°C	-	-	-	+/-	+/-	+/-
Growth on:						
Cellobiose	-	l/-	+	-	-	-
D-Xylose	-	-	-	-	-	+
L-Rhamnose	l	l/w	l/w	-	-	-
D-Mannitol	l/w/-	l/-	l	-/+	+	-
Salicin	-	+	+	-	+	-
D-Gluconate	-	-	-	+	+	-
Citrate	+	+	-	-	-	+/-
Requires L-methionine	-	-	-	+	+	-
Growth in the presence of 1 µg of cycloheximide per ml	+	-	-	-	-	-
Growth in the presence of 10 µg of cycloheximide per ml	-	-	-	-	-	-
Ascus dehiscence	rp	rp	rp	rp	rp	sl
G+C content (mol%)	33.6 ± 0.14 ^c	33.6 ± 0.25	33.1 ± 0.23	33.1 ± 0.05	33.1 ± 0.32	32.5 ± 0.14

^a The following compounds are assimilated by all four species: glucose, ethanol, glycerol, lactate, and succinate. No other compound used in standard assimilation tests (16) is assimilated by these four species.

^b +, Positive; -, negative; l, latent growth; w, weak growth; +/-, some strains negative; -/+, some strains positive; l/w-, strain dependent; rp, rapid; sl, slow (well after maturation).

^c Mean ± standard deviation.

ences known between *P. antillensis* and *P. opuntiae* var. *opuntiae* are the ability of the latter to utilize salicin as a carbon source and the ability of *P. antillensis* to grow in the presence of 1 µg of cycloheximide per ml. The assimilation of L-rhamnose by *P. antillensis* might be easily overlooked. On yeast nitrogen base agar it usually takes from 7 to 9 days before small colonies appear. However, when rhamnose-grown cells are reinoculated into fresh yeast nitrogen base medium containing rhamnose (solid or liquid), full growth takes place in 2 to 5 days, suggesting that the enzymes involved in rhamnose metabolism are inducible.

The DNA-DNA reassociation data show (Table 3) that *P. antillensis* shares approximately 50% of its base sequences with *P. opuntiae* var. *opuntiae*. DNA-DNA reassociation values of about 50% are very rare among yeast species (10), but this relatively high DNA complementarity between the two yeasts indicates a significant relatedness. The complete lack of conjugation in laboratory experiments between mating types of the two species could be due to mutations in one or more genes that control the formation of zygotes (prezygotic breakdown). Such changes may have developed as a result of the reproductive isolation of *P. antillensis* and *P. opuntiae* (8). So far, *P. opuntiae* var. *opuntiae* has been found only in necrotic tissues of Australian opuntias (1, 14), the exact origin of which is not known in detail (7), although it is known that they were imported from the Americas into Australia within the last 200 years (1, 7). Extensive sampling of *Opuntia* species in Arizona and Texas (12), as well as on a number of islands in the Caribbean Sea (Starmer and Phaff, unpublished data), including those on which we discovered *P. antillensis*, failed to yield typical strains of *P. opuntiae* var. *opuntiae*. The lack of discovery of the latter in the New World may be due to insufficient sampling, such as the omission of South American opuntias. We postulate that *P. antillensis* and *P. opuntiae* var. *opuntiae* originated from a common ancestor and that both allopatry and differences in host plant chemistry (*C. royerii* [*P. antillensis*] and *Opuntia*

inermis [*P. opuntiae* var. *opuntiae*]) have played a role in their evolutionary divergence. A resolution of the problem of the relationship between the two species will require further study of characters, such as analysis of allozymes (4) and specific DNA sequences or both.

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