4-1-2010

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Recommended Citation

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A novel species of ascomycetous yeast, *Pichia insulana* sp. nov., is described from necrotic tissue of columnar cacti on Caribbean islands. *P. insulana* is closely related to and phenotypically very similar to *Pichia cactophila* and *Pichia pseudocactophila*. There are few distinctions between these taxa besides spore type, host preference and locality. Sporogenous strains of *P. insulana* that produce asci with four hat-shaped spores have been found only on Curaçao, whereas there was no evidence of sporogenous *P. cactophila* from that island. In addition, sequences of the D1/D2 fragment of the large-subunit rDNA from 12 Curaçao strains showed consistent differences from the sequences of the type strains of *P. cactophila* and *P. pseudocactophila*. The type strain of *P. insulana* is TSU00-106.5[T] (=CBS 11169[T] =UCD-FST 09-160[T]).

INTRODUCTION

The necrotic tissue of some cacti is home to a diverse yeast community. Many of the spore-forming species produce either two or four hat-shaped ascospores and are oligotrophic, assimilating fewer than 20 % of the carbon sources tested (Starmer *et al.*, 1990). Similarities among many of the species can make them difficult to distinguish, and the first investigators classified many cactophilic strains as *Pichia membranifaciens*, a species now known to be rare in this habitat (Heed *et al.*, 1976). Repeated collection of variants from similar habitats led to the recognition that they were separate species, and the habitat is now known to contain over a dozen endemic species with hat-shaped spores in at least three different genera (two endemic).

A particularly subtle example of this similarity is *Pichia pseudocactophila*. Cactophilic yeast identification is typically based on tests of a minimum of 63 physiological characters. *Pichia cactophila*, the worldwide dominant yeast from cactus tissue (in terms of frequency of isolation), grows strongly in 13 physiological tests and variably in four others (Starmer *et al.*, 1978). *P. cactophila* was itself initially identified as *P. membranifaciens*. Sporogenous *P. cactophila* strains form two spores almost exclusively [see Shen & Lachance (1993) for an example of a four-spored *P. cactophila*]. This observation, the appearance of spores in colonies grown from single spores and the large size of the spores support the conclusion that

the spores are diploid. Some of the strains collected from cacti of the Pachycereinae from the Sonoran Desert had asci with four smaller spores. Although rare in their only known habitat, the four-spored strains proved to represent a separate species, subsequently named *P. pseudocactophila* (Holzschu *et al.*, 1983). Determination of specific status for *P. pseudocactophila* was based on low DNA–DNA relatedness with *P. cactophila* and allozyme frequency variation. Subsequent sequencing of the D1/D2 fragment of the large-subunit (LSU) rDNA also found consistent differences between the two species (Kurtzman & Robnett, 1998). There is no physiological character known that will separate the two species consistently, and spore number and source (location and host plant) of the strain remain the accepted way to differentiate the two species without resorting to DNA sequencing. A potential problem for identification lies in the tendency of cactophilic yeast species to have non-sporulating strains, as is known for *P. cactophila* (described as an anamorphic species, *Candida cactophila*). It is not known whether *P. pseudocactophila* has non-sporulating lineages.

In early 2000, yeasts were isolated from necrotic cacti growing on the island of Curaçao, one of the Netherlands Antilles in the Caribbean. The dominant yeast was physiologically identical to *P. cactophila*, but all sporulating strains produced asci with four small hat-shaped spores. Here, we present data that demonstrate that the strains from Curaçao are neither *P. cactophila* nor *P. pseudocactophila*, and we propose the name *Pichia insulana* sp. nov. for the new taxon. A search of strains housed at the Phaff Yeast Collection at the University of California Davis revealed

Abbreviation: LSU, large subunit.

The GenBank/EMBL/DDBJ accession number for the partial LSU rDNA sequence of strain TSU00-106.5[T] is EU747339.

several strains from the Caribbean region that had originally been identified as *P. cactophila* but did not have the expected partial LSU rDNA sequence, and we added those strains to this study.

METHODS

Strain isolation and determination of physiological abilities. Strains used in this study are listed in Table 1. Samples of necrotic cactus stem tissue were collected from five sites on Curaçao in January 2000. Methods for isolation of yeast from cactus samples have been described by Starmer & Phaff (1983). Morphological and physiological tests were done as described by Yarrow (1998), as modified by Lachance *et al.* (1988). Over 380 strains were isolated from 49 rots and 99 of these had physiological profiles consistent with *P. cactophila*. Of those 99 strains, 24 produced four-spored asci, one produced asci with either two or three spores and no asci were detected from the remaining 74 strains. Asci from strain TSU00-106.5^T (the designated type strain) were dissected and mating types were determined for all spore clones. In addition, matings were attempted between *P. insulana* mating types, the asporogenous strains of *P. insulana* strains from Curaçao, the six strains identified as *P. cactophila* from the UC Davis collection and the mating types of *P. pseudocactophila*.

rDNA sequence determination. LSU rDNA sequences were amplified using primers NL1 and NL4 (Kurtzman & Robnett, 1998) with the following PCR program: 95 °C for 2 min, 36 cycles consisting of 1 min at 94 °C, 1 min at 52 °C and 2 min at 72 °C and a final extension of 8 min at 72 °C. After purification with the Jet Quick kit (Genomed GmbH), sequencing was carried out by the MWG service (MWG Biotech AG). Electrophoregrams were read with TraceViewer (http://www.codoncode.com).

Phylogenetic analysis of the sequences. The analysis involved 12 *P. insulana* sequences (Table 1) plus the published sequences for the type strains of *P. cactophila* and other closely related strains as determined through rDNA sequence similarity (from Kurtzman & Robnett, 1998). The clade is composed of cactophilic species plus *Pichia kudriavzevii* (formerly *Issatchenkia occidentalis*; Kurtzman *et al.*, 2008), *Pichia nakasei* and *Candida inconspicua* [the sister taxon of *P. cactophila* in Kurtzman & Robnett (1998)]. *P. nakasei* was the outgroup for this analysis. There are now multiple ways to align nucleotide sequences and to construct a phylogeny based on the aligned sequences. Rather than employ a single combination of alignment/phylogeny construction algorithms and be subject to the assumptions of both, we chose to employ multiple alignment/phylogeny construction pairings and to report the results common to all. Sequences were aligned using CLUSTAL_X, DIALIGN 2 (which maximizes the number of aligned segments; Morgenstern, 1999) and SOAP (which uses CLUSTAL_X as its alignment algorithm but varies the gap and extension penalties and presents a consensus alignment; Löytynoja & Milinkovitch, 2001). Phylogenies involving just the unique *P. insulana* sequences plus the *P. cactophila* and *C. inconspicua* type strain sequences were constructed using PAUP* (Swofford, 1999) under a variety of tree comparison criteria, including parsimony (default settings except that gaps were treated as both missing data and a separate character state), neighbour-joining and maximum-likelihood (using default PAUP* settings). Neighbour-joining phylogenies were based on uncorrected *p*, F84, Tamura–Nei, Tajima–Nei and maximum-likelihood Jukes–Cantor distances. A second phylogeny was constructed with the addition of 10 more D1/D2 fragment sequences from type strains of species in the same clade as *P. cactophila* (Kurtzman & Robnett, 1998). The increase in

Table 1. Strain numbers, collection location and host of sequenced *P. insulana* sp. nov. isolates

Collections: TSU, first author's culture collection at Tennessee State University, Nashville, TN, USA; UCD-FST, Phaff Yeast Culture Collection at the University of California, Davis, CA, USA. Yeasts with TSU identification numbers are from sites on the island of Curaçao, Netherlands Antilles. Strains with UCD-FST numbers were originally identified as (possibly) *P. cactophila* but have LSU rDNA sequences similar to those of *P. insulana*. Spore data were obtained from strains on YM plates inoculated at the same time that the identification series of plates was inoculated. The search for spores was done at or after 5 days post-inoculation.

| Strain	Location	Host*	Spores
TSU00-101.1	Weg naar Westpunt	*Stenocereus griseus*	Two- and three-spored asci
TSU00-106.5^T	Weg naar Westpunt	*Cereus repandus*	Four-spored asci
TSU00-115.4	Weg naar Westpunt	*Stenocereus griseus*	None
TSU00-125.3	Wacawa	*Opuntia* sp.	None
TSU00-133.1	Wacawa	*Opuntia* sp.	None
TSU00-135.2A	Wacawa	*Opuntia* sp.	Four-spored asci
TSU00-139.4	Savonet	*Cereus repandus*	Four-spored asci
TSU00-142.6	Savonet	*Stenocereus griseus*	None
TSU00-144.2	Savonet	*Stenocereus griseus*	None
TSU00-149.3	Westpunt	*Stenocereus griseus*	None
TSU00-151.3	Westpunt	*Stenocereus griseus*	Four-spored asci
TSU00-153.2A	Westpunt	*Stenocereus griseus*	None
UCD-FST 81-111	Coastal Venezuela	*Stenocereus griseus*	None
UCD-FST 82-472.1	Hispaniola	*Pilosocereus* sp.	None
UCD-FST 82-527.3	Hispaniola	*Stenocereus hystrix*	None
UCD-FST 82-608.1	British Virgin Islands	*Pilosocereus royenii*	None
UCD-FST 83-1065.2	Cayman Islands	*Opuntia stricta*	None
UCD-FST 83-1117.1	Cayman Islands	*Opuntia stricta* fruit	None

***Stenocereus griseus* = *Lemairerocereus griseus*; *Opuntia* sp. is probably *O. caracassana*; *Pilosocereus* sp. is probably *P. royenii*.

computation time resulting from the addition of taxa meant that maximum-likelihood analysis was dropped from the larger analysis, but the results reported here are those common to all algorithms used. Bootstrap values were determined using only distance and parsimony criteria.

RESULTS

Sequencing of the rDNA from the 12 strains produced eight unique sequences (there were four pairs of identical sequences: TSU00-101.1/TSU00-125.3, TSU00-106.5^T/ TSU00-139.4, TSU00-115.4/TSU00-151.3 and TSU00-135.2A/TSU00-153.2). Among the eight unique sequences, there were no indels, but they differed at a mean of 3.15 sites (ranging from 1 to 6 sites). Comparison of the sequences to the *P. cactophila* and *P. pseudocactophila* sequences revealed five indels, with the longest being a 3 bp indel unique to *P. pseudocactophila*. Only one indel was unique to *P. insulana*. There were a mean of 9.88 substitutions between the eight sequences from *P. insulana* strains and the sequence of the type strain of *P. cactophila* (ranging from 8 to 13) and a mean of 18.25 substitutions for the comparison between *P. insulana* and the type strain of *P. pseudocactophila* (ranging from 16 to 21). The six strains from the UC Davis collection that were not collected on Curaçao all had D1/D2 rDNA sequences identical to that of TSU00-125.3 and TSU00-101.1 (these six sequences were not deposited in GenBank or added to the dataset used to construct phylogenies). This establishes that the six strains (all asporogenous) from the UC Davis collection are members of *P. insulana*, not *P. cactophila*.

Analysis of the set of eight D1/D2 LSU rDNA sequences from *P. insulana* and the type strains of *P. cactophila* and *C. inconspicua* produced a tree that placed all 12 strains of *P. insulana* from Curaçao in a single clade. The *P. insulana* clade was joined to a second clade containing both *P. cactophila* and *C. inconspicua*. Both the *P. insulana* and the *P. cactophila*/*C. inconspicua* clades had bootstrap values of nearly 100 % (for parsimony as well as distance criteria), supporting the conclusion that *P. insulana* and *P. cactophila* are separate taxa. The essential topology of the tree did not differ among algorithms and the bootstrap values described above were common to all analyses (some minor rearrangements were found within the *P. insulana* clade). When the type strains from related species were included in the analysis, the topology of the tree and bootstrap analysis once again supported the separation of the *P. insulana* and *P. cactophila*/*C. inconspicua* clades (Fig. 1). As in the case of the smaller dataset, the optimal tree or trees found were identical in topology except for minor rearrangements within the *P. insulana* clade irrespective of which tree-building algorithm was used, although the actual tree presented is based on distance. Bootstrap values for parsimony were also similar to those presented in Fig. 1. Another topological feature common to all trees is the small clade consisting of strains TSU00-142.6 and TSU00-144.2,
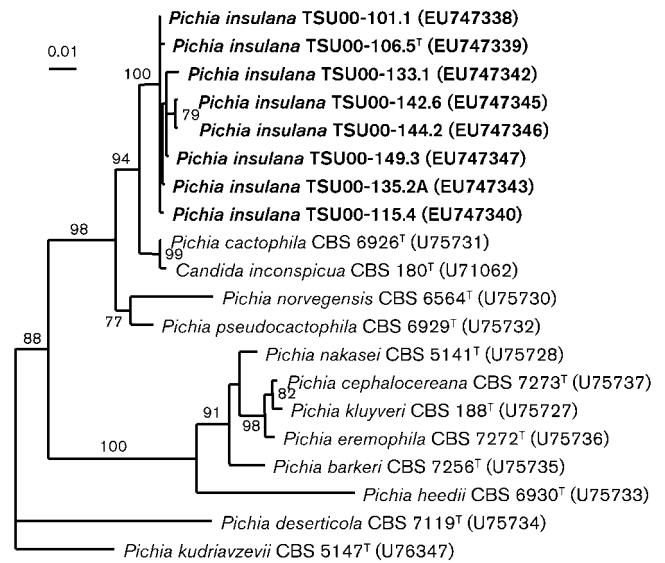
Fig. 1. Neighbour-joining tree based on Tajima–Nei distances calculated from aligned D1/D2 LSU rDNA sequences representing the eight unique *P. insulana* sequences and sequences from related type strains. GenBank accession numbers are given. Bootstrap values (%) are placed on or at the most convenient end of the branch leading to the clade to which the value pertains (values below 70 % omitted). Bar, 0.01 substitutions per site.

both from *Stenocereus griseus* rots at the Savonet site on Curaçao. Bootstrap support varied from 76 to 88 %. Neither strain produced spores. Given the small number of strains and the slight magnitude of the variation involved, it is impossible to come to any conclusions about the taxonomic importance of this clade.

Latin diagnosis of *Pichia insulana* Ganter, Cardinali *et* Boundy-Mills sp. nov.

In YM (Difco) liquido post dies 3 in 30 °C, cellulae rotundae vel ovoideae, 1.3–2.5 × 2.5–5.0 μm. In agaro farinae Zea mays post dies 10 pseudomycelium paucum. Cultura in agaro-malti post dies 21 in 25 °C cana-cremea, butyrosa vel mollis, glabra, nitida, margine glabro. In agaro YM (Difco) post 6–14 dies asci ovoidei, 4 ascosporas continentes. Fermentatio fere nulla; in tribus stipitibus exigua glucosi fermentatio. Glucosum, ethanolum, D-glucosamina, acidum lacticum, succinicum acidum, citricum acidum et malicum acidum assimilantur. Lente vel exigue crescit in glicerolo. Non crescit in D-galactoso, L-sorboso, maltoso, sucroso, cellobioso, trehaloso, lactoso, melibioso, raffinosio, melezitoso, inulino, solubile amylo, D-xyloso, D- vel L-arabinoso, D-riboso, L-rhamnoso, erythritolo, adonitolo (ribitolo), dulcitolo (galactitolo), D-mannitolo, D-sorbitolo (D-glucitolo), methyl-α-glucosido, salicino, glucono-δ-lactono, 5 keto-gluconato, inositolo, N-acetylglucosamino, glucuronico acido, hexadecano, methanolo, ethyl-acetato, 2-propanolo, vel acetono. Crescere potest in 42 °C, et in 5 % NaCl/0.5 % glucoso, sed non in 10 % NaCl/0.5 % glucoso. Non

crescit in digitonino et in cycloheximido at 0.1 p.p.m. *Habitatio in cacti tribus* Opuntieae, *stipitibus subtribus* Stenocereinae *et stipitibus tribus* Cereeae.

Typus: stirps TSU00-106.5^T *ex tabidosus sacculis cacti* Cereus repandus *isolata est. Stirps* TSU00-106.5^T *in collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum sub no.* CBS 11169^T *et Phaff Yeast Culture Collection, Davis, CA, USA, sub no.* UCD-FST 09-160^T *deposita est.*

Description of *Pichia insulana* Ganter, Cardinali & Boundy-Mills sp. nov.

Pichia insulana (in.su.la′na. L. fem. adj. *insulana* of or belonging to an island, referring to the isolation of strains from the island of Curaçao).

Carbon sources assimilated are glucose, ethanol, D-glucosamine, lactic acid, succinic acid, citric acid and malic acid. Growth is delayed on glycerol and sometimes weak. None of the known strains grows on D-galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, D- or L-arabinose, D-ribose, L-rhamnose, erythritol, adonitol (ribitol), dulcitol (galactitol), D-mannitol, D-sorbitol (D-glucitol), methyl α-glucoside, salicin, glucono-δ-lactone, 5-ketogluconate, inositol, *N*-acetylglucosamine, glucuronic acid, hexadecane, methanol, ethyl acetate, 2-propanol or acetone. Most strains demonstrate no ability to ferment, but three strains ferment glucose weakly (some gas in the Durham tubes) after 14 days. All strains grow at 42 °C and on 5 % NaCl/0.5 % glucose medium, but not at 10 % NaCl. The only nitrogen source assimilated strongly is ethylamine, but most strains grow weakly on lysine. Nitrogen sources not assimilated include KNO_3 and KNO_2. All known strains are susceptible to digitonin and 0.1 p.p.m. cycloheximide. No tested strain shows any killer activity against *Candida glabrata* strain Y-55 (Starmer *et al.*, 1987). After 3 days growth on YM agar (Difco), cells are spherical to ellipsoid and approximately 2–3 × 4–7 µm (Fig. 2). Short pseudohyphae are rare on cornmeal agar after 10 days of growth. Three days' growth in liquid YM medium (Difco) at 30 °C produces spherical to ovoid cells, 1.3–2.5 × 2.5–5 µm. Colonies grown on malt agar are cream-coloured, with a smooth, glistening surface and an even margin. Spores can be observed on YM agar (Difco) after 6–14 days. Six asci from strain TSU00-106.5^T were dissected, which yielded 18 spore clones. All four spores germinated from three asci but only two germinated from the remaining three asci. Mating among the spore clones confirmed that the species is heterothallic, as all three complete asci segregated in the expected 2h$^+$: 2h$^-$ ratio (h$^+$ and h$^-$ symbolizing opposite mating types). Once opposite mating types are mixed, changes in the shape of vegetative cells (the appearance of subequatorial bulges characteristic of shmooing) and conjugation tube formation are observed within 1–7 days (reasons for the wide variation in the response time are not known). Spore
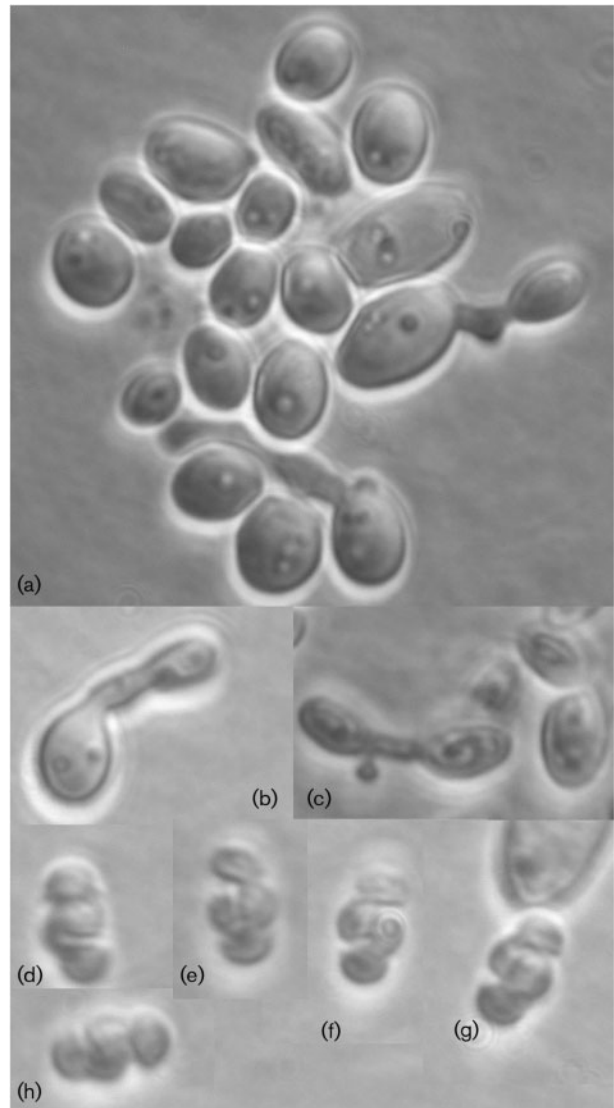
Fig. 2. Cells and dehisced asci of *Pichia insulana* sp. nov. TSU00-106.5^T after 8 days on YM agar. (a) Vegetative cells; (b, c) isolated conjugants with conjugation tube visible; (d–h) dehisced asci with four hat-shaped spores.

clones TSU00-106.5V and TSU00-106.5W were designated mating types h$^+$ and h$^-$, respectively. No mating between either *P. insulana* mating type and the asporogenous strains from Curaçao, the six UC Davis strains or the mating types of *P. pseudocactophila* produces spores or gives any indication (shmooing or conjugation tube formation) of sexual activity.

Strain TSU00-106.5^T (=CBS-11169^T =UCD-FST 09-160^T), collected from a rot pocket in a *Cereus repandus* cactus on the island of Curaçao, is the type strain. The mating type spore clones of TSU00-106.5^T are deposited at the Phaff Yeast Culture Collection, University of California Davis, Davis, CA, USA, as UCD-FST 09-161 (h+ clone) and UCD-FST 09-162 (h− clone).

Table 2. Composition of the cactophilic yeast community found on Curaçao

Opuntia sp. was probably *Opuntia caracassana*. Sites: Hat, Hato Airport; Sav, Savonet; Wac, Wacawa; WNW, Weg naar Westpunt; Wes, Westpunt. Numbers given for yeast species are the number of rots in which that species was found, and should be compared with the total number of rots to assess how common a species was at a particular collection site. Overall proportions of rots containing a given species are given on the right.

Yeast species	*Opuntia* sp.		*Stenocereus griseus*					*Cereus repandus*			Total	
	Wac	Sav	Hat	WNW	Wac	Sav	Wes	WNW	Sav	Wes	*n*	%
Pichia insulana sp. nov.	11	1	1	5	1	5	4	11	3	1	43	32
Galactomyces geotrichum	9	2	0	1	0	1	2	2	1	0	18	14
Candida sonorensis	3	1	0	2	0	3	1	1	2	0	13	10
Pichia kluyveri	0	1	0	1	0	2	1	4	1	0	10	8
Starmera 'curacao'	3	2	0	1	0	1	0	1	0	0	8	6
Pichia deserticola	0	0	1	0	0	2	2	2	0	0	7	5
Clavispora opuntiae	1	0	0	0	0	0	2	2	1	0	6	5
Sporopachydermia cereana complex	0	0	0	0	0	2	0	2	1	0	5	4
Rhodotorula graminis	1	0	0	0	0	0	0	2	0	0	3	2
Cryptococcus albidus	0	0	0	0	0	0	1	1	0	0	2	2
Geotrichum clavatum	1	0	0	0	0	1	0	0	0	0	2	2
Prototheca sp.	0	0	0	0	0	1	1	0	0	0	2	2
Aureobasidium pullulans	0	0	0	0	0	0	0	1	0	0	1	1
Candida sonorensis-like	0	0	0	0	0	0	0	0	1	0	1	1
Pichia mexicana curacao	1	0	0	0	0	0	0	0	0	0	1	1
Rhodotorula minuta	0	0	0	0	0	1	0	0	0	0	1	1
Unknown curacao 2	0	0	0	0	0	1	1	2	1	0	5	4
Unknown curacao 7	1	0	0	0	0	0	0	1	0	0	2	2
Unknown curacao 6	0	0	0	0	0	0	1	0	0	0	1	1
Unknown curacao 8	1	0	0	0	0	0	0	0	0	0	1	1
Unknown curacao 10	1	0	0	0	0	0	0	0	0	0	1	1
Site parameters												
Mean rot pH	7.6	7.0	8.3	6.1	8.5	6.7	6.5	6.5	6.3	4.5	–	–
Mean species per rot	2.36	3.50	2.00	1.67	1.00	4.00	3.20	2.91	3.67	1.00	–	–
Number of rots	14	2	1	6	1	5	5	11	3	1	–	–
Total isolates	33	7	2	10	1	20	16	32	11	1	133	–

DISCUSSION

The addition of *P. insulana* to the cactophilic yeasts is consistent with our changing perceptions of the systematics of this interesting yeast community, although it raises questions about our perceptions of the community's ecology. As mentioned in the introduction, our concept of the cactophilic yeast community has changed since the inception of its scientific study. The number of endemic species has grown steadily, mostly through examination of clusters of strains exhibiting undescribed physiological profiles. As many of the species were originally classified as members of the genus *Pichia*, it appeared at one time that an oligotrophic member of that genus had undergone a minor radiation upon colonizing cacti, a rather recent plant family (Starmer *et al.*, 1986). However, molecular evidence does not support this scenario, and the present picture is of several invasions by yeast lineages from separate clades within the Saccharomycetales (Starmer *et al.*, 2003). Given the diverse origins of the yeasts in the habitat, the yeasts that arrived became isolated from their relatives and many have undergone subsequent speciation within the habitat.

Originally, the cactophilic species appeared to exhibit two distinct geographical distributions: those with restricted local distributions and those with global distributions. Most cactophilic species belonged to the local group. For instance, *Candida orba* has been found only in the region around Brisbane, Australia (Starmer *et al.*, 2001). *Starmera* and *Phaffomyces* are both endemic genera that consist of locally distributed species (Yamada *et al.*, 1999). Most local species are sexual. Three asexual (or effectively asexual) species made up the global group: *P. cactophila*, *Cryptococcus cereanus* and *Candida sonorensis*. The global species were often the most commonly isolated yeasts within a given habitat, so that there seemed little advantage to local specialization for the global subset of cactophilic yeasts, in contrast to those with local distributions, many of which do specialize yet never dominate their communities.

This picture of three globally dominant species began to change with the discovery of sexuality in *C. cereanus*, which became *Sporopachydermia cereana* (Rodrigues De Miranda, 1978) not long after it was first described. *C. cereanus* strains (often referred to in publications as belonging to the *C. cereanus* 'complex') were noted for their physiological variability (Lachance, 1998; and our unpublished data), and reassessment of this variability and examination of partial LSU rDNA sequences revealed this global species to be another example of a cluster of related cactophilic species (Lachance *et al.*, 2001). Much less physiological variability has been reported from the two remaining global species, even when considerable local genetic variability was uncovered (Ganter & Quarles, 1997; Ganter *et al.*, 2004). The description of *P. insulana*, along with the previous description of *P. pseudocactophila*, makes it possible that the most 'global' of the global species, *P. cactophila*, is also a cluster of local, cryptic, endemic species. An observation and an absence of observations support this possibility. Some physiological variation has been found in strains collected from north-eastern Brazil, and they may represent another species in the complex. The absence of observations results from the fact that strains identified as *P. cactophila* from most regions have not yet been examined for consistent patterns of molecular variation, a means of uncovering cryptic speciation when standard identification methods do not suffice. The global species group may not survive close scrutiny.

Comparison of the distributions of *P. insulana*, *P. cactophila* and *P. pseudocactophila* raises questions that illustrate our lack of understanding of yeast ecology. All are restricted to the cactophilic habitat and are phenotypically indistinguishable. With this degree of overlap, how do all three coexist within this habitat? There is no consistent pattern to the interactions between the widespread, generalist species (*P. cactophila*) and the sexual species with restricted distributions and/or host ranges. All of the sequences from strains collected on Curaçao belonged to the *P. insulana* clade, whether or not they produced spores, so it is likely that *P. cactophila* is not found on Curaçao, making it the first cactophilic community investigated that does not include *P. cactophila*. *P. cactophila* is found throughout the Caribbean and in Venezuela, so chance does not seem a likely reason for its absence from Curaçao. The cactophilic yeast community of Curaçao was dominated by *P. insulana* (Table 2), much in the way that *P. cactophila* dominates the cactophilic yeast community at many locales (Starmer *et al.*, 1990). The six strains found in the Phaff Yeast Culture Collection show that *P. insulana* is not confined to Curaçao, but that it is not the dominant species on other islands, although the cacti found there are closely related to those on Curaçao, so host differences are not the most likely explanation for the switch from dominance to rarity. The situation of *P. insulana* on islands other than Curaçao is similar to that of *P. pseudocactophila* in the Sonoran Desert. *P. pseudocacto-phila*, which is restricted to cacti of the Pachycereinae, is rarer on its only hosts than is *P. cactophila*. Although the standard assimilation tests were developed to utilize substrates found in a generalized yeast habitat, our lack of insight into cactophilic ecology might simply result from the fact that these tests do not characterize the most ecologically relevant characteristics for this habitat. Other possibilities abound. We may need to include larger-scale killer factor screenings (the ability to kill *Candida glabrata* Y-55 was the only killer screening done) or regularly include information on a species' animal vectors before we can discern ecologically relevant differences between these species.

We have found no single physiological test that will allow one to distinguish *P. insulana* from *P. cactophila* and *P. pseudocactophila*. As part of the search for such a distinguishing physiological character, we tried growing strains of each at 45 °C. The results came as close to success as any character we tried. All *P. insulana* strains from Curaçao grew strongly at that temperature and none of the several *P. cactophila* strains we tested were able to grow at 45 °C, although there have been reports of some variability among *P. cactophila* strains in growth at this elevated temperature. There were two problems with this character as a means of separating these three species: the type strain of *P. pseudocactophila* also grew strongly at 45 °C and, of the six *P. insulana* strains identified from the Phaff Culture Collection, four did not grow at 45 °C, one grew weakly and the other strongly. These six strains were all collected over 25 years ago, and the failure of four to grow at elevated temperature may be an artefact of their long laboratory residence, but we cannot resolve this discrepancy here. However, growth at 45 °C, if combined with collection locale, may help with preliminary identification of strains of these species.

ACKNOWLEDGEMENTS

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