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Pichia lachancei sp. nov., associated with several Hawaiian plant species

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A description is given of *Pichia lachancei* sp. nov., a new species of yeast that occurs in association with several Hawaiian plant species of the genera *Tetraplasandra*, *Cheirodendron* and *Clermontia*. The new species is heterothallic and occurs in nature in the haploid as well as the diploid state. Upon conjugation of complementary mating types, zygotes are formed that reproduce by budding as diploid cells. When placed on sporulation medium, four hat-shaped spores are produced which are rapidly released from the ascus. Phylogenetic analysis showed that *P. lachancei* is most closely related to *Pichia rhodanensis* and *Pichia jadinii*. The diploid type strain of *P. lachancei*, isolated from rotting bark of *Tetraplasandra hawaiiensis* on the island of Hawaii, is strain UCD-FST 79-9^T (= ATCC 201914^T = CBS 8557^T = NRRL Y-27008^T).

Keywords: *Pichia lachancei* sp. nov., phylogenetic analysis, large subunit rDNA analysis

INTRODUCTION

During explorations in 1973 and 1978 of the yeast biota associated with native Hawaiian plants, 18 strains of a species representative of the yeast genus *Pichia* Hansen emend. Kurtzman were recovered from rotting bark and fruits of several endemic plant species on the island of Hawaii. Attempts to identify these strains by means of the available keys of Kurtzman (1984, 1998) and Barnett *et al.* (1990) failed to give satisfactory matches with known species.

Further studies involving rDNA analysis confirmed that the isolates represented a new species. Phylogenetic placement of the new species was made by comparing the nucleotide sequence of the species-specific large subunit (LSU) rDNA region D1/D2 for the type strain with sequences from all other currently recognized ascomycetous yeasts (Kurtzman & Robnett, 1997, 1998).

We propose to name the new species *Pichia lachancei*, honouring Marc-André Lachance for his many contributions to the knowledge of natural habitats of yeasts.

Abbreviations: LSU, large subunit; YM, yeast extract/malt extract agar.

The GenBank accession numbers for the sequences reported in this paper are shown in Table 2.

METHODS

Strain isolation. Samples of moist rotting bark or exudate were collected in sterile containers from branches of *Tetraplasandra hawaiiensis* Gray, *Cheirodendron* (Araliaceae) Nutt. ex Seem sp. and from decaying fruit of *Clermontia* (Campanulaceae) Gaud. sp. along Wright Road through the Olaa Tract of Hawaii Volcanoes National Park, Hawaii and several other locations on the island of Hawaii. Samples were streaked on the same day as they were collected on acidified (to pH 3·8 with 1 M HCl) yeast extract/malt extract agar (YM; Difco) and incubated at approximately 25 °C. Pure cultures were obtained by re-streaking on YM agar. Morphological and physiological characteristics of the isolates were determined by methods currently used in yeast taxonomy (Yarrow, 1998). Among the many strains isolated from the above sources, 18 strains were representative of *P. lachancei* (Table 1).

Ascospore isolation. Single ascospores were isolated from individual four-spored asci with the aid of a micromanipulator.

DNA base composition. Nuclear DNA base composition determination of the type strain was carried out by the buoyant density method in CsCl as described by Price *et al.* (1978).

DNA isolation, PCR, sequencing reactions and sequence analysis. Protocols for nuclear DNA isolation, symmetrical

Table 1. Strain numbers, host plants and ploidy of *P. lachancei* isolates

All strains were collected at various locations on the island of Hawaii, Hawaii.

Strain*	Host plant	Ploidy
UCD 73-506.1	*Cheirodendron* rotting bark with larvae	Unknown
UCD 73-507.1	*Cheirodendron* rotting bark with larvae	Unknown
UCD 73-591.1	*Cheirodendron* rotting bark with larvae	Unknown
UCD 73-593.1	*Cheirodendron* rotting bark	Unknown
UCD 73-738.2	*Tetraplasandra* rotting bark	Diploid
UCD 73-744.1	*Cheirodendron* rotting bark	Unknown
S 78-352.1 (= UCD 79-2)	*Tetraplasandra* rotting bark	h^+
S 78-353.1 (= UCD 79-3)	*Tetraplasandra* rotting bark	h^+
S 78-354.1 (= UCD 79-4)	*Tetraplasandra* rotting bark	h^-
S 78-354.2 (= UCD 79-5)	*Tetraplasandra* rotting bark	h^-
S 78-376.2^T (= UCD 79-9^T)	*Tetraplasandra* rotting bark	Diploid
UCD 79-10	Single spore isolate from UCD 79.9^T	h^+
UCD 79-11	Single spore isolate from UCD 79.9^T	h^-
UCD 79-12	Single spore isolate from UCD 79.9^T	h^-
UCD 79-13	Single spore isolate from UCD 79.9^T	h^+
S 78-385.1	*Clermontia* decaying fruit	h^+
S 78-347.1	*Cheirodendron* rotting bark	Unknown
S 78-383.1	*Cheirodendron* rotting bark	Unknown
S 78-441.1	*Cheirodendron* rotting bark	Unknown
S 78-361.2	*Tetraplasandra* rotting bark	Unknown
S 78-387.3	*Clermontia* decaying fruit	Unknown
S 78-362.3	*Tetraplasandra* rotting bark	Unknown

* UCD (= UCD-FST), Culture collection of the Department of Food Science and Technology, University of California, Davis, CA, USA. Designations that begin with S were assigned by W. T. Starmer.

amplification of LSU region D1/D2 (nucleotides 63–642 for *Saccharomyces cerevisiae*) by PCR and cycle sequencing of both strands of this region with the *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) were previously given in detail (Kurtzman & Robnett, 1997). Sequence data were visually aligned with QEDIT 2.15 (SemWare). Phylogenetic relationships were calculated with a Power Macintosh 8500/120 by the maximum-parsimony program of PAUP 3.1.1 (Swofford, 1993) with a heuristic search employing both simple and random sequence additions. Confidence limits for phylogenetic trees were estimated from bootstrap analyses (100 replications). *Schizosaccharomyces pombe* was the designated outgroup in the analyses.

Nucelotide sequence accession numbers. GenBank accession numbers are given in Table 2.

RESULTS AND DISCUSSION

On the basis of our phylogenetic analysis of nucleotide sequences from LSU region D1/D2, *P. lachancei* represents a novel species of the genus *Pichia*. It is a member of the *Pichia bimundalis* clade and is located basal to *P. rhodanensis* and *P. jadinii* (Fig. 1). *P. lachancei* can be differentiated phenotypically from *P. rhodanensis* and *P. jadinii* by its inability to assimilate maltose, trehalose and melezitose and by its lack of growth at 37 °C.

Latin diagnosis of *Pichia lachancei* sp. nov.

In YM (*Difco*) *liquido post dies* 5 *ad* 30 °C, *cellulae ovoideae vel elongatae*, 3–5 × 4–12 μm, *singulae*, *binae aut catenis brevis*; *sedimentum*; *pellicula tenuis*. *Cultura in agaro malti post unem mensem ad* 25 °C *griseola*, *butyrosa vel mollis*, *umbonata*, *rugosa*, *semi nitida*, *margo pseudomycelialis*. *In agaro farinae Zea mays post dies* 14 *pseudomycelium eumorphum*. *Cultura heterothallica*. *Asci habentes* 4 *sporos pileiformae in quoque asco*; *asci rumpunter*. *Fermentatio glucosi*, *saccharosi et raffinosi* (*tarda*). *Glucosum*, *saccharosum*, *cellobiosum*, *raffinosum*, D-*xylosum*, L-*rhamnosum*, *ethanolum*, *glycerolum*, D-*mannitolum* (*tarde*), D-*glucitolum*, *salicinum*, *methyl β*-D-*glucosidum*, *gluconoδ-lactonum*, *acidum lacticum*, *acidum succinicum*, *acidum citricum*, *ethyl acetas assimilantur at non galactosum*, L-*sorbosum*, *maltosum*, *trehalosum*, *lactosum*, *melibiosum*, *melezitosum*, *inulinum*, *amylum solubile*, L-*arabinosum*, D-*arabinosum*, D-*ribosum*, *erythritolum*, *ribitolum*, *galactitolum*, *methyl* α-D-*glucosidum*, 2-*ketogluconatum*, 5-*ketogluconatum*, *meso-inositolum*, *glucosaminum*, *N-acetyl-glucosaminum*, *hexadecanum*, 2-*propanolum*, *nec acetonum*. *Kalium nitricum*, *natrium nitrosum non assimilantur*; *ethyl aminum*, *lysinum*, *cadaverinum assimilantur*. *Ad crescentiam vitaminae additae necessariae sunt*. *Crescere potest in* 30 °C *at non in* 37 °C. *G* + *C acidi deoxyribo-*

Table 2. Strains of *Pichia lachancei* and reference species compared

T, Type strain; NT, neotype strain.

Species	Strain designation*			GenBank no.
	CBS	NRRL	UCD-FST	
'*Hansenula misumaiensis*'	8062^T	Y-17389^T		U73581
Pichia americana	5644^T	Y-2156^T		U73575
Pichia amylophila	7020^T	YB-1287^T		U73577
Pichia bimundalis	5642^T	Y-5343^T		U73574
Pichia euphorbiae	8033^T	Y-17232^T		U73580
Pichia euphorbiiphila	8083^T	Y-12742^T		U73582
Pichia fabianii	5640^T	Y-1871^T		U73573
Pichia jadinii	1600^T	Y-1542^T		U73570
Pichia japonica	7209^T	YB-2750^T		U73579
Pichia lachancei	8557^T	Y-27008^T	79-9^T	AF017412
		Y-27009	79-3h$^+$	
		Y-27010	79-4h$^-$	
Pichia meyerae	7076^T	Y-17236^T		U73578
Pichia mississippiensis	7023^T	YB-1294^T		U74597
Pichia petersonii	5555^T	YB-3808^T		U73572
Pichia rhodanensis	5518^T	Y-7854^T		U73571
Pichia veronae	6591^T	Y-7818^T		U73576
Saccharomyces cerevisiae	1171^{NT}	Y-12632^{NT}		U44806
Schizosaccharomyces pombe	356^T	Y-12796^T		U40085

*CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA; UCD-FST, Department of Food Science and Technology, University of California, Davis, CA, USA.

nucleati 40·3 mol %. *Habitatio ad corticem putrescens arborum Hawaiiensis*. *Typus*: *stirps* UCD-FST 79-9^T *ex cortex Tetraplasandrae sp. isolata est*. *In collectione zymotica Centraaalbureau voor Schimmelcultures, Delphi Batavorum sub no*. CBS 8557^T *deposita est*.

Description of the type strain of *Pichia lachancei* sp. nov.

Pichia lachancei (la.chan′cei. L. masc. gen. *lachancei* of Lachance, honouring M.-A. Lachance, a systematist and biologist of yeasts).

In YM (Difco) liquid medium after 5 d at 30 °C, the cells are ovoid to elongate or short cylindrical, 3–5 × 4–12 (sometimes 16) μm, single, in pairs and small clusters, reproducing by multilateral budding; a sediment is formed and a very thin, creeping pellicle. The streak culture on malt agar after 1 month at 25 °C is greyish, butyrous to pasty, umbonate, rugose, semi-glossy, pseudomycelial border. On cornmeal agar after 2 weeks, a well-developed pseudomycelium is present. Cells are heterothallic. On dilute vegetable agar (1:4), four hat-shaped spores are formed which are rapidly liberated from the asci upon maturity. Mating type segregation results in two h$^+$ and two h$^-$ spores. Glucose and sucrose are fermented; raffinose is very slowly fermented. The following carbon compounds are assimilated: glucose, sucrose, cellobiose, raffinose, D-xylose, L-rhamnose, ethanol, glycerol, D-mannitol (slowly), D-glucitol, salicin, methyl *β*-D-glucoside, glucono-*δ*-lactone, lactate, succinate, citrate and ethyl acetate. The following are not assimilated: galactose, L-sorbose, maltose, trehalose, lactose, melibiose, melezitose, inulin, soluble starch, L-arabinose, D-arabinose, D-ribose, i-erythitol, ribitol, galactitol, methyl α-D-glucoside, 2-ketogluconate, 5-ketogluconate, *meso*-inositol, glucosamine, *N*-acetyl-D-glucosamine, hexadecane, 2-propanol and acetone. KNO_3 and $NaNO_2$ are not utilized as sole sources of nitrogen; ethylamine, cadaverine and L-lysine are utilized. Does not grow in vitamin-free medium. Grows in amino-acid-free medium. Does not grow in the presence of 100 μg cycloheximide ml^{-1}. Good growth on YM agar containing 5% NaCl; weak growth on YM agar containing 7·5% NaCl and no growth at 10% NaCl. Does not grow in the presence of 50% (w/w) glucose. Grows at 30 °C; no growth at 37 °C. Does not hydrolyse casein or gelatin. Does not produce urease or lipolytic activity. Diazonium Blue B reaction is negative. Nuclear DNA base composition 40·3 mol % (buoyant density method). Habitat: moist rotting bark of *Tetraplasandra hawaiiensis* on the island of Hawaii. The type strain is UCD-FST 79-9^T which has been

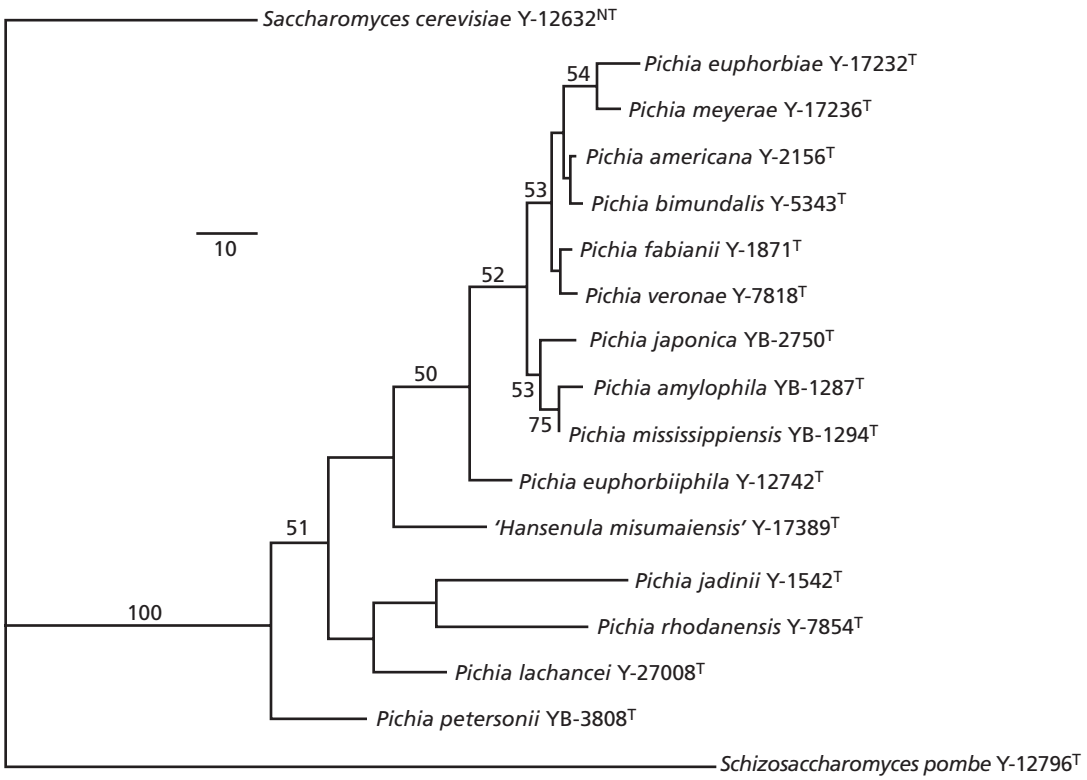

Fig. 1. Phylogenetic tree of *Pichia lachancei* and related species. The phylogram was calculated from divergence in LSU region D1/D2 and represents the single most parsimonious tree derived from maximum-parsimony analysis. Branch lengths are proportional to nucleotide differences as indicated on the marker bar. Numbers given on branches are the percentage of frequencies with which a given branch appeared in 100 bootstrap replicates. Frequencies under 50% are not given. Tree length = 389, consistency index (CI) = 0·697, homoplasy index (HI) = 0·303, retention index (RI) = 0·516 and rescaled consistency index (RC) = 0·360. Each species is represented by the type (T) or neotype (NT) strain. Strain designations are NRRL numbers. The outgroup species in the analysis was *Schizosaccharomyces pombe*.

deposited in the American Type Culture Collection as strain ATCC 201914^T, in the Centraalbureau voor Schimmelcultures, Delft, The Netherlands as strain CBS 8557^T and in the ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois, USA as NRRL Y-27008^T. Strains of *Pichia lachancei* isolated during two expeditions to Hawaii and their sources are given in Table 1. Based on rearing records of Montgomery (1975), it is assumed that *P. lachancei* is vectored by one or several picture-winged *Drosophila* species that use the three substrates indicated in Table 1 as larval breeding sites or feeding sites on the island of Hawaii. Most of the isolates of *P. lachancei* were asporogenous upon isolation, which was attributed to their haploid heterothallic state. From a single diploid ascus of strain UCD-FST 79-9^T, four spores (two h^+ and two h^-) were isolated which are represented by strains 79-10 to 79-13 (Table 1). By using these mating types, other strains were identified as haploid mating types among the isolates (strains 79-2 to 79-5) but many strains did not react with either of the two mating types (indicated by unknown in Table 1). These could represent diploid strains that had lost the ability to sporulate, a common phenomenon with *Pichia* species in culture collections. The phenotype of the asporogenous strains was identical to that of the type strain.

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