International Journal of Systematic and Evolutionary Microbiology (2001), **51**, 1201–1207 Printed in Great Britain

Candida cleridarum, *Candida tilneyi* and *Candida powellii*, three new yeast species isolated from insects associated with flowers

Marc-André Lachance,[1] Jane M. Bowles,[1] María Marta Chavarría Díaz[2] and Daniel H. Janzen[3]

[1] Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada N6A 5B7

[2] Area de Conservación Guanacaste, Apartado 169-5000, Liberia, Costa Rica

[3] Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA

Author for correspondence: Marc-André Lachance. Tel: +1 519 661 3752. Fax: +1 519 661 3935. e-mail: lachance@julian.uwo.ca

Three new asexual yeast species were isolated from various floricolous insects. *Candida cleridarum* sp. nov. was the dominant species in clerid beetles collected in flowers of various cacti in Arizona and Southern California. The sequence of the D1D2 domains of the large-subunit rDNA showed that it is a sister species to *Candida fragi* (0·9% base difference), a yeast isolated once from fermenting strawberries. *Candida tilneyi* sp. nov. and *Candida powellii* sp. nov. were recovered from bees and from nitidulid beetles in flowers of two species of morning glory (*Ipomoea*) in north-western Costa Rica. *C. tilneyi* sp. nov. is most closely related to *Candida geochares*, but differs in the D1D2 sequence by 4·7% base substitutions. *C. powellii* sp. nov. is a relative of *Candida batistae* and *Candida floricola*, showing sequence differences of 5·9 and 6·9%, respectively. In all cases, the new species are phenotypically similar to their nearest relatives, but are sufficiently different to allow conventional identification. The type strains are *C. cleridarum* strain UWO(PS) 99-101.1^T (= CBS 8793^T), *C. tilneyi* strain UWO(PS) 99-325.1^T (= CBS 8794^T) and *C. powellii* strain UWO(PS) 99-325.3^T (= CBS 8795^T).

Keywords: *Candida cleridarum* sp. nov., *Candida tilneyi* sp. nov., *Candida powellii* sp. nov., insects, flowers

INTRODUCTION

Beetles that feed in ephemeral flowers often carry a unique yeast community made up of species that are not found in any other habitats (Lachance *et al*., 1990, 1998a, b, c, 1999; Rosa *et al*., 1999a). Cactus flowers examined in the Rio de Janeiro coastal sand plain are no exception, as their yeast biota is dominated by *Kodamaea nitidulidarum*, *Candida restingae* and *Wickerhamiella cacticola*. *C. restingae* was also found in insects from a flower of *Hylocereus costaricensis*, a Costa Rican cactus (M.-A. Lachance, unpublished results), but none of these species has been reported in any other substrate. Clerid beetles found in the flowers of several cactus species distributed across the desert regions of Arizona and southern California contained, almost exclusively, an asexual ascomycetous yeast related to *Candida fragi*, which was isolated in Japan from fermenting strawberries (Suzuki *et al*., 1991). We propose to name the new species *Candida cleridarum* sp. nov.

The yeast community of morning glories (*Ipomoea* species) is dominated by *Metschnikowia* spp. (Lachance *et al*., 1990, 1998a), *Wickerhamiella* spp. (Lachance *et al*., 1998c), *Kodamaea* spp. (Rosa *et al*., 1999a; Lachance *et al*., 1999) or their asexual relatives (Lachance *et al*., 1998b, c; Rosa *et al*., 1999a), according to geography and the dominant insect species. Other yeasts may also occur in these flowers because of visits by other pollinating insects (Rosa & Lachance, 1998). *Conotelus* sp. (Nitidulidae), another nitidulid beetle, and bees collected in three *Ipomoea* species in northern Guanacaste Province, Costa Rica, carried yeasts typical of the morning-glory community in North and South America, as well as two new asexual species. We propose to name these new species *Candida tilneyi* sp. nov. and *Candida powellii* sp. nov. *C. tilneyi* is named in honour of Professor Lew Tilney of the University of Pennsylvania, in grateful rec-

Abbreviations: ACG, Area de Conservación Guanacaste; CTAB, cetyltrimethylammonium bromide.

The GenBank accession numbers for the sequences reported in this paper are AF251552–AF251554.

ognition of his generous assistance with the acquisition of the Rincón Rainforest lands for the Area de Conservación Guanacaste (ACG). *C. powellii* is named after Professor Jerry Powell of the University of California at Berkeley, in appreciation of his enthusiastic support of science in the ACG for the past two decades and his influencing of the Rainmaker Foundation in California to decide to support the Rincón Rainforest Project in the ACG.

METHODS

Isolation and characterization of yeasts. The origins of the strains considered in this study are described in Tables 1 and 2. The yeasts were isolated by allowing insects taken from flowers to walk for 15 min over plates of YM agar, supplemented with chloramphenicol (100 μg ml^{-1}). The cultures are preserved in liquid nitrogen using the Microbank system (Pro-Lab Diagnostics).

The yeasts were characterized by standard methods (Yarrow, 1998). Phenetic similarity to other described yeasts was examined by using the computer program YEASTCOMPARE (C. Ciriello & M.-A. Lachance, unpublished results), which compares the nutritional characteristics of any yeast with those of known species.

DNA sequence analysis. The D1 and D2 variable domains of the large-subunit rDNA were amplified, by using a PCR, from whole cells as described previously (Lachance *et al.*, 1999). The amplified DNA was concentrated and cleaned on QIAquick PCR columns (Qiagen) and sequenced in an ABI sequencer at the John P. Robarts Research Institute,

Table 1. Origin of strains of *Candida cleridarum* and *Candida fragi*

Strain(s)	Host plant species	Locality
Candida cleridarum		
99-101.1^T	*Opuntia phaeacantha*	Lost Dutchman State Park, AZ, USA
99-103.1, 99-104.1	*Opuntia echinocarpa*	Lost Dutchman State Park, AZ, USA
99-105.1, 99-106.1, 99-109.1	*Opuntia phaeacantha*	Pinal Hwy (Florence/Oracle Junction), AZ, USA
99-107.1, 99-108.2.1, 99-110.1	*Opuntia echinocarpa*	Pinal Hwy (Florence/Oracle Junction), AZ, USA
99-111.1, 99-112.1	*Opuntia phaeacantha*	Gilbert Ray Campground, Saguaro National Monument, AZ, USA
99-113.1, 99-114.1	*Opuntia echinocarpa*	Gilbert Ray Campground, Saguaro National Monument, AZ, USA
99-116.1	*Carnegiea gigantea*	Alamo Canyon, Organ Pipe National Monument, AZ, USA
99-117.1, 99-118.1, 99-119.1, 99-120.1	*Opuntia basilaris*	Joshua Tree National Monument, CA, USA
99-122.1	*Opuntia basilaris*	Amboy, Mojave Desert, CA, USA
Candida fragi		
NRRL-Y-17910^T	*Fragaria* sp.	Japan

Table 2. Origin of strains of *Candida tilneyi* and *Candida powellii*

Strain(s)	Plant	Insect	Locality
Candida powellii			
99-325.3^T, 99-328.1	*Ipomoea carnea*	*Conotelus* sp.	Intersection of Cuajiniquil Road and Interamerican Highway, Guanacaste, Costa Rica
99-332.2, 99-334.2	*Ipomoea carnea*	Unidentified Nitidulid beetle	Intersection of Cuajiniquil Road and Interamerican Highway, Guanacaste, Costa Rica
99-331.1	*Ipomoea carnea*	*Trigona* sp.	Intersection of Cuajiniquil Road and Interamerican Highway, Guanacaste, Costa Rica
00-109.2	*Ipomoea trifida*	*Trigona* sp.	Vicinity of Playa Naranjo, Sector Santa Rosa, ACG
00-116.2	*Ipomoea carnea*	*Conotelus* sp.	Vicinity of Playa Naranjo, Sector Santa Rosa, ACG
00-118.1	*Ipomoea carnea*	Halictid bee	Vicinity of Playa Naranjo, Sector Santa Rosa, ACG
00-191.1	*Ipomoea batatoides*	(Flower)	Sube baja, Bosque San Emilio, Sector Santa Rosa, ACG
Candida tilneyi			
99-325.1^T	*Ipomoea carnea*	*Conotelus* sp.	Intersection of Cuajiniquil Road and Interamerican Highway, Guanacaste, Costa Rica
99-331.2	*Ipomoea carnea*	*Trigona* sp.	Intersection of Cuajiniquil Road and Interamerican Highway, Guanacaste, Costa Rica
00-118.2	*Ipomoea carnea*	Halictid bee	Vicinity of Playa Naranjo, Sector Santa Rosa, ACG

London, Ontario, Canada. The sequences were edited with the program DNAMAN, version 4.0 (Lynnon BioSoft). Existing sequences for other yeasts were retrieved from GenBank. The CLUSTAL W (Thompson *et al.*, 1994) algorithm provided in the DNAMAN package was used to align the sequences and construct a neighbour-joining tree with 1000 bootstrap iterations.

RESULTS AND DISCUSSION

Phylogenetic placement

The relationship between the new *Candida* species and their closest relatives is shown in Fig. 1. The D1D2 sequence of *C. cleridarum* differed by 0·9% (5 substitutions over 561 bp) from that of *C. fragi*. On the basis of the generalizations formulated by Kurtzman & Robnett (1998) as well as comparisons between closely related heterothallic species (Lachance *et al.*, 1998a), and in the absence of sexuality, valid cases could be made either for regarding these yeasts as conspecific varieties or for viewing them as distinct species. In view of the strong habitat specificity of *C. cleridarum* and some clear differences in growth characteristics, as outlined below, we regard them as distinct species. These yeasts are part of a larger clade (Kurtzman & Robnett, 1998; not shown in Fig. 1) containing many yeasts that are often found in association with plants or insects. Moderately related teleomorphs are principally in the ascogenous genus *Debaryomyces*.

The species from morning glory belong to two sister clades (shown in their entirety in Fig. 1), in which the only known teleomorph is *Starmerella bombicola*. The D1D2 region of *C. tilneyi* differed by 4·7% substitutions from that of *Candida geochares*, its closest relative, although the neighbour-joining analysis placed the new species in a basal position with respect to four close relatives. Such a degree of sequence divergence is construed unequivocally as evidence that the yeasts are different species. Similarly, *C. powellii* is clearly distinct from its neighbours. Its closest relative in terms of pair-wise sequence similarity is *Candida batistae* (5·9% substitutions), although the neighbour-joining analysis suggests that it is a sister species to *Candida floricola*. It is probable that both yeasts are haploid mating types of a heterothallic species in the genus *Starmerella*.

Ecology and habitat specificity

The abundance and exclusivity of *C. cleridarum* in terms of its habitat is remarkable. Of 22 beetle samples examined, 19 contained this yeast, usually in large numbers. Of these, only four samples contained other yeast species, all in smaller numbers. The beetles were collected over an area of approximately 200×400 km and from five cactus species (Table 1). The single beetle isolated from a flower of *Echinocereus triglochidiana* did not yield *C. cleridarum*. Other members of the same clade (Kurtzman & Robnett, 1998) occur mostly in association with fungi, flowers and decaying trees (Meyer *et al.*, 1998).

C. tilneyi and *C. powellii* have not been observed in floricolous beetles examined in major collections from North and South America, Hawaii, Australia and some islands of the south-west Pacific (M.-A. Lachance and co-workers, unpublished results). We therefore hypothesize that the bees visiting *Ipomoea* spp. flowers may be instrumental in the occurrence of these yeasts (Table 2). In particular, the flowers of *Ipomoea batatoides* occurred near the tops of tall trees and may not have been visited by the low-flying nitidulids. Other members of the *Starmerella* clade (Fig. 1) occur in flowers, solitary and social bees, bumblebees, beetles and other insects, and have been reported in less-specific substrates including clinical and domestic materials (Meyer *et al.*, 1998; Rosa & Lachance, 1998; Rosa *et al.*, 1999b).

Our recent studies in north-western Guanacaste Province have revealed the presence, in bees that visit morning glories, of several other relatives of all three species described here. Although their description will await the isolation of additional specimens, it is noteworthy that the careful study of tropical floricolous insects is indeed broadening our knowledge of the yeast biodiversity of the insect–flower ecosystem.

Identification

C. cleridarum is very similar to its sister species, *C. fragi*, but enough differences exist to allow conventional identification. *C. fragi* assimilates cellobiose, has a lower maximum growth temperature (maximum, 31–32 °C; Suzuki *et al.*, 1991; weak growth only at 30 °C) and does not grow in the presence of 50% glucose or cetyltrimethylammonium bromide (CTAB) (75 μg ml^{-1}). Suzuki *et al.* (1991) discussed the general similarity between *C. fragi* and other *Candida* species, in particular *Candida natalensis*, *Candida oleophila* and *Candida sake*. The same observations were made by Meyer *et al.* (1998) in their most recent treatment of the genus *Candida*. Our examination of type strains of several species in the *C. cleridarum* clade (Fig. 1) revealed that there is no difficulty in differentiating the new species from its neighbours on the basis of growth tests. In particular, no other member of the clade can grow at 37 °C (nor does *C. sake*). Many yeasts, whether related to *C. sake* or not, are easily misidentified as *C. sake* because of the heterogeneity of that taxon as currently defined. As recent rDNA sequencing efforts (Kurtzman & Robnett, 1998) eventually achieve their full impact on the classification of *Candida* species, these difficulties should disappear, or at least be substantially alleviated.

The yeasts in the *Starmerella* clade are generally very similar to one another in their phenotypic properties. *C. tilneyi* most closely resembles *C. geochares*, but is easily differentiated on the basis of the assimilation of sucrose, cellobiose, salicin and ribose by the latter. *C. powellii* is distinguished from *S. bombicola* principally by its growth on maltose and at 37 °C, from *C. floricola* by the lack of growth on sucrose and raffinose

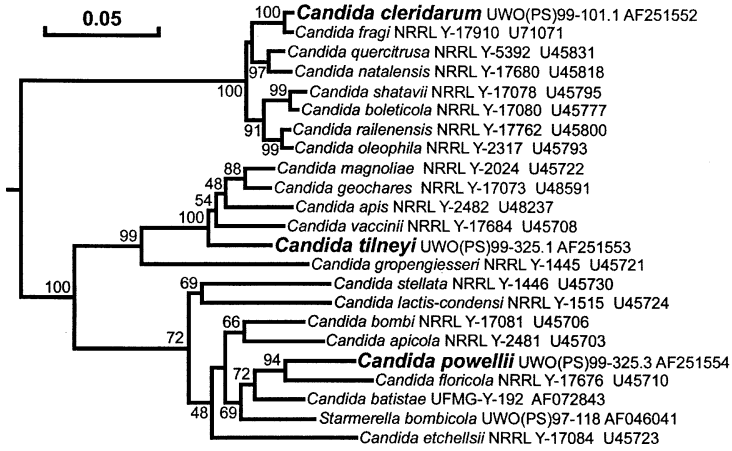

Fig. 1. Neighbour-joining tree showing the phylogenetic placement of *Candida cleridarum*, *Candida tilneyi* and *Candida powellii*, based on the sequence of the D1D2 divergent domains of the large-subunit rDNA. Note that the clade containing *C. cleridarum* is not a sister clade to that containing the other two new species. The percentage bootstrap values were obtained from 1000 iterations. The scale bar shows 5% sequence divergence. All strains shown are type strains.

and from *C. batistae* by growth on maltose and succinic acid.

Latin diagnosis of *Candida cleridarum* Lachance sp. nov.

In medio liquido post dies tres cellulae singulae, binae, aut in catenis brevis, ovoidae aut bacilliformes (3–5 × 3–8 μm). *Cellulae longiores formari possunt. Post unum mensem annulus tenuis et sedimentum formantur. Cultura in agaro malti post dies* 14 (17 °C), *infimo-convexa, tumulosa, glabra, candida et butyrosa. In agaro farinae Zea mays post dies* 14 *pseudomycelium formatur. Asci non formantur. Glucosum et sucrosum* (*exigue et lente*) *fermentantur. Sucrosum, galactosum* (*lente*), *maltosum, melezitosum, methyl* α-D-*glucosidum* (*exigue*), *salicinum,* L-*sorbosum,* D-*xylosum,* D-*ribosum* (*variabile et exigue*), *ethanolum* (*exigue*), *glycerolum, ribitolum* (*lente*), *xylitolum* (*lente*), *mannitolum, glucitolum, acidum lacticum* (*exigue*), *acidum succinicum, acidum citricum* (*lente*), *acidum malicum* (*lente*), *acidum gluconicum* (*lente*), *glucono-δ-lactonum* (*lente*), 2-*ketogluconatum, glucosaminum* (*exigue*), *N-acetylglucosaminum et hexadecanum* (*exigue*) *assimilantur, at non inulinum, raffinosum, melibiosum, lactosum, trehalosum* (*aliquando exigue*), *amylum solubile, cellobiosum,* L-*rhamnosum,* L-*arabinosum,* D-*arabinosum, methanolum,* 1-*propanolum,* 2-*propanolum,* 1-*butanolum, erythritolum, galactitolum, meso-inositolum, acetonum nec ethyl acetas. Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum. Ad crescentiam vitaminae externae necessariae sunt. Augmentum in* 37 °C. *Habitat floras cactorum et scarabeos junctes Cleridae in* Arizona *et* California. *Typus* UWO(PS) 99-101.1^{T}. *In collectione zymotica* Centraalbureau voor Schimmelcultures, *Delphi Batavorum, sub* no. CBS 8793^{T} *deposita est.*

Description of *Candida cleridarum* Lachance sp. nov.

Candida cleridarum (cle.ri.da′rum. N.L. gen. pl. fem. n. *cleridarum* of Cleridae, referring to the family of beetles that act as vectors for this yeast species).

In yeast extract (0·5%) glucose (2%) broth after 3 d at 25 °C, cells are ovoid to bacilliform, occur singly or in parent–bud pairs and measure 3–5 × 3–8 μm (Fig. 2). Some elongated cells, up to 15 μm long, may be formed. A thin ring may be formed. A sediment is formed after 1 month. On malt agar after 2 weeks at 17 °C, colonies are low-convex, umbonate, glabrous, smooth, white and butyrous. Undifferentiated pseudohyphae consisting of elongated cells are formed after 2 weeks in Dalmau plate cultures on cornmeal agar. The pseudohyphal cells may be swollen. Conjugation and ascus formation are not observed, even after mixing strains in pairs. Fermentation: in glucose, gas production begins after 3–4 d and a full tube of gas develops after 6–7 d. Sucrose is fermented weakly and slowly. Assimilation of carbon compounds: sucrose, galactose (slow), maltose, melezitose, methyl α-D-glucoside (weak), salicin, L-sorbose, D-xylose, D-ribose (variable and weak), ethanol (weak), glycerol, ribitol (slow), xylitol (slow), mannitol, glucitol, lactic acid (weak), succinic acid, citric acid (slow), malic acid (slow), D-gluconic acid (slow), glucono-δ-lactone (slow), 2-ketogluconic acid, D-glucosamine (weak), *N*-acetylglucosamine and hexadecane (weak) are assimilated; no growth occurs on inulin, raffinose, melibiose, lactose, trehalose (rarely weak), starch, cellobiose, L-rhamnose, L-arabinose, D-arabinose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, galactitol, *meso*-inositol, acetone or ethyl acetate. Assimilation of nitrogen compounds: positive for ethylamine, lysine and cadaverine; negative for sodium nitrate and sodium nitrite. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Gelatin liquefaction and casein hydrolysis are negative. Lipolytic activity on Tween 80 agar is positive. Acid formation on chalk agar is negative. Growth on YM agar with 5% sodium chloride is positive; with 10% sodium chloride, negative (rarely weak). Growth on 50% glucose/yeast extract agar is slow. Growth in the presence of 10 μg cycloheximide ml^{-1} is negative. Growth in the presence of 8 μg digitonin ml^{-1} at 25 °C is negative. Growth in the presence of 75 μg CTAB ml^{-1} is positive (usually slow). Production of starch-like compounds is nega-

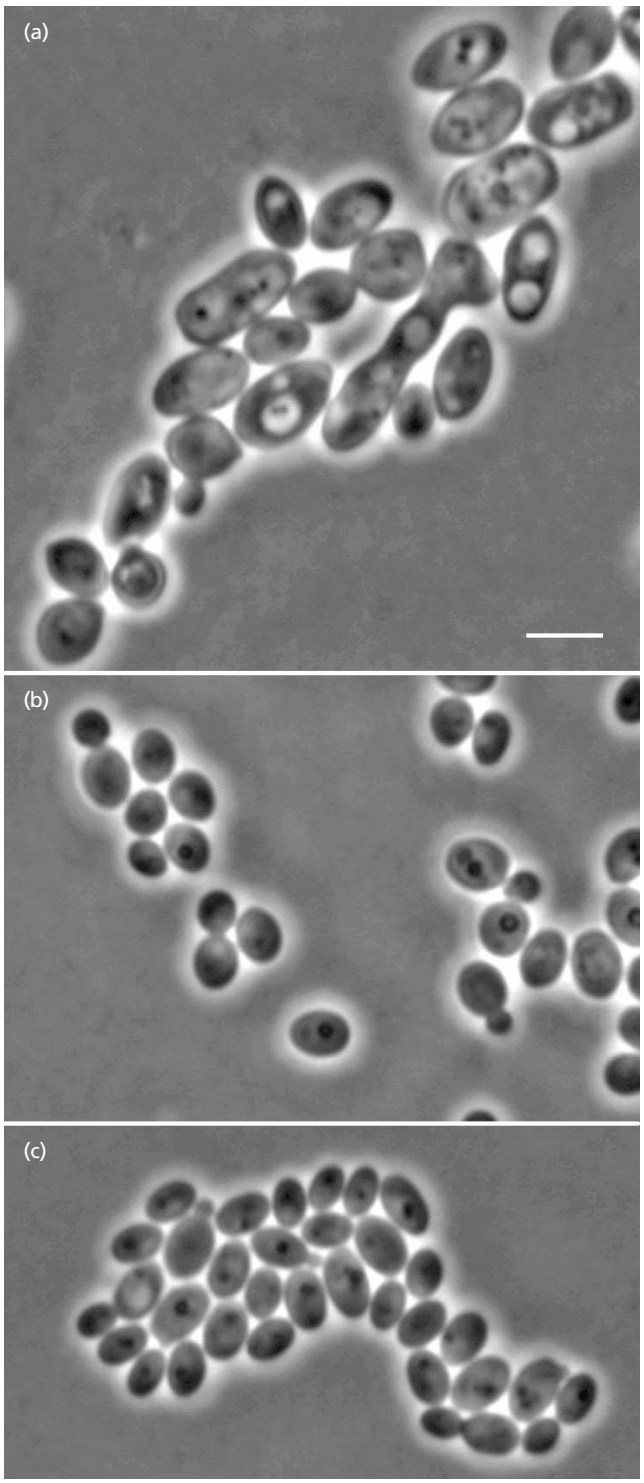

Fig. 2. Phase-contrast photomicrographs of *C. cleridarum* (a), *C. tilneyi* (b) and *C. powellii* (c). Bar, 5 μm.

tive. Urease activity is negative. The Diazonium Blue B reaction is negative. The habitat is in flowers of cacti and associated clerid beetles in the south-western USA. The type strain of *Candida cleridarum* is strain UWO(PS) 99-101.1^T. It was isolated from a clerid beetle collected in a flower of *Opuntia phaeacantha* in Lost Dutchman State Park, AZ, USA. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, the Netherlands, as strain CBS 8793^T.

Latin diagnosis of *Candida tilneyi* Lachance sp. nov.

In medio liquido post dies tres cellulae singulae, *binae*, *aut in catenis brevis*, *globosae aut ovoidae* (1–3 × 2–4 μm). *Post unum mensem velum nitidum et sedimentum formantur*. *Cultura in agaro malti post dies* 14 (17 °C), *infimo-convexa*, *tumulosa*, *glabra*, *candida et butyrosa*. *In agaro farinae Zea mays post dies* 14 *pseudomycelium aut mycelium verum non formantur*. *Asci non formantur*. *Glucosum fermentatur*. *Galactosum*, L-*sorbosum*, *glycerolum*, *ribitolum* (*lente*), *xylitolum* (*lente*), *mannitolum* (*aliquando lente*), *glucitolum*, *acidum succinicum* (*lente*), *acidum citricum* (*lente*), *acidum malicum* (*lente*), *acidum gluconicum*, *glucono-δ-lactonum et* 2-*ketogluconatum* (*lente*) *assimilantur*, *at non inulinum*, *sucrosum*, *raffinosum*, *melibiosum*, *lactosum*, *trehalosum*, *maltosum*, *melezitosum*, *methyl* α-D-*glucosidum*, *amylum solubile*, *cellobiosum*, *salicinum*, L-*rhamnosum*, D-*xylosum*, L-*arabinosum*, D-*arabinosum*, D-*ribosum*, *methanolum*, *ethanolum*, 1-*propanolum*, 2-*propanolum*, 1-*butanolum*, *erythritolum*, *galactitolum*, *meso-inositolum*, *acidum lacticum*, *glucosaminum*, *N-acetylglucosaminum*, *acetonum*, *ethyl acetas nec hexadecanum*. *Ethylaminum* (*lente*), *lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum*. *Ad crescentiam vitaminae externae necessariae sunt*. *Augmentum in* 30 °C, *at non* 37 °C. *Habitat floras Ipomoea* spp. *et insectos junctes in* Costa Rica. *Typus* UWO(PS) 99-325.1^T. *In collectione zymotica* Centraalbureau voor Schimmelcultures, *Delphi Batavorum*, *sub* no. CBS 8794^T *deposita est*.

Description of *Candida tilneyi* Lachance sp. nov.

Candida tilneyi (til′ney.i. L. gen. sing. masc. n. *tilneyi* of Tilney, referring to Lew Tilney of the University of Pennsylvania, in whose honour the species is named).

In yeast extract (0·5 %) glucose (2 %) broth after 3 d at 25 °C, cells are spherical to ovoid, occur singly or in parent–bud pairs and measure 1–3 × 2–4 μm (Fig. 2). A very thin pellicle is formed. A sediment is formed after 1 month. On malt agar after 2 weeks at 17 °C, colonies are low-convex, glabrous, smooth, white and butyrous. In Dalmau plate cultures on cornmeal agar after 2 weeks, hyphae or pseudohyphae are not formed. Conjugation and ascus formation are not observed, even after mixing strains in pairs. Fermentation: in glucose, gas production begins after 3–4 d and a full tube of gas develops after 7–8 d. Assimilation of carbon compounds: galactose, L-sorbose, glycerol, ribitol (weak), xylitol (slow), mannitol (slow or positive), glucitol, succinic acid (slow), citric acid (slow), malic acid (slow), D-gluconic acid, glucono-δ-lactone and 2-ketogluconic acid (slow) are assimilated; no

growth occurs on inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, melezitose, methyl α-D-glucoside, salicin, D-xylose, D-ribose, starch, cellobiose, L-rhamnose, L-arabinose, D-arabinose, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, erythritol, galactitol, *meso*-inositol, lactic acid, D-glucosamine, *N*-acetylglucosamine, acetone, ethyl acetate or hexadecane. Assimilation of nitrogen compounds: positive for ethylamine (slow), lysine and cadaverine; negative for sodium nitrate and sodium nitrite. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 30 °C is positive; growth at 37 °C is negative. Gelatin liquefaction is weak. Casein hydrolysis is negative. Lipolytic activity on Tween 80 agar is negative. Acid formation on chalk agar is negative. Growth on YM agar with 5% sodium chloride is positive; with 10% sodium chloride, slow. Growth on 50% glucose/yeast extract agar is slow. Growth in the presence of 10 μg cycloheximide ml^{-1} is slow; growth with 100 μg cycloheximide ml^{-1} is negative. Growth in the presence of 8 μg digitonin ml^{-1} at 25 °C is negative. Growth in the presence of 75 μg CTAB ml^{-1} is negative. Production of starch-like compounds is negative. Urease activity is negative. The Diazonium Blue B reaction is negative. The habitat is in flowers of morning glories and associated insects in Costa Rica. The type strain of *Candida tilneyi* is strain UWO(PS) 99-325.1^T. It was isolated from a beetle (*Conotelus* sp.) of a flower of *Ipomoea carnea* in Guanacaste, Costa Rica. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, the Netherlands, as strain CBS 8794^T.

Latin diagnosis of *Candida powellii* Lachance sp. nov.

In medio liquido post dies tres cellulae singulae, *binae*, *aut in catenis brevis*, *globosae aut ovoidae* (1–3 × 2–4 μm). *Post unum mensem annulus tenuis et sedimentum formantur*. *Cultura in agaro malti post dies* 14 (17 °C), *infimo-convexa*, *tumulosa*, *glabra*, *candida et butyrosa*. *In agaro farinae Zea mays post dies* 14 *pseudomycelium aut mycelium verum non formantur*. *Asci non formantur*. *Glucosum et maltosum fermentantur*. *Galactosum* (*lente*), *maltosum*, L-*sorbosum*, D-*ribosum* (*lente*), *glycerolum*, *xylitolum* (*lente aut exigue*), *mannitolum*, *glucitolum*, *acidum succinicum* (*lente*), *acidum citricum* (*lente*), *acidum malicum* (*lente*) *et glucono-δ-lactonum* (*lente*) *assimilantur*, *at non inulinum*, *sucrosum*, *raffinosum*, *melibiosum*, *lactosum*, *trehalosum*, *melezitosum*, *methyl* α-D-*glucosidum*, *amylum solubile*, *cellobiosum*, *salicinum*, L-*rhamnosum*, D-*xylosum*, L-*arabinosum*, D-*arabinosum*, *methanolum*, *ethanolum*, 1-*propanolum*, 2-*propanolum*, 1-*butanolum*, *erythritolum*, *ribitolum*, *galactitolum*, *meso-inositolum*, *acidum lacticum*, *acidum gluconicum*, 2-*ketogluconatum*, *glucosaminum*, *N-acetylglucosaminum*, *acetonum*, *ethyl acetas nec hexadecanum*. *Ethylaminum*, *lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum*. *Ad crescentiam vitaminae externae necessariae sunt*. *Augmentum in* 37 °C. *Habitat floras Ipomoea* spp. *et insectos junctes in* Costa Rica. *Typus* UWO(PS) 99-325.3^T. *In collectione zymotica* Centraalbureau voor Schimmelcultures, *Delphi Batavorum*, *sub* no. CBS 8795^T *deposita est*.

Description of *Candida powellii* Lachance sp. nov.

Candida powellii (po′wel.li.i. L. gen. sing. masc. n. *powellii* of Powell, referring to Jerry Powell of the University of California at Berkeley, in whose honour the species is named).

In yeast extract (0·5%) glucose (2%) broth after 3 d at 25 °C, cells are spherical to ovoid, occur singly or in parent–bud pairs and measure 1–3 × 2–4 μm (Fig. 2). A thin ring may be formed. A sediment is formed after 1 month. On malt agar after 2 weeks at 17 °C, colonies are low-convex, glossy, smooth, white and butyrous. In Dalmau plate cultures on cornmeal agar after 2 weeks, hyphae or pseudohyphae are not formed. Conjugation and ascus formation are not observed, even after mixing strains in pairs. Fermentation: in glucose, gas production begins after 3–4 d, and a full tube of gas develops after 6–7 d. In maltose, gas production begins after 5–6 d, and a full tube of gas develops after 9–10 d. Assimilation of carbon compounds: galactose (slow), maltose, L-sorbose, D-ribose (slow), glycerol, xylitol (slow or weak), mannitol, glucitol, succinic acid (slow), citric acid (slow), malic acid (weak) and glucono-*δ*-lactone (slow) are assimilated; no growth occurs on inulin, sucrose, raffinose, melibiose, lactose, trehalose, melezitose, methyl α-D-glucoside, starch, cellobiose, salicin, L-rhamnose, D-xylose, L-arabinose, D-arabinose, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, erythritol, ribitol, galactitol, *meso*-inositol, lactic acid, D-gluconic acid, 2-ketogluconic acid, D-glucosamine, *N*-acetylglucosamine, acetone, ethyl acetate or hexadecane. Assimilation of nitrogen compounds: positive for ethylamine, lysine and cadaverine; negative for sodium nitrate and sodium nitrite. Growth in vitamin-free medium is negative (background growth may be red-pigmented). Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Gelatin liquefaction and casein hydrolysis are negative. Lipolytic activity on Tween 80 agar is positive. Acid formation on chalk agar is negative. Growth on YM agar with 5% sodium chloride is weak; with 10% sodium chloride, negative. Growth on 50% glucose/yeast extract agar is positive. Growth in the presence of 10 μg cycloheximide ml^{-1} is positive; growth with 100 μg cycloheximide ml^{-1} is negative. Growth in the presence of 8 μg digitonin ml^{-1} at 25 °C is negative. Growth in the presence of 75 μg CTAB ml^{-1} is negative or slow. Production of starch-like compounds is negative. Urease activity is negative. The Diazonium Blue B reaction is negative. The habitat is in flowers of morning glories and associated insects in Costa Rica. The type strain of *Candida powellii* is strain UWO(PS) 99-325.3^T. It was isolated from a beetle (*Conotelus* sp.)

of a flower of *Ipomoea carnea* in Guanacaste, Costa Rica. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, the Netherlands, as strain CBS 8795^T.

ACKNOWLEDGEMENTS

This work was funded by the Natural Science and Engineering Research Council of Canada (M.-A. L.) and by the National Science Foundation, USA (D. H. J.). We thank C. P. Kurtzman for the gift of strains and S. Caveney for his help with beetle identification. Sample collection in the south-western United States took place during the 1999 Ontario Universities Field Course in desert ecology. We acknowledge the cooperation of the personnel of the Area de Conservación Guanacaste, Sector Santa Rosa, and the permission to conduct research in the conservation area. The sequencing expertise of P. Campbell and H. Schneider is gratefully acknowledged.

REFERENCES

Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Leeuwenhoek* **73**, 331–371.

Lachance, M.-A., Starmer, W. T. & Phaff, H. J. (1990). *Metschnikowia hawaiiensis* sp. nov., a heterothallic haploid yeast from Hawaiian morning glory and associated drosophilids. *Int J Syst Bacteriol* **40**, 415–420.

Lachance, M.-A., Rosa, C. A, Starmer, W. T, Schlag-Edler, B., Barker, J. S. F. & Bowles, J. M. (1998a). *Metschnikowia continentalis* var. *borealis*, *Metschnikowia continentalis* var. *continentalis*, and *Metschnikowia hibisci*, new heterothallic haploid yeasts from ephemeral flowers and associated insects. *Can J Microbiol* **44**, 279–288.

Lachance, M.-A., Rosa, C. A, Starmer, W. T. & Bowles, J. M. (1998b). *Candida ipomoeae*, a new yeast species related to large-spored *Metschnikowia* species. *Can J Microbiol* **44**, 718–722.

Lachance, M.-A., Rosa, C. A, Starmer, W. T, Schlag-Edler, B., Barker, J. S. F. & Bowles, J. M. (1998c). *Wickerhamiella australiensis*, *Wickerhamiella cacticola*, *Wickerhamiella occidentalis*, *Candida drosophilae* and *Candida lipophila*, five new related yeast species from flowers and associated insects. *Int J Syst Bacteriol* **48**, 1431–1443.

Lachance, M.-A., Bowles, J. M, Starmer, W. T. & Barker, J. S. F. (1999). *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian *Hibiscus* flowers. *Can J Microbiol* **45**, 172–177.

Meyer, S. A, Payne, R. W. & Yarrow, D. (1998). *Candida* Berkhout. In *The Yeasts*, *a Taxonomic Study*, 4th edn, pp. 454–573. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

Rosa, C. A. & Lachance, M.-A. (1998). The yeast genus *Starmerella* gen. nov. and *Starmerella bombicola* sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow. *Int J Syst Bacteriol* **48**, 1413–1417.

Rosa, C. A, Lachance, M.-A., Starmer, W. T, Barker, J. S. F., Bowles, J. M. & Schlag-Edler, B. (1999a). *Kodamaea nitidulidarum*, *Candida restingae* and *Kodamaea anthophila*, three new related yeast species from ephemeral flowers. *Int J Syst Bacteriol* **49**, 309–318.

Rosa, C. A, Viana, E. M, Martins, R. P, Antonini, Y. & Lachance, M.-A. (1999b). *Candida batistae*, a new yeast species associated with solitary digger nesting bees in Brazil. *Mycologia* **91**, 428–433.

Suzuki, M., Nakase, T. & Fukazawa, Y. (1991). *Candida fragi*, a new species of anamorphic yeast isolated from fermenting strawberry. *J Gen Appl Microbiol* **37**, 423–429.

Thompson, J. D, Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Yarrow, D. (1998). Methods for the isolation and identification of yeasts. In *The Yeasts*, *a Taxonomic Study*, 4th edn, pp. 77–100. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.