A novel species of ascomycetous yeast, Candida coquimbonensis sp. nov., from the necrotic tissue of cacti in Chile and Australia is described. C. coquimbonensis sp. nov. is closely related and phylogenetically similar to Phaffomyces opuntiae. There is no overlap in the geographical distribution between C. coquimbonensis and any species in the Phaffomyces clade. However, this is the first member of the clade to be collected in both native (Chile) and non-native (Australia) cactus habitats. The type strain of C. coquimbonensis sp. nov. is TSU 00-206.4B$^T$ (=CBS 12348$^T$=USCFST 12-103$^5$).

Necrotic cactus tissue harbours several interesting lineages of yeast. One such clade includes all three members of the genus Phaffomyces and the species Candida orba. Kurtzman et al. (2008) found 100% bootstrap support for this clade based on concatenated sequence data from three loci. In addition, the clade is split into two subclades, again with 100% bootstrap support (grouping also supported by DNA–DNA reassociation data, Starmer et al., 2001). One subclade includes Phaffomyces thermotolerans and C. orba, and the other subclade includes Phaffomyces opuntiae and Phaffomyces antillensis. P. opuntiae and C. orba have been found only in Australia, where cacti were introduced a little more than 200 years ago (Dodd, 1940). Because no other cactophilic yeast species is confined to Australia (or to any other introduced populations of cacti), the distributions of P. opuntiae and C. orba are problematic. Since the Australian cacti are so recent, neither species is likely to have arisen there. However, no member of either species has been isolated from native cactus habitats. North American cacti have been sampled for yeast far more often than South American cacti and for this reason, cactus populations in Peru, Chile, Argentina and Brazil were sampled in 1990, 2000 and 2002. Although these collections failed to isolate any members of the previously mentioned clade, the collection in 2000 produced five isolates of a fifth member of the clade, which had a physiological profile that matched that of an isolate collected from Australia in the same year. These six isolates represent a novel species, Candida coquimbonensis sp. nov., which we describe here.

**Strain collection and isolation and determination of physiological and morphological characteristics**

The strains used in this study were isolated from necrotic cactus tissue by standard methods (Table 1; Starmer & Phaff, 1983). Morphological and physiological tests were performed as described in Yarrow (1998) and, for tests specific to cactophilic yeast, Lachance et al. (1988). After initial identification (within 2 months after collection, Table 1), strains were lyophilized and stored at room temperature. To complete the work for this study, strains were revived and stored at −80°C in YEPD broth/glycerol, 5:1, v/v. Because the original isolation and testing took place at Tennessee State University, USA, in 2000, we restested each strain at the University of Perugia, Italy, in 2010 to confirm the original profile. No significant differences were found between the two sets of tests.

Strains were examined for ascospore formation, twice. The first time was during the initial identification of each strain from colonies grown on yeast extract–malt extract agar (after 3 days, 1 and 2 weeks). The second time was during the recent re-identification on McClary’s acetate agar (after 5 days at 25°C). Matings were attempted of all possible pairs of strains. Each strain and attempted mating was examined microscopically for mating activity and ascospores.

**rDNA sequence determination**

Genomic DNA was extracted from each strain (Bolano et al., 2001; Cardinali et al., 2001). Amplification of domain D1/
D2 of 26S rDNA was performed using the primers NL-1 (5’-GCATATCAATAAGCGGAGGAAAG-3’) and NL-4 (5’-GGTCCTGTGTGCTTAAGAGGG-3’) (O’Donnell, 1993) with EurowTaq (Euroclone) and a PTC-100 Peltier Thermal Cycler (MJ Research). Amplification was performed as in Kurtzman & Robnett (1998) with the following specifics: initial denaturation at 95 °C for 4 min, 35 amplification cycles (94 °C for 1 min, 53 °C for 1 min and 72 °C for 1 min) and a final extension at 72 °C for 10 min. Amplicons were purified using a GFX PCR DNA purification kit (GE Healthcare) with electrophoresis on 1.5% agarose gel (Gellyphor; EuroClone).

Sequences were aligned using CLUSTAL_X (Larkin et al., 2007), DIALIGN 2 (Morgenstern, 1999), and SOAP (Löytynoja & Milinkovitch, 2001). Consensus was used to resolve differences between alignments. Pairwise differences among sequences were determined using ARLEQUIN 2.00, 2000) and phylogenies were constructed with PAUP* (Swofford, 1999).

Latin diagnosis of Candida coquimbonensis sp. nov.

Candida coquimbonensis (co.quim.bo.nen’sis. N. L. fem. ex nomine ‘Coquimbo’, regionis Chileanae qua quinque stipites isolati sunt).


Description of Candida coquimbonensis sp. nov.

Candida coquimbonensis (co.quim.bo.nen’sis. N.L. fem. adj. coquimbonensis of or pertaining to Coquimbo, the Chilean region from which five of the six strains were collected).

Glucose is not fermented and pellicles do not form in the fermentation tubes. Carbon sources strongly utilized by all strains are D-glucose, ethanol, glycerol, D-sorbitol, DL-lactic acid, succinic acid and citric acid. All strains exhibit delayed growth on salicin and weak growth on malic acid. Carbon sources to which the response of the strains varied are maltose, cellobiose and glucono-D-lactone. Carbon sources not utilized by any strain are D-galactose, L-sorbosum, sucrose, trehalose, lactose, melibiose, raffinose, melezitose, inulins, soluble starch, D-xylose, L- or D-arabinose, D-ribose, L-rhamnose, i-erythritol, adonitol, dulcitol, D-mannitolum, methyl 2-D-glucoside, ketogluconate, glucosamine, myo-inositol, glucuronic acid, N-acetylglucosamine, methanol, ethyl acetate, acetone and 2-propanol.

Nitrogen sources weakly utilized are ethylamine and lysine. Nitrate, nitrite and glucosamine are not utilized. All strains grow at 4 °C. Growth at 37 °C is variable and no strain grows at 42 °C. All strains show delayed growth on glucose with 5% NaCl (w/v) but none grow in the presence of 10% or 12% NaCl, or 50% glucose. No strain grows on 0.1 p.p.m. cycloheximide or on digitonin. There is no evidence of the production of extracellular protease, lipase or urease. After 3 days at 25 °C on YM agar, streak cultures produce small, round colonies with sharp edges with a

<table>
<thead>
<tr>
<th>TSU collection no.</th>
<th>UP collection no.</th>
<th>Locale</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSU 00-205.3</td>
<td>LCF 1581</td>
<td>Pichidanguis, Chile</td>
<td>Echinopsis chiloensis necrosis</td>
</tr>
<tr>
<td>TSU 00-206.1</td>
<td>LCF 1561</td>
<td>Pichidanguis, Chile</td>
<td>Echinopsis chiloensis necrosis</td>
</tr>
<tr>
<td>TSU 00-206.4B</td>
<td>LCF 1562</td>
<td>Pichidanguis, Chile</td>
<td>Echinopsis chiloensis necrosis</td>
</tr>
<tr>
<td>TSU 00-214.2A</td>
<td>LCF 1565</td>
<td>Ovalle, Chile</td>
<td>Eulychnia acida necrosis</td>
</tr>
<tr>
<td>TSU 00-214.2B</td>
<td>LCF 1566</td>
<td>Ovalle, Chile</td>
<td>Eulychnia acida necrosis</td>
</tr>
<tr>
<td>TSU 00-437.1</td>
<td>LCF 1607</td>
<td>Angorachina Station, SA, Australia</td>
<td>Opuntia stricta fruit</td>
</tr>
</tbody>
</table>
glossy white colour. After growth in YM broth at 25 °C for 3 days, the cells are spherical (3–4 × 2.5–3 μm) and occur singly or in pairs (Fig. 1). Vegetative reproduction occurs by multilateral budding. After 7 days, sediment is present in the tubes.

Strain TSU 00-206.4BT, collected from a necrotic Echinopsis chilensis cactus near the Chilean town of Pichidangui, is designated the type strain of Candida coquimbonensis (=CBS 12348T = USCFST 12-103T). The binomial has been registered with MycoBank (MB800643 at www.mycobank.org).

Discussion

The LSU rDNA sequences of the strains listed in Table 1 were deposited in GenBank. The alignment yielded a 540 bp segment, excluding gaps. Analysis of the sequences as pairwise differences between strains demonstrates the separation of the six sequenced C. coquimbonensis strains from other members of the Phaffomyces clade. Pairwise sequence differences between C. coquimbonensis strains range from 0 to 1.4 % (mean=0.8 %). Differences between the P. opuntiae type strain, the Phaffomyces taxon most similar to C. coquimbonensis, and the six C. coquimbonensis strains ranged from 2.2 to 3.6 % (mean=2.9 %). Fig. 2 shows the neighbour-joining tree (based on Tajima–Nei distances) for the Phaffomyces clade. Parsimony analysis (exhaustive search) produced three trees. One tree was identical to that shown in Fig. 2 and the other two trees differed only in how TSU 00-205.3, TSU 00-437.1 and TSU 00-206.4BT were arranged (data not shown). Bootstrap support was high (70 % or higher) for all branches of the tree. There was 100 % bootstrap support for the node that splits P. opuntiae from all C. coquimbonensis strain sequences (85 % for bootstrap based on parsimony).

Unlike many yeast species, there was some within-species variation in the portion of the LSU rDNA genes of the strains sequenced for this study. The largest distance between C. coquimbonensis strains exceeds the rule-of-thumb minimum between-species distance of 1 %. However, because each of the six C. coquimbonensis strains have at least one other C. coquimbonensis strain with which the pairwise distance is less than 1 %, it is impossible to separate the cluster based on LSU rDNA gene sequence alone. Given the consistent differences in physiologies between C. coquimbonensis and P. opuntiae (its sister taxon), it is best to describe these strains as a single taxon. It is possible that asexuality has allowed greater variation within the clade than within sexual lineages and may indicate a somewhat greater age for the clade, although rDNA is not an ideal molecular clock for within-species variation.

C. coquimbonensis has a physiological profile that is distinct from all other described Phaffomyces clade taxa (Table 2). It is the only member of the clade to assimilate sorbitol. That unique characteristic and its growth on citric acid medium distinguish it from its closest relative, P. opuntiae. Although all strains of C. coquimbonensis collected thus far have failed to mate or produce spores, it is not certain that the lineage is asexual. It is possible that all known strains are of the same mating type. P. thermotolerans is normally collected from the environment as a haploid (Starmer et al., 1979). The situation for C. coquimbonensis is similar to that for C. orba. It, too, is represented by only a few
strains and all C. orba strains conjugated with the same h+ strain of P. antillensis, leaving the nature of sexuality in C. orba in doubt as well (Starmer et al., 2001). At this point, it is not even possible to calculate the probability of failing to collect at least one strain of each C. coquimbobensis mating type, as the distribution of mating types can deviate from 50:50% (Lachance & Starmer, 2008; Lachance et al., 2001). At this point, it

The addition of C. coquimbobensis to the Phaffomyces clade does not resolve the origins of either P. opuntiae or C. orba, the Phaffomyces taxa confined to regions only recently occupied by their cactus hosts. However, it does support the hypothesis that P. opuntiae is South American in origin. During the successful attempt to identify biological control agents to control invasive members of the cactus genus Opuntia in eastern Australia (Dodd, 1940), cacti from both North and South America were transported to Australia. C. coquimbobensis is the first member of the Phaffomyces clade to be isolated from both native and introduced cactus habitats.

The disjunct nature of the distributions of the members of the Phaffomyces clade is consistent with the biogeography of cactophilic yeast in general (Ganter, 2011; Starmer et al., 2006). The habitat has been invaded by several lineages of ascomycetous yeast (Starmer et al., 2003) and each lineage has either speciated within the cactophilic habitat or shows genetic variation tied to geographical origin (Ganter, 2011). The Phaffomyces clade has perhaps the most disjunct distribution of all cactophilic lineages, without overlap among any populations from native cactus habitats. Phaffomyces clade taxa never dominate collections in any locale within the native distribution of cacti and show host differentiation as well. They contradict the hypothesis that microbes must be numerous within appropriate habitats to survive over evolutionary timescales. In Australia, where

their hosts and, presumably, the yeast are introduced, the distribution of C. orba is confined to a small region around Brisbane and P. opuntiae is a dominant species throughout most of north-east Australia. This is the only known overlap in distributions for Phaffomyces taxa. The single isolation of C. coquimbobensis came from the Flinders Range in South Australia, where P. opuntiae did not occur among the 95 isolates obtained from this area (the closest isolation of P. opuntiae came from Smithfield, SA, almost 500 km from the Finders locales).

Table 2. Physiological characteristics, as read from agar plates, that differ among the five named taxa in the Phaffomyces clade

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltose</td>
<td>-</td>
<td>d/-</td>
<td>-</td>
<td>d/-</td>
<td>d/-</td>
</tr>
<tr>
<td>Celloobiose</td>
<td>+</td>
<td>d/-</td>
<td>-</td>
<td>+</td>
<td>d/-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>D</td>
<td>d/-</td>
<td>D/-</td>
<td>W</td>
<td>-</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Methyl x-D-glucoside</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucono-D-lactone</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>D</td>
<td>D/-</td>
</tr>
<tr>
<td>Citric acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 37 °C</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Growth at 42 °C</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Species: 1, P. opuntiae; 2, P. thermotolerans; 3, P. antillensis; 4, C. orba; 5, C. coquimbobensis. +, Strong growth; D, delayed growth; W, weak growth; −, no growth; and when strains differ in response, all responses are given divided by forward slashes.

References


