Candida bracarensis sp. nov., a novel anamorphic yeast species phenotypically similar to Candida glabrata

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Two yeast strains, 153MT and NCYC 3133, isolated from clinical sources in separate hospitals were found to be almost identical in the sequences of the D1/D2 domain of large-subunit rDNA, the PCR fingerprinting profiles and physiological characteristics. The isolates were phenotypically similar, although not identical, to Candida glabrata and Kluyveromyces delphensis (recently renamed Nakaseomyces delphensis). Sequence analysis of the 26S rDNA D1/D2 gene variable region revealed that the two clinical isolates were closely related phylogenetically to C. glabrata and K. delphensis, but differed sufficiently to justify their assignment as representatives of a separate species. The name Candida bracarensis sp. nov. is proposed for the novel species with the type strain 153MT (=CBS 10154=NCYC D3853=CECT 12000).

The genus Candida belongs to the ascomycetous yeasts and includes some of the most common human pathogens [e.g. Candida albicans and Candida tropicalis (Ahearn, 1998)]. About 20 species of Candida have been shown to cause disease in humans, but the list of medically important yeasts continues to grow, mostly due to developments in medical interventions and to the increasing number of immunodeficient patients. Improved molecular methods for detecting and differentiating yeasts are also able to distinguish closely related species, providing evidence for the existence of new Candida species (e.g. Candida dubliniensis, Candida orthopsilosis) previously misidentified by conventional chemotaxonomic criteria and which may represent new emerging pathogens (Sullivan et al., 1995; Tavanti et al., 2005).

Following an epidemiological study of candidiasis in the north of Portugal (Correia et al., 2004), one of the clinical isolates (strain 153M1), identified originally as Candida glabrata, was found to have a different and quite unique T3B fingerprinting pattern. In order to clarify its taxonomic position, conventional physiological and biochemical characterization (Yarrow, 1998) as well as rDNA sequencing was performed. A search for similar sequences in the GenBank sequence database revealed the existence of a second strain [NCYC 3133 (formerly NCYC D3411)], isolated from a patient in a UK hospital, with high sequence similarity in the 26S rDNA D1/D2 region. The two clinical isolates shared identical physiological and molecular characteristics and were related to C. glabrata, a species of growing medical concern (Fidel et al., 1999), and to Kluyveromyces delphensis [recently renamed Nakaseomyces delphensis (Kurtzman, 2003)]. However, the extent of sequence divergence was large enough to propose the assignment of these isolates to a novel species, described in this paper as Candida bracarensis sp. nov.

Yeast isolates and their characterization

The strains under study were isolated from patients suffering from candidiasis. Strain 153M1 was collected from a vaginal exudate in a Portuguese hospital and strain NCYC 3133 was isolated from a blood culture from a UK hospital.

The isolates were characterized by using standard chemotaxonomic methods (Yarrow, 1998) and by PCR fingerprinting with primer T3B (Correia et al., 2004).

rDNA sequencing and sequence analysis

The D1/D2 domain of the 26S rDNA was amplified and sequenced according to the procedures described by...
Sampaio et al. (2001). The entire ribosomal internal transcribed spacer (ITS) region, including the 5.8S rDNA, was amplified using the forward primer ITS1 (5'-TCCGTAG-GTGAACCTGGC-3') and reverse primer ITS4 (5'-TCCT-CGGTTATATGC-3') and sequenced using the amplification primers (ITS1 and ITS4) as well as two additional internal primers, ITS2 (5'-GCTGCGTCTTCTCATGAG-3') and ITS3 (5'-GCATCGATGAAAGGCGC-3') (White et al., 1990). In the case of NCYC 3133, PCR amplification of both rDNA regions was carried out directly from whole yeast cells using the protocol described by James et al. (2005). Sequencing was performed with an ABI 310 Genetic Analyzer (Applied Biosystems) using standard protocols. Forward and reverse sequence alignments were made with MegAlign (DNASTAR) and corrected visually.

The 26S rDNA D1/D2 sequences determined in this study were compared with sequences from the GenBank database and aligned with CLUSTAL_X version 1.81 (Thompson et al., 1997). Phylogenetic analysis was carried out using the PAUP* version 4.0b8 software package (Swofford, 2000) and the 26S rDNA D1/D2-derived tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) with the Kimura two-parameter distance measure. Confidence limits were estimated from bootstrap analysis (1000 replicates) and only values of 50% or greater were recorded on the tree.

**Molecular fingerprinting profiles**

When analysed by PCR fingerprinting using primer T3B, strains 153MT and NCYC 3133 were found to have very similar profiles which were quite distinct from those of C. glabrata and K. delphensis strains (Fig. 1).

**Physiological and biochemical characterization**

The physiological and biochemical characteristics of strains 153MT and NCYC 3133 were found to be similar, but not identical, to those of C. glabrata and K. delphensis. In fact, the two strains can be separated from C. glabrata, their nearest known relative (Fig. 2), on the basis of L-lysine assimilation. Both novel strains are able to utilize L-lysine as a sole nitrogen source, whereas strains of C. glabrata assimilate this compound either weakly or not at all (Barnett et al., 2000; Meyer et al., 1998). Similarly, strains 153MT and NCYC 3133 can be readily separated from K. delphensis based on their ability to assimilate both L-lysine and α,α-trehalose, to ferment α,α-trehalose, to grow at temperatures of 40 °C and by an inability to assimilate ethanol (Barnett et al., 2000; Lachance, 1998).

**Phylogenetic analysis**

Sequence analysis of the D1/D2 domain of 26S rDNA for strain 153MT showed the highest similarity with strain NCYC 3133 (differing by a single nucleotide substitution). Strain Candida cf. glabrata UWO(PS)98-110.4 and three clinical strains, designated JAF-2004 isolates 2960 to 2962, also shared a high similarity with strain 153MT, showing 14 nucleotide differences and 14 nucleotide substitutions and two gaps, respectively. Analysis of the phylogenetic tree derived from the alignment of D1/D2 sequences showed that the two closest species to strains 153MT and NCYC 3133 were C. glabrata and K. delphensis (Fig. 2). Sequence similarity values were estimated from an alignment of the D1/D2 sequences of the respective type strains with strain 153MT. The similarity between C. glabrata and K. delphensis was 92%, corresponding to 38 nucleotide differences and four gaps. When compared with these species, strain 153MT displayed 94.8% sequence similarity with C. glabrata (corresponding to 30 nucleotide differences) and 93.8% similarity with K. delphensis (corresponding to 32 nucleotide differences and four gaps). The pairwise alignment of 26S rDNA D1/D2 sequences is presented in Supplementary Fig. S1 available in IJSEM Online).

Sequence analysis was also carried out on the entire ribosomal ITS region (i.e. ITS1/5.8S rDNA/ITS2) of strains 153MT and NCYC 3133. Both strains were found to have ITS1 sequences of identical length (305 bp), which differed from one another by a total of 13 base substitutions, corresponding to 95.7% sequence similarity. Likewise, the ITS2 sequences of the two novel strains were found to be 252 bp (153MT) and 253 bp (NCYC 3133) in length and differed from one another by a single base substitution and a single nucleotide gap (99.2% sequence similarity). Such levels of ITS1 and ITS2 sequence divergence probably reflect the fact that the strains were isolated from patients in separate hospitals, one in Portugal (153MT) and the other in the UK (NCYC 3133). Comparison of the complete ITS...
sequences revealed that 153MT displayed greater sequence similarity to *K. delphensis* (68 %) than to *C. glabrata* (65 %), while 153MT and NCYC 3133 displayed 97.9 % sequence similarity to each other. Despite being related to *C. glabrata*, the number of nucleotide substitutions in the D1/D2 region (30 nucleotides over 581) of the novel strains is much higher than the 0–3 nucleotide differences typically observed between either conspecific or sister species (Kurtzman & Robnett, 1997, 1998).

Indeed, as the 26S rDNA D1/D2-derived tree in Fig. 2 illustrates, strains 153MT and NCYC 3133 clearly belong to a phylogenetically distinct species, a fact supported by both ITS sequencing and PCR fingerprint profiling. Consequently, based on the collective results of this study, a novel species, *Candida bracarensis* sp. nov., is proposed. As the novel species exhibited an API 32C profile identical to *C. glabrata*, it seems possible that 153MT and NCYC 3133 may not be unique isolates of this species and similar clinical isolates may well exist, but may have been misidentified as *C. glabrata*.

This novel species adds to the overall knowledge of yeast biodiversity and represents an additional reference in its phylogenetic clade. This is particularly significant for yeast groups impacting on human health, since modern medical therapy and improved methods for detecting and differentiating yeasts have shown that many novel and unusual species have become clinically important.

Latin diagnosis of *Candida bracarensis* Correia, Sampaio, James et Pais sp. nov.

In medio liquido malti post dies tres ad 30 °C, cellulae sunt globosae (3–0–3.5 μm) ad ellipsoideae (3–0–4.0 × 4.0–4.5 μm) singulae aut binae (Fig. 3). In agaro malti post 7 dies ad 30 °C, cultura albida cremea, centrum colonia altum, butyrosa et margine glabro. In agaro farinae Zea mays post dies 21 ad 25 °C, pseudohyphae et hyphae verae absentes. Asci non formantur. Glucosum et trehalosum fermentantur. Galactosum, maltosum, sucrosum, lactosum, cellobiosum, melezitosum, raffinosum et inulunum non fermentantur. Assimilantur glucosum, trehalosum, glycerolum, D-glucono-1,5-lactonum, D-gluconatum et L-lysinum. Non assimilantur galactosum, L-sorbosum, sucrosum, maltosum, cellobiosum, melezitosum, D-xilosum, L-arabinosum, D-arabinosum, D-ribosum, D-glucosaminum, ethanolum, erythritolum, ribitolum, xylitolum, D-mannitolum, D-glucitolum, methyl-D-glucosidum, salicinum, acidum lacticum, arbutinum, acidum succinicum, acidum citricum, lactosum, inulunum, raffinosum, melibiosum, amyllum soluble, L-rhamnosum, methylglucosum, galactitolum, D-glucuronatum, cadaverinum, ethylaminum, D-glucosaminum (nitrogenium), imidazolium, creatininum, creatininum, kalium nitratum, natrium nitritum et D-tryptophanum. Temperatura 42 °C crescit. Materia amyloidea et acidum aceticum non formantur. Diazonium coeruleum B negativum.

Typus CBS 10154T isolatus ex vaginae. Depositus et
Description of Candida bracarensis Correia, Sampaio, James & Pais sp. nov.

Candida bracarensis [brac’ar.en.sis. L. fem. adj. bracarensis pertaining to Bracara Augusta, the roman name of the city (Braga, Portugal) from where the type strain was isolated.]

In YM broth, after 3 days at 30 °C, the cells are spherical (3-0–3-5 μm) to ellipsoidal (3-0–4-0 x 4-0–4-5 μm) and occur singly or in pairs (Fig. 3). On YM agar, culture colonies are umbonate, pale cream, glistening and butyrous with an entire margin. Neither pseudohyphae nor true hyphae are formed under the coverglass in Dalmau plate culture on cornmeal agar after 21 days at 25 °C. Ascospores are not detected in the type strain when grown for up 3 months on YM agar or sodium acetate agar incubated at 25 °C. Glucose and α,α-trehalose are fermented. Carbon compounds sucrose, α,α-trehalose, glycerol, D-glucono-1,5-lactone and D-glucuronate are assimilated. No growth occurs on D-galactose, L-sorbose, D-glucosamine, D-ribose, D-xyllose, D-arabinose, L-arabinose, L-rhamnose, maltose, methyl α-D-glucoside, cellobiose, arbutin, salicin, melibiose, lactose, raffinose, melezitose, inulin, starch, erythritol, ribitol, xylitol, D-glucitol, D-mannitol, galactitol, D-glucuronate, DL-lactate, sucinate, citrate, methanol or ethanol. The only nitrogen compound assimilated is L-lysine. Tests for the Diazonium blue B reaction, urea hydrolysis and starch and acetic acid formation are negative. Growth occurs on 0-1 % cycloheximide and at 42 °C.

The type strain, 153M\textsuperscript{T} (CBS 10154\textsuperscript{T} = CECT 12000\textsuperscript{T} = NCYC D3853\textsuperscript{T}), was isolated from a case of vaginal candidiasis in a medical institution, Braga, Portugal.

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References


