***Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals**

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Atypical oral *Candida* isolates were recovered from 60 HIV-infected and three HIV-negative individuals. These organisms were germ-tube-positive and produced abundant chlamydospores which were frequently arranged in triplets or in contiguous pairs. They belonged to *C. albicans* serotype A and had atypical carbohydrate assimilation profiles. Fingerprinting the genomic DNA of a selection of these organisms with the *C. albicans*-specific probe 27A and five separate oligonucleotides, homologous to eukaryotic microsatellite repeat sequences, demonstrated that they had a very distinct genomic organization compared to *C. albicans* and *C. stellatoidea*. This was further established by random amplified polymorphic DNA (RAPD) and karyotype analysis. Comparison of 500 bp of the V3 variable region of the large ribosomal subunit genes from nine atypical isolates and the corresponding sequences determined from *C. albicans*, *C. stellatoidea*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. kefyr* and *C. krusei* showed that the atypical organisms formed a homogeneous cluster (100% similarity) that was significantly different from the other *Candida* species analysed, but was most closely related to *C. albicans* and *C. stellatoidea*. These genetic data combined with the phenotypic characteristics of these atypical organisms strongly suggest that they constitute a novel species within the genus *Candida* for which the name *Candida dubliniensis* is proposed.

Keywords: *Candida dubliniensis* sp. nov., rRNA gene sequences, phylogenetic position, oral candidosis, AIDS

INTRODUCTION

Fungal taxonomy is problematic because of reliance on phenotypic and morphological characteristics for classification. Increasingly the speciation of fungi with many

morphological and phenotypic characteristics in common is likely to depend on genetic characterization (Check, 1994). In this regard, molecular analysis has yielded some surprising findings; e.g. until recently *Pneumocystis carinii* was considered to be a protozoan but rRNA gene sequence analysis has revealed that it is more closely related to fungi (Edman *et al.*, 1988; Wakefield *et al.*, 1992). Species of the genus *Candida* have been classically identified on the basis of biochemical reactions and morphological features because of the lack of sexual reproduction. However, phenotypic parameters such as substrate assimilation, cell wall composition and production of extracellular proteolytic enzymes can vary greatly within some species such as *Candida albicans* (Williamson *et al.*, 1986; Ghannoum *et al.*, 1990; Martinez *et al.*, 1990; Allen & Beck, 1983; Odds, 1988a), making

Abbreviations: RAPD, random amplified polymorphic DNA; RAT agar, rice-agar-Tween agar.

The EMBL and GenBank accession numbers for the nucleotide sequences reported in this paper are X83716 (*Candida stellatoidea* ATCC 20408), X83717 (*Candida albicans* 132A), X83718 (*Candida dubliniensis* NCPF 3949), Z48340 (*Aspergillus fumigatus* NCPF 2109), Z48341 (*Candida glabrata* 11088A), Z48342 (*Candida krusei* 105-1), Z48343 (*Candida parapsilosis* 44), Z48344 (*Candida kefyr* NCPF 3234) and Z48346 (*Candida tropicalis* NCPF 3111).

The British National Collection of Pathogenic Fungi accession number for *Candida dubliniensis* sp. nov. described in this paper is NCPF 3949.

the concept of species unclear to some extent. This has led to much confusion in the classification of *Candida* species. The taxonomic relationship between *C. albicans* and the closely related *C. stellatoidea* is illustrative of the present position, with many researchers now accepting the latter as a variant or synonym of *C. albicans* (Odds, 1988b; Barnett *et al.*, 1990). Similarly, *C. pseudotropicalis* is now considered to be a synonym of *C. kefyr* (Odds, 1988b). However, there is an ongoing debate as to whether the two genera *Candida* and *Torulopsis* should be merged (Odds, 1998b). It is therefore not surprising that several recent studies described isolates of *Candida* whose properties did not conform precisely with conventional species definitions (Akisada *et al.*, 1983; Mahrous *et al.*, 1990; Schmid *et al.*, 1992; Sullivan *et al.*, 1993; McCullough *et al.*, 1995).

The present study was originally prompted by the recovery of unusual isolates of oral *Candida* from 20 separate HIV-infected and AIDS patients with a history of recurrent oral candidosis attending the Dublin Dental Hospital between March 1991 and September 1992. These isolates produced chlamydospores, but were not readily identifiable as *C. albicans* or *C. stellatoidea* on the basis of carbohydrate assimilation profiles. Furthermore, restriction-endonuclease-digested genomic DNA from these isolates yielded weak hybridization profiles consisting of a small number of bands when probed with the cloned *C. albicans*-specific mid-repeat sequence probe 27A, compared to the large number of strongly hybridizing bands obtained with DNA from *C. albicans* isolates. DNA from these atypical isolates also yielded distinctive fingerprint patterns when probed with oligonucleotides (Coleman *et al.*, 1993; Sullivan *et al.*, 1993). Since our original study reporting these atypical *Candida* isolates, we have identified a significant number of similar isolates from additional HIV-infected and AIDS patients attending the Dublin Dental Hospital.

Here we report a detailed phenotypic and genotypic analysis of representative atypical *Candida* isolates recovered in Dublin and similar isolates recovered from AIDS patients in Australia, which establishes them as a unique group within the genus *Candida* for which we propose the name *Candida dubliniensis*.

METHODS

Yeast strains and clinical isolates. Sixty-four atypical oral *Candida* isolates which were recovered between August 1988 and September 1994 were studied: 55 were isolated from separate HIV-infected patients attending the Dublin Dental Hospital, and of these, 47 were intravenous drug users, six were homosexuals and two were haemophiliacs. Thirty-seven of these patients had full blown AIDS at the time of isolate recovery. Two atypical oral *Candida* isolates were recovered from HIV-negative intravenous drug users attending the Dublin Dental Hospital and one isolate was recovered from a HIV-negative healthy Irish subject. The remaining six isolates were recovered from five separate AIDS patients attending the Fairfield Hospital, Melbourne, Australia between November 1989 and April 1992. Two isolates were recovered from one of these patients at separate clinical evaluations. Other yeast strains and representative atypical isolates used for detailed analysis in

the study are listed in Table 1. In a previous study, we found that *C. stellatoidea* NCPF 3108 was phenotypically and genotypically indistinguishable from the atypical *Candida* isolates (Sullivan *et al.*, 1993), and therefore it has been included with them throughout the text. A group of 20 non-oral clinical isolates of *C. albicans* were also included in the study for comparison in some experiments. These were epidemiologically unrelated isolates recovered from separate HIV-negative individuals. Seven of these isolates were recovered from blood cultures, eight from vaginal specimens, three from faecal specimens and two from urine samples.

The yeasts were grown routinely on Potato Dextrose Agar (PDA, Oxoid) at pH 5.6 for 48 h at 37 °C. For liquid culture, isolates and strains were routinely grown in Yeast Peptone Dextrose Broth [YPD; (l⁻¹) 10 g yeast extract (Oxoid), 20 g peptone (Difco), 20 g glucose, pH 5.5] at 37 °C in an orbital incubator (Gallenkamp) set at 180 r.p.m. Germ tube tests were performed by inoculating 2.0 ml fresh, pooled, normal human serum with a fresh colony of yeast and incubating at 37 °C for 2 h in a waterbath. Chlamydospore, pseudohyphal and hyphal production were assessed on rice-agar-Tween agar (RAT agar; bioMérieux) and cornmeal agar (Oxoid), supplemented with 1% (v/v) Tween 80 (Sigma), following inoculation with a 48-h-old yeast colony. The inoculum was applied in several shallow parallel grooves in the agar, covered with a sterile coverslip and incubated for 2–3 d at 25–27 °C in the dark. Germ tubes were observed under bright-field illumination using a Nikon microscope (model 71610). Inoculated RAT agar plates were examined for chlamydospore production by microscopy with bright-field illumination using a Nikon microscope. Samples from RAT and cornmeal agar plates were also examined after staining with lactophenol cotton blue stain. A drop of 1% (w/v) lactophenol cotton blue stain (Larone, 1993) was spotted directly onto an inoculated RAT agar plate, having gently prised up the glass cover slip covering the culture growth, and recovering the stained area by replacing the cover slip. Plates were examined by microscopy 30 min after staining. Estimates of the relative ability of atypical and reference *Candida* to produce chlamydospores on RAT agar were made using an arbitrary scale of (+) to (++++) where (+) and (++) denote low-level chlamydospore production and (++++) denotes hyper-chlamydospore production. Atypical isolates were further tested for their ability to form true hyphae using *N*-acetylglucosamine-yeast nitrogen base-proline (NYP) liquid medium (Marichal *et al.*, 1986). This medium promotes growth of *C. albicans* in the hyphal form in a humidified atmosphere of 10% (v/v) CO₂ at 37 °C. Following incubation for 24 h, plates containing inoculated NYP medium were examined for hyphal formation by light microscopy.

The ability of the atypical isolates to assimilate a variety of carbohydrate-source compounds was determined using the API ID 32C yeast identification system (bioMérieux), according to the manufacturer's instructions. The assimilation profiles of each isolate were examined on three separate occasions using different batches of ID 32C kits. Nitrate assimilation was assayed according to the method described by Buckley (1989).

Chemicals, enzymes, radioisotopes and oligonucleotides. Analar-grade or molecular biology-grade chemicals were purchased from Sigma, BDH or Boehringer Mannheim. [γ -³²P]dATP (5000 Ci mmol⁻¹; 185 TBq mmol⁻¹) and [α -³²P]dATP (3000 Ci mmol⁻¹; 110 TBq mmol⁻¹) were purchased from Amersham. Enzymes were purchased from the Promega Corporation or from Boehringer Mannheim and were used according to the manufacturer's instructions.

Serotyping. *Candida* isolates were serotyped on the basis of

Table 1. Reference strains and clinical isolates

Strain/isolate*	Source	Reference	rRNA gene V3 sequence accession no.
<i>C. albicans</i>			
132A	Irish oral isolate from HIV ⁺ patient	Gallagher <i>et al.</i> (1992)	X83717†
179B	Irish oral isolate from HIV ⁺ patient	Gallagher <i>et al.</i> (1992)	—
CM3	Australian oral isolate from HIV ⁺ patient	This study	—
WO-1	American isolate from blood culture	Slutsky <i>et al.</i> (1987)	L28817
Unnamed	Canadian vaginal isolate	Mercure <i>et al.</i> (1993)	X70659
<i>C. stellatoidea</i>			
11006 (type I)	American Type Culture Collection (MD, USA)	Kwon-Chung <i>et al.</i> (1989)	—
20408 (type II)	American Type Culture Collection (MD, USA)	Kwon-Chung <i>et al.</i> (1989)	X83716‡
Atypical <i>Candida</i> (<i>C. dubliniensis</i> sp. nov.)			
CD33	Irish oral isolate from HIV ⁺ patient	This study	—
CD34	Irish oral isolate from HIV ⁺ patient	This study	—
CD36	Irish oral isolate from HIV ⁺ patient	NCPF 3949, this study	X83718§
CD37	Irish oral isolate from HIV ⁺ patient	This study	—
CD38	Irish oral isolate from HIV ⁺ patient	This study	—
CD40	Irish oral isolate from HIV ⁺ patient	This study	—
CD41	Irish oral isolate from HIV ⁻ healthy subject	This study	—
CM1	Australian oral isolate from HIV ⁺ patient	This study	—
CM2	Australian oral isolate from HIV ⁺ patient	This study	—
CM4	Australian oral isolate from HIV ⁺ patient	This study	—
CM5	Australian oral isolate from HIV ⁺ patient	This study	—
CM6	Australian oral isolate from HIV ⁺ patient	This study	—
CM7	Australian oral isolate from HIV ⁺ patient	This study	—
3108	National Collection of Pathogenic Fungi (UK)	NCPF 3108; Sullivan <i>et al.</i> (1993)	—
<i>C. tropicalis</i>			
3111	National Collection of Pathogenic Fungi (UK)	NCPF 3111	Z48346
<i>C. parapsilosis</i>			
44	CXWMS, London	This study	Z48343
<i>C. glabrata</i>			
11088A	CXWMS, London	This study	Z48341
<i>C. kefyr</i>			
3234	National Collection of Pathogenic Fungi (UK)	NCPF 3234	Z48344
<i>C. krusei</i>			
105-1	CXWMS, London	This study	Z48342
<i>Aspergillus fumigatus</i>			
2109	National Collection of Pathogenic Fungi (UK)	NCPF 2109	Z48340

**C. stellatoidea* is currently considered a synonym of *C. albicans* (Odds, 1988b; Barnett *et al.*, 1990) and *C. kefyr* is currently considered a synonym of *C. pseudotropicalis* (Odds, 1988b).

† The rRNA gene V3 region sequences obtained from *C. albicans* strains 132A (serotype B) and 179B (serotype A) were identical.

‡ The rRNA gene V3 region sequences obtained from the *C. stellatoidea* type II and type I strains ATCC 20408 and ATCC 11006, respectively, were identical.

§ The rRNA gene V3 region sequence obtained from the *C. dubliniensis* isolate CD36 was identical to the corresponding sequences of the *C. dubliniensis* isolates CD34, CD37, CD38, CD40, CM2, CM5, CM7 and NCPF 3108.

|| Clinical isolates recovered during 1993 at the Charing Cross and Westminster Medical School (CXWMS).

Table 2. Oligonucleotide primers used in the analysis of atypical *Candida* isolates and reference *Candida* strains

Oligonucleotide	Nucleotide sequence 5'-3'	Used for	Reference
1	(GGAT) ₄	Hybridization probe	Sullivan <i>et al.</i> (1993)
2	(GACA) ₄	Hybridization probe	Sullivan <i>et al.</i> (1993)
3	(GATA) ₄	Hybridization probe	Sullivan <i>et al.</i> (1993)
4	(GT) ₈	Hybridization probe	Sullivan <i>et al.</i> (1993)
5	(GTG) ₅	Hybridization probe	Sullivan <i>et al.</i> (1993)
6	GCGATCCCCA	RAPD	Akopyanz <i>et al.</i> (1992)
7	AGTGAATTCGCGGTGAGATGCCA	RAPD	Akopyanz <i>et al.</i> (1992)
8	AACGCGCAAC	RAPD	Akopyanz <i>et al.</i> (1992)
9	ATTACTGACGCTGATTGTGC	RAPD	Akopyanz <i>et al.</i> (1992)
10	CCGAGCCA	RAPD	Akopyanz <i>et al.</i> (1992)
11	GCATATCAATAAGCGGAGGAAAAG	rRNA gene analysis	Fell (1993)
12	GGTCCGTGTTTCAAGACG	rRNA gene analysis	Fell (1993)

agglutination reactions with commercially available antiserum raised against *Candida* antigenic factor number 6 (Iatron Laboratories). Isolates which agglutinated with the antiserum were classified as serotype A and isolates which did not agglutinate were classified as serotype B (non-serotype A). *C. albicans* strains 179B (serotype A) and 132A (serotype B) (Gallagher *et al.*, 1992) were used in control reactions. Isolates were serotyped on at least two separate occasions with different batches of antisera.

Isolation of genomic DNA and DNA fingerprinting. Total cellular DNA from *Candida* isolates was prepared from spheroplasts by using a modification of the methods described by Durkacz *et al.* (1985) and Pearce & Howell (1991) as described by Gallagher *et al.* (1992). The procedure used for *Eco*RI digestion of genomic DNA was as described by Coleman *et al.* (1986). Restriction fragments were separated by electrophoresis through 0.8% horizontal agarose gels using 0.5 × TBE buffer (Sambrook *et al.*, 1989) as the electrophoresis buffer for 18 h at 3 V cm⁻¹. Following electrophoresis, DNA fragments were transferred to MagnaGraph nylon membrane filters (MSI) by the method of Southern (1975). These filters were hybridized consecutively with the *C. albicans*-specific mid-repeat sequence probe 27A (Scherer & Stevens, 1988) and five separate synthetic oligonucleotide probes (oligonucleotides 1–5, Table 2) as described by Sullivan *et al.* (1993). Following hybridization with each probe, filters were stripped of bound probe by incubation in a boiling solution of 0.5% SDS for 5 min followed by brief washing in distilled water. Experiments were repeated on at least two occasions with separate preparations of genomic DNA.

Randomly amplified polymorphic DNA (RAPD) analysis. Five additional oligonucleotide primers (oligonucleotides 6–10, Table 2) were used in RAPD experiments as described previously (Sullivan *et al.*, 1993). Briefly, the conditions used were as follows: approximately 10 ng of *Candida* total cellular DNA was added to a 0.5 ml microfuge tube (Eppendorf) containing 20 pmol of the oligonucleotide primer, 250 µM (each) dATP, dTTP, dCTP and dGTP (Boehringer Mannheim), 3 mM MgCl₂, 2.5 U *Taq* DNA polymerase and 1 × *Taq* buffer (Promega) in a final volume of 25 µl. Amplification reactions were performed in a Perkin Elmer Cetus DNA thermal cycler using the following cycling conditions: 4 cycles of 94 °C for 5 min, 36 °C for 5 min and 72 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 36 °C for 1 min and 72 °C for 2 min.

This was followed by a final incubation at 72 °C for 10 min. Reaction products were separated in 2.0% (w/v) agarose gels containing 0.5 µg ethidium bromide ml⁻¹ and viewed on a UV transilluminator (UVP). Experiments were repeated on at least two occasions with separate preparations of target genomic DNA.

Pulsed-field gel electrophoresis (PFGE). Yeast chromosomes were prepared in agarose plugs as described by Vazquez *et al.* (1991) and separated in 1.4% (w/v) agarose gels using the CHEF-Mapper PFGE system (Bio-Rad). The electrophoresis buffer used was 0.5 × TBE and was maintained at 14 °C using buffer recycling through a Bio-Rad minichiller (model 1000). Gels were run for 72 h with an initial switch time of 40 s and a final switch time of 600 s with a ramping factor of –2.379. Following electrophoresis, gels were stained with 0.5 µg ethidium bromide ml⁻¹ for 20 min, destained for between 20 and 90 min in distilled water and viewed on a UV transilluminator.

rRNA gene nucleotide sequence analysis. A PCR product of approximately 600 bp was amplified from yeast isolates using universal primers (primers 11 and 12, Table 2) specific for the V3 variable region of the large ribosomal subunit gene as described by Fell (1993). Reactions were performed in a final reaction mixture (50 µl) containing 10 pmol each of primer; 200 µM each of dATP, dTTP, dGTP and dCTP (Promega); 2.5 mM MgCl₂; 2.5 U *Taq* DNA polymerase (Promega); 1 × *Taq* reaction buffer (Promega) and approximately 10 ng *Candida* total cellular DNA. Amplification reactions were performed in a Perkin Elmer Cetus DNA thermal cycler (model 9600) using the following cycling parameters: 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 66 °C for 90 s and 72 °C for 15 s with a final extension at 72 °C for 4 min. Amplified PCR products were purified by adding 300 µl filtered sterile water and centrifuging at 3000 r.p.m. through a Microcon 100 tube (Amicon) for 15 min using a Microcentaur microfuge (MSE). The DNA was washed twice with 400 µl sterile water by centrifugation as above and eluted by incubation with 50 µl TE buffer (10 mM Tris/HCl, pH 7.5, 1 mM EDTA) for 15 min at room temperature and centrifugation as described above for 30 s. Aliquots (2 µl) of each sample were then electrophoresed on a 1% (w/v) agarose gel and their DNA concentrations estimated. The amplified DNA products were then sequenced in both directions using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) ac-

cording to the manufacturer's instructions using PCR primers 11 and 12 as sequencing primers (Table 2). Completed sequencing reactions were electrophoresed on a ABI 373A Sequencer as described by the manufacturer and the data analysed using the computer programme SEQED (Applied Biosystems). Multiple sequence alignments were performed on a 500 bp internal region of each amplified product using the CLUSTAL W sequence analysis program (Thompson *et al.*, 1994) and the data used to construct a genetic distance matrix incorporating corrections for multiple base changes according to the method of Jukes & Cantor (1969). A phylogenetic tree was generated from the data in this matrix using the neighbour-joining method of Saitou & Nei (1987) and was further tested using bootstrap analysis (Felsenstein, 1985).

RESULTS

Phenotypic characterization

Although all 64 atypical oral *Candida* isolates and NCPF 3108 grew well at 30 and 37 °C, neither they, nor the type I *C. stellatoidea* reference strain ATCC 11006, were able to grow at 42 °C on PDA or in YPD. This was contrary to the results obtained with the three oral *C. albicans* strains 132A, 179B and CM3, the 20 non-oral *C. albicans* control clinical isolates and the type II *C. stellatoidea* strain ATCC 20408, all of which grew at this temperature.

All of the atypical isolates exhibited one of three very similar carbohydrate-source assimilation profiles with the API ID 32C yeast identification system (Table 3), none of which corresponded exactly with any known *Candida* species profile in the API APILAB database. All of the isolates showed positive assimilation reactions for the carbohydrate substrates galactose, sucrose, maltose, N-acetylglucosamine, sorbitol, mannitol, 2-keto-gluconate, glucose and glucosamine, but were unable to utilize 18 other carbon-source and nitrogen-source substrates tested (Table 3). Twenty-six of the isolates did not assimilate trehalose, and in addition six of these did not assimilate palatinose (Table 3). All of the atypical isolates were able to grow in the presence of cycloheximide and none of them could hydrolyse aesculin. In addition, none of the atypical isolates, nor the reference *Candida* strains tested could assimilate nitrate.

All 65 atypical isolates were found to be germ-tube-positive, to produce pseudohyphae and thick-walled chlamydospores on RAT and cornmeal agar, and to be of *C. albicans* serotype A. However, the pattern of chlamydospore production by the atypical isolates was quite distinct compared to reference *C. albicans* and *C. stellatoidea* strains. The former produced chlamydospores in copious amounts (+ + + + +; see Methods). Characteristically, these were attached to short pseudohyphae with abundant lateral branching and frequently occurred terminally in triplets or in contiguous pairs (Fig. 1b, c). Staining with 1% (w/v) lactophenol cotton blue preferentially stained the chlamydospores intensely, allowing them to be distinguished from suspensor cells, pseudomycelium and blastospores, which stained poorly or not at all (Fig. 1d). This phenomenon was observed on both RAT and cornmeal agar. In contrast, the oral *C. albicans* strains

132A (serotype B) and 179B (serotype A), and the *C. stellatoidea* strains ATCC 11006 (type I) and ATCC 20408 (type II) produced very much smaller numbers of chlamydospores (+ to + +), which occurred singly and were attached terminally to pseudohyphae (Fig. 1a), although in a small number of preparations contiguous pairs of chlamydospores were observed. Similar low-level production of chlamydospores (+ to + +) was observed for the 20 non-oral *C. albicans* control isolates and for 418 separate oral isolates of *C. albicans* from Irish HIV-infected ($n = 310$) and age- and gender-matched healthy control subjects ($n = 108$) which were recovered during the same period as the atypical isolates. The predominant mycelial form produced by the atypical isolates on RAT and cornmeal agar was pseudohyphae, although a few true hyphae were observed following prolonged incubation (72 h). All of eight *C. albicans* reference isolates tested were also found to produce true hyphae in NYP medium at 37 °C in a humidified atmosphere of 10% (v/v) CO₂.

Genomic DNA fingerprinting analysis

Thirty-four of the atypical isolates, including 28 Irish and the 6 Australian isolates, were chosen for DNA fingerprinting analysis. Genomic DNA was digested with the restriction enzyme *EcoRI* and following separation of the fragments, direct visual analysis of the gross patterns obtained allowed the atypical isolates to be readily differentiated from the oral *C. albicans* strains 132A and 179B, from the 20 non-oral *C. albicans* control isolates and from the *C. stellatoidea* strains ATCC 11006 (type I) and ATCC 20408 (type II) (data not shown). However, these differences were more pronounced when the digested DNA preparations were transferred to nylon membrane filters and hybridized with the ³²P-labelled *C. albicans*-specific, mid-repeat sequence DNA probe 27A. The probe hybridized efficiently to the *EcoRI*-digested DNA isolated from the *C. albicans* (oral and non-oral) and from the *C. stellatoidea* reference strains, yielding 10–15 bands ranging in size between approximately 500 bp and 20 kb (Fig. 2a, lanes 1–4). The same probe, however, bound poorly to DNA purified from the atypical isolates, yielding hybridization profiles containing only 4–7 bands, most of which were weak and ranging in size between 5 and 20 kb (Fig. 2a, lanes 5–11). Identical results were obtained in repeat experiments with separate preparations of genomic DNA.

Genomic DNA from 17 of the atypical isolates (11 Irish and 6 Australian) and the reference *C. albicans* (oral) and *C. stellatoidea* strains described above was examined in more detail. DNA preparations were digested with the restriction endonuclease *HinfI*, which cleaves DNA more frequently than *EcoRI*, and the fragments separated by agarose gel electrophoresis. The atypical isolates were characterized by the presence of a very high molecular mass *HinfI* fragment (approximately 15 kb), which was not detected in the *HinfI* digests of DNA from the *C. albicans* strains 132A and CM3 or from the *C. stellatoidea* strains ATCC 11006 (type I) and ATCC 20408 (type II)

Table 3. Substrate assimilation by atypical *Candida* isolates and reference strains of *C. albicans* and *C. stellatoidea*

Substrate utilization was determined using the API ID 32C yeast identification system. Each isolate was tested on three separate occasions with three different batches of ID 32C kits.

Substrate	Atypical isolates*			<i>C. albicans</i> *		<i>C. stellatoidea</i> *	
	A <i>n</i> = 33	B 26	C 6	132A	179B	Type I ATCC 11006	Type II ATCC 20408
Pentoses							
L-Arabinose	—	—	—	—	—	—	—
D-Xylose	—	—	—	—	+	+	+
Ribose	—	—	—	—	—	—	—
Hexoses							
α-Methyl-D-glucoside	—	—	—	+	+	—	—
Galactose	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Sorbose	—	—	—	—	—	—	—
Rhamnose	—	—	—	—	—	—	—
Disaccharides							
Cellobiose	—	—	—	—	—	—	—
Maltose	+	+	+	+	+	+	+
Lactose	—	—	—	—	—	—	—
Melibiose	—	—	—	—	—	—	—
Sucrose	+	+	+	+	+	—	—
Trehalose	+	—	—	+	+	—	+
Palatinose	+	+	—	+	+	—	—
Trisaccharides							
Melezitose	—	—	—	—	—	—	—
Rafinose	—	—	—	—	—	—	—
Alcohols							
Glycerol	—	—	—	—	—	—	—
Erythritol	—	—	—	—	—	—	—
Mannitol	+	+	+	+	+	+	+
Inositol	—	—	—	—	—	—	—
Sorbitol	+	+	+	+	+	+	+
Organic acids							
Glucuronate	—	—	—	—	—	—	—
DL-Lactate	—	—	—	+	+	—	—
2-Keto-gluconate	+	+	+	+	+	+	+
Levulinate	—	—	—	—	—	—	—
Gluconate	—	—	—	—	—	—	—
Amino acids							
N-Acetylglucosamine	+	+	+	+	+	+	+
Glucosamine	+	+	+	+	+	+	+
Nitrate†							
Potassium nitrate	—	—	—	—	—	—	—

*ID 32C assimilation profile codes: A = 7143140015 (no definitive identification obtained using the APILAB database; includes NCPF 3108); B = 7142140015 (no definitive identification obtained using the APILAB database); C = 7142100015 (no definitive identification obtained using the APILAB database); 132A = 7347140015 (very good identification, *C. albicans*); 179B = 7347340015 (excellent identification, *C. albicans*); ATCC11006 = 3142300015 (excellent identification, *C. stellatoidea*); ATCC 20408 = 3143300015 (excellent identification, *C. stellatoidea*). All of the isolates tested grew in the presence of cyclohexamide and were unable to hydrolyse aesculin. Seventeen of the 20 non-oral *C. albicans* control clinical isolates yielded ID 32C assimilation profiles identical to that obtained with the *C. albicans* oral isolate 179B and the remaining three yielded profiles identical to that obtained with the *C. albicans* oral isolate 132A.

† Nitrate assimilation tests were performed as described by Buckley (1989).

Fig. 1. For legend see page 1515.

Fig. 1. For legend see facing page.

Fig. 2. Southern blot analysis of *EcoRI*-digested total genomic DNA from *C. albicans* and *C. dubliniensis* isolates probed with (a) ^{32}P -labelled *C. albicans*-specific probe 27A and (b) ^{32}P -labelled oligonucleotide (GGAT)₄. The fingerprints shown correspond to *C. albicans* isolates 132A (lane 1) and CM 3 (lane 2), *C. stellatoidea* type II strain ATCC 20408 (lane 3), *C. stellatoidea* type I strain ATCC 11006 (lane 4), *C. dubliniensis* isolates CD33 (lane 5), CD36 (lane 6), CD38 (lane 7), CM2 (lane 8), CM5 (lane 9), CM7 (lane 10) and NCPF 3108 (lane 11). Size reference markers are indicated in kb on the left of the figure.

Fig. 3. (a) Agarose gel electrophoresis of total genomic DNA from *Candida* isolates digested with *Hinfi*. DNA was from organisms as follows: *C. albicans* isolates 132A (lane 1) and CM3 (lane 2), *C. stellatoidea* type II strain ATCC 20408 (lane 3), *C. stellatoidea* type I strain ATCC 11006 (lane 4), *C. dubliniensis* isolates CD33 (lane 5), CD36 (lane 6), CD38 (lane 7), CM2 (lane 8), CM5 (lane 9), CM7 (lane 10) and NCPF 3108 (lane 11). Size reference markers are indicated in kb on the left of the figure. The digested DNA shown in (a) was transferred onto a nylon membrane filter and probed with ^{32}P -labelled *C. albicans*-specific probe 27A, generating the autoradiogram shown in (b).

(Fig. 3a). When hybridized with 27A, the probe bound almost exclusively to the high molecular mass *Hinfi* fragment present in the atypical isolate genomic DNA digests (Fig. 3b).

All of the results described above strongly suggested that the genomic structure of the atypical isolates differed substantially from the reference *C. albicans* and *C. stellatoidea* strains examined. This suggestion was con-

firmed following hybridization analysis of *EcoRI*-cleaved genomic DNA from seven representative atypical isolates (three Irish, three Australian and NCPF 3108), from the oral *C. albicans* strains 132A (Irish) and CM3 (Australian) and from the *C. stellatoidea* strains ATCC 11066 and ATCC 20408 with each of the five synthetic oligonucleotide probes (GGAT)₄, (GACA)₄, (GATA)₄, (GT)₈ and (GTG)₅; (Table 2). In these experiments, the same nylon-membrane-bound DNA samples used in the

Fig. 1. Light micrographs showing chlamydospore production on RAT agar plates incubated at 25–27 °C for 72 h. (a) Chlamydospores and pseudohyphae produced by *C. albicans* strain 132A. (b) Production of abundant chlamydospores by the *C. dubliniensis* isolate CD33. Examples of contiguously arranged chlamydospores (arrowheads) and terminal pairs (arrows) are indicated. (c) Terminal triplet arrangements of chlamydospores (arrows) produced by *C. dubliniensis* isolate CD33. (d) Chlamydospores produced by *C. dubliniensis* isolate CD33 stained with lactophenol cotton blue. Bars: 10 µm (a, b, d); 5 µm (c).

Fig. 4. Agarose gel electrophoresis of amplified DNA products generated using primer 6 (Table 2) in RAPD analysis of *Candida* isolates. The fingerprints shown correspond to *C. albicans* isolates 132A (lane 1) and CM3 (lane 2), *C. stellatoidea* type II strain ATCC 20408 (lane 3), *C. stellatoidea* type I strain ATCC 11006 (lane 4), *C. dubliniensis* isolates CD33 (lane 5), CD36 (lane 6), CD38 (lane 7), CM2 (lane 8), CM5 (lane 9), CM7 (lane 10) and NCPF 3108 (lane 11). A negative control in which no template DNA was used in the PCR reaction was also included (lane 12). Size reference markers are indicated in kb on the left of the figure.

hybridization experiments described above with probe 27A were sequentially hybridized with each oligonucleotide probe, generating five distinct additional fingerprint profiles. In each case, the overall fingerprint profiles of the atypical isolates were very similar to each other, but quite distinct from the profiles obtained with the *C. albicans* isolates 132A and CM3, and the *C. stellatoidea* strains ATCC 11006 and ATCC 20408. An example of the fingerprint profiles generated with the oligonucleotide probe (GGAT)₄ is shown in Fig. 2b. Identical results were obtained in repeat experiments with separate preparations of genomic DNA.

Further supporting data for the distinctive genomic organization of the atypical isolates was obtained by applying RAPD analysis to genomic DNA from the same organisms examined by hybridization analysis with the five oligonucleotide probes described above. An additional oligonucleotide primer (primer 6, Table 2) was used to generate RAPD profiles of each organism investigated. The overall RAPD profiles of the atypical isolates tested were very similar to each other (Fig. 4, lanes 5–11), but very distinct from the profiles obtained with the reference *C. albicans* and *C. stellatoidea* strains tested (Fig. 4, lanes 1–4). Identical results were obtained in repeat experiments with separate preparations of template genomic DNA from the same organisms. Further RAPD experiments employing four different oligonucleotide primers (primers 7–10, Table 2) and template DNA isolated from *C. albicans* strain 132A, *C. stellatoidea* strain ATCC 20408 (type II) and atypical isolates CD33 (Irish), CM2 (Australian) and NCPF 3108, also resulted in the generation of very distinct RAPD patterns for the atypical isolates which were readily discernible from those generated from the reference strains (data not shown).

Fig. 5. Electrophoretic karyotypes of *Candida* isolates. Lanes: 1, *C. albicans* isolate 132A; 2, *C. albicans* isolate CM3; 3, *C. stellatoidea* type II strain ATCC 20408; 4, *C. stellatoidea* type I strain ATCC 11006; 5–14, *C. dubliniensis* isolates CM1, CM2, CM4, CM5, CM6, CD33, CD36, CD38, NCPF 3108 and CD41, respectively. Chromosomes from *Saccharomyces cerevisiae*, used as molecular mass standards (Bio-Rad), are on the left of the figure and the molecular masses indicated are in Mb.

Karyotype analysis

The karyotype profiles of a selection of 10 atypical isolates (four Irish, five Australian and NCPF 3108) were compared with those of the oral *C. albicans* isolates 132A (Irish) and CM3 (Australian), and both *C. stellatoidea* strains ATCC 11006 (type I) and ATCC 20408 (type II). The atypical isolates yielded karyotype profiles which were quite distinct from those obtained for the reference strains (Fig. 5). *C. albicans* 132A and CM3 yielded almost identical karyotypes containing seven distinct chromosome-sized bands. Interestingly, this pattern was almost indistinguishable from the karyotype pattern obtained with the reference type II *C. stellatoidea* strain ATCC 20408 (Fig. 5, lane 3). This type of pattern, however, is significantly different from that obtained with the reference type I *C. stellatoidea* strain ATCC 11006, which contained nine chromosome-sized bands (Fig. 5, lane 4). Similarly, the karyotype patterns obtained with the atypical isolates were quite distinct from the *C. albicans* patterns, with most of the profiles containing 9 or 10 individual chromosome-sized bands (Fig. 5, lanes 5–14). One apparent characteristic feature of the atypical isolates karyotype profiles was the presence of one or more chromosome-sized bands of < 1 Mb, a feature also displayed by the reference type I *C. stellatoidea* strain ATCC 11006.

Analysis of ribosomal RNA gene nucleotide sequences

Approximately 600 bp of DNA from the V3 variable region of the large ribosomal subunit genes were amplified by PCR from genomic DNA of nine representative atypical isolates (five Irish, three Australian and NCPF 3108; see Table 1) from the *C. albicans* strains 132A and 179B, and from the *C. stellatoidea* strains ATCC 11006

132A	AATTGAAAT	CTGGGCTCT	TGGGCTCGA	GTGTAAATT	GAAGAAGTA	TCTTTGGGC	CGGCTCTGT	70
CD36	-----	-----	-----	-----	-----	-----	-----	
11006	-----	-----	-----	-----	-----	-----	-----	
132A	CTATGTTCT	TGGAACAGA	CGTCACAG	GGTGAGAAT	CCGTGCGAT	AGATGACCG	GCTCTGTGA	140
CD36	-----	-----	-----	-----	-----	-----	-----	
11006	-----	-----	-----	-----	-----	-----	-----	
132A	AAGTTCCTC	GACGATCGA	GTGTGTTGG	AATGACGCT	TAAGTGGTG	GTAATTCGA	TCTAAGCTA	210
CD36	-----	-----	-----	-----	-----	-----	-----	
11006	-----	-----	-----	-----	-----	-----	-----	
132A	AATATTGGC	AGAGACCGAT	AGCAACAAG	TACAGTGAT	GAAAGATGA	AAGAATTTC	AAAAGAGAT	280
CD36	-----	-----	-----	-----	-----	-----	-----	
11006	-----	-----	-----	-----	-----	-----	-----	
132A	GAAGAAGTAC	GTGAATTTGT	TGAAGGGAA	GGGCTTGAG	TCAGACTTG	TATTTTGCAT	GCTGCTCTC	350
CD36	-----	-----	-----	-----	-----	-----	-----	
11006	-----	-----	-----	-----	-----	-----	-----	
132A	CGGGGGCGC	CGCTGGGTT	TACCGGCCA	GCATGGGTT	GGAGCGGAG	GATATGGCG	GAGGAATGT	420
CD36	-----	-----	-----	-----	-----	-----	-----	
11006	-----	-----	-----	-----	-----	-----	-----	
132A	GCACGGCTC	TGCTGTGTG	TATAGCTCT	GACGATCTG	CCAGCTAGA	CCGAGGACTG	CGGTTTTCAT	490
CD36	-----	-----	-----	-----	-----	-----	-----	
11006	-----	-----	-----	-----	-----	-----	-----	
132A	CTAGATGTT	-----	-----	-----	-----	-----	-----	500
CD36	-----	-----	-----	-----	-----	-----	-----	
11006	-----	-----	-----	-----	-----	-----	-----	

Fig. 6. Comparison of partial nucleotide sequences of the V3 region of the large ribosomal subunit genes of *C. albicans* 132A, *C. dubliniensis* CD36 and *C. stellatoidea* type I strain ATCC 11006. Sequence matches are indicated by dashes. The base adenine shown in bold at position 457 was replaced by a guanine in the corresponding sequence of *C. albicans* strain WO-1.

(type I) and ATCC 20408 (type II). In each case the nucleotide sequence of the amplified DNA was determined on both strands and the data compared using the same 500 bp of completely unambiguous sequence. These sequences have been submitted to the EMBL nucleotide sequence database (Table 1). The sequences obtained from the oral *C. albicans* strains 132A and 179B were identical, but differed at a single base, at position 457 (Fig. 6), from the corresponding sequences of *C. albicans* strain WO-1 (isolated from blood) and two *C. albicans* vaginal isolates, the sequences of which were obtained from the GenBank and EMBL nucleotide sequence databases, respectively (Srikantha *et al.*, 1994; Mercure *et al.*, 1993; Table 1). The sequences of the corresponding regions of

the *C. stellatoidea* strains ATCC 11006 (type I) and ATCC 20408 (type II) were also found to be identical to each other, but differed from the sequence of *C. albicans* strain 132A by only a single base at position 342 and from that of strain WO-1 at two positions (i.e. bases 342 and 457, see Fig. 6). Analysis of the corresponding sequences from the nine atypical isolates tested (Table 1) revealed that they were all identical to each other, but that they differed from the *C. albicans* 132A sequence at the same 14 positions in each case, from the *C. albicans* WO-1 sequence at an additional position (i.e. a 15 bp difference in total) and from the *C. stellatoidea* strains ATCC 11066 and ATCC 20408 sequences at the same 13 positions. Alignments of a representative sequence from an atypical isolate, and one sequence from each of the *C. albicans* and *C. stellatoidea* reference strains tested are shown in Fig. 6.

For comparison, since there was no corresponding sequence data in the databases for species of *Candida* other than *C. albicans*, DNA fragments were also amplified from the corresponding region of the ribosomal genes from one isolate each of five other *Candida* species, including *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. kefyr*, and from that of a single isolate of *Aspergillus fumigatus*. These sequences have been submitted to the GenBank nucleotide sequence database (Table 1). In each case the amplified products were sequenced on both strands and aligned with the corresponding sequences obtained from a representative atypical isolate and from reference strains of *C. albicans* and *C. stellatoidea*. The sequence data obtained from the five 'non-*albicans*' *Candida* species and the strain of *Aspergillus fumigatus* differed considerably from the *C. albicans* sequence data. An evolutionary distance matrix (Table 4) based on these sequences was compiled from the 10 aligned sequences, correcting for multiple base changes according to Jukes & Cantor (1969). Using this data, an evolutionary tree was generated employing the neighbour-joining method of Saitou &

Table 4. Genetic distance matrix based on comparison of sequences of the V3 region of the large ribosomal subunit

Values correspond to percentages of difference corrected for multiple base changes by the method of Jukes & Cantor (1969).

	<i>C. du.</i>	<i>C. al.</i> (132A)	<i>C. al.</i> (WO-1)	<i>C. st.</i>	<i>C. tr.</i>	<i>C. pa.</i>	<i>C. ke.</i>	<i>C. gl.</i>	<i>A. fu.</i>	<i>C. kr.</i>
<i>C. dubliniensis</i> (CD36)*	—									
<i>C. albicans</i> (132A)	2.25	—								
<i>C. albicans</i> (WO-1)†	2.48	0.02	—							
<i>C. stellatoidea</i> (20408)‡	2.25	0.00	0.02	—						
<i>C. tropicalis</i> (3111)	6.71	7.70	7.95	7.70	—					
<i>C. parapsilosis</i> (44)	7.96	8.97	9.23	8.97	5.26	—				
<i>C. kefyr</i> (3234)	15.76	16.32	16.60	16.32	17.09	15.23	—			
<i>C. glabrata</i> (11088A)	20.29	20.27	20.57	20.27	20.78	21.52	12.58	—		
<i>A. fumigatus</i> (2109)	27.53	26.56	26.23	26.56	29.93	29.36	30.46	29.95	—	
<i>C. krusei</i> (105-1)	29.60	29.93	29.60	29.93	32.62	32.67	33.84	35.92	37.53	—

*All eight isolates tested, in addition to NCPF 3108, yielded identical sequences.

†The sequence for *C. albicans* strain WO-1 was obtained from the GenBank database (accession no. L28817).

‡The type I and type II *C. stellatoidea* strains (ATCC 11006 and 20408, respectively) yielded identical sequences.

Fig. 7. An unrooted phylogenetic neighbour-joining tree generated from the alignment of a 500 bp section of the V3 region of the large ribosomal genes from *C. dubliniensis* CD36 and species listed in Table 1. The scale bar represents a 5% difference in nucleotide sequence. Numbers at each node were generated by bootstrap analysis and represent the percentage of times the arrangement occurred in 1000 randomly generated trees.

Nei (1987) (Fig. 7). When a bootstrap analysis of the 10 aligned sequences was performed, the cluster of *C. dubliniensis*, *C. albicans* WO-1, *C. albicans* 132A and *C. stellatoidea* occurred in 987 of 1000 trees. Thus, in 98.7% of the trees generated *C. dubliniensis* was grouped separately from *C. albicans* and *C. stellatoidea*.

These findings provided strong evidence that *C. dubliniensis* constitutes a discrete taxon within the genus *Candida*.

DISCUSSION

We have identified a novel yeast which produces abundant chlamydo spores from the oral cavities of HIV-infected individuals presenting with oral candidosis and from a small number of HIV-negative subjects and have described its phenotypic and genotypic properties. It remains to be elucidated whether these yeasts occur in other anatomical sites and other subject populations. Having considered the characteristics of these isolates in

relation to the genus *Candida*, we consider them to constitute a previously undescribed species of *Candida*, for which we propose the name *Candida dubliniensis*. Two phenotypic characteristics, including the ability to produce chlamydo spores and germ tubes, were those specifically associated with only two *Candida* species, *C. albicans* and *C. stellatoidea* (Odds, 1988c). The inability to grow at 42 °C is also a characteristic feature of type I *C. stellatoidea* but not of *C. albicans* (Kamiyama *et al.*, 1989) or type II *C. stellatoidea* (sucrose-negative variants of *C. albicans*, Kwon-Chung *et al.*, 1988) both of which grow at this temperature. *C. dubliniensis* could be distinguished from type I *C. stellatoidea* by its ability to assimilate sucrose, although it should be noted that a recent study reported that prolonged exposure to sucrose can induce type I *C. stellatoidea* to form sucrose-positive variants (Wickes *et al.*, 1991). *C. dubliniensis*, which belongs to serotype A, could also be distinguished from type I *C. stellatoidea* on the basis of serotype, the latter of which belong to serotype B (Kwon-Chung *et al.*, 1989). In addition, *C. dubliniensis* could also be readily distinguished from *C. albicans* and *C. stellatoidea* by its consistent production of abundant chlamydo spores on RAT and cornmeal agar. Furthermore, chlamydo spores were often observed in triplets or in contiguous pairs attached to a single suspensor cell, a feature that was rarely observed in over 400 oral isolates of *C. albicans* from separate Irish HIV-infected and control subjects or in other reference strains and control isolates of *C. albicans* or *C. stellatoidea* tested. As far as we are aware, production of contiguous chlamydo spores has only been described once before, in a study of oral isolates of *C. albicans* (Akisada *et al.*, 1983). To ensure that the reference *C. albicans* isolates used in this study were representative of the species from a taxonomic viewpoint, both oral and non-oral isolates were examined and the data obtained with both groups were found to be entirely consistent.

Genotypic studies clearly reinforced the suggestion obtained from phenotypic tests of the unique nature of *C. dubliniensis* relative to other species of the genus *Candida*, with particular reference to *C. albicans* and *C. stellatoidea*. *C. dubliniensis* genomic DNA hybridized poorly with the cloned *C. albicans*-specific, dispersed mid-repeat sequence probe 27A, relative to genomic DNA from *C. albicans* and *C. stellatoidea*. Since 27A and similar probes hybridize well with repetitive DNA sequences dispersed throughout the genome of *C. albicans* (including oral and non-oral isolates) and *C. stellatoidea* (Scherer & Stevens, 1988; Kwon-Chung *et al.*, 1989; this study), this finding suggested that the genomic organization of *C. dubliniensis* is significantly different. Further evidence establishing the distinctive genomic organization of *C. dubliniensis* was obtained following DNA fingerprint analysis using a range of five different oligonucleotide probes homologous to eukaryotic microsatellite DNA sequences, and by RAPD analysis with an additional five oligonucleotides. Furthermore, this distinctive genomic organization was paralleled by the patterns of chromosome-sized DNA bands exhibited by *C. dubliniensis* following PFGE. A feature shared with type I *C. stellatoidea*, but not with the

majority of *C. albicans* isolates (Kwon-Chung *et al.*, 1988), was the presence of chromosome-sized molecules of < 1 Mb.

Previous studies involving sequence analysis of the V3 variable regions of large ribosomal RNA gene sequences have provided very useful information concerning the phylogenetic relationships between a variety of marine yeasts (Fell *et al.*, 1992). Analysis of a 500 bp sequence from the corresponding region of the large ribosomal subunit rRNA genes of *C. dubliniensis* and seven other *Candida* species tested, including *C. albicans* and *C. stellatoidea*, revealed significant differences (Table 4, Figs 6 and 7). Analysis of sequence data obtained from nine epidemiologically unrelated *C. dubliniensis* isolates (five Irish, three Australian and NCPF 3108; Table 1) demonstrated that *C. dubliniensis* formed a homogeneous cluster (100% similarity) representing a unique taxon within the genus *Candida*, and clearly separated from the other *Candida* species tested (Table 4, Figs 6 and 7). The closest species linked to the cluster were *C. stellatoidea* (2.25% sequence divergence) and *C. albicans* (2.25–2.48% sequence divergence) (Table 4, Figs 6 and 7). The almost identical sequences obtained with the *C. albicans* and *C. stellatoidea* strains examined in the present study reinforces the concept that both of these groups of organisms are probably not separate species. However, the significant genetic divergence found between *C. albicans* and *C. dubliniensis* indicates strongly that *C. dubliniensis* should be considered a separate species.

Description of *Candida dubliniensis* sp. nov.

Candida dubliniensis [dubliniensis; after Dublin, capital city of the Republic of Ireland].

Dimorphic yeast consisting of ovoid or spherical blastospores (3–7 µm × 3–14 µm). Grows as round convex cream-coloured colonies on Sabouraud dextrose agar (Oxoid) and cream-white-coloured colonies on Potato Dextrose agar (Oxoid). On RAT agar (bioMérieux) or cornmeal-Tween 80 agar at 25–27 °C for 48–72 h, produces pseudohyphae (and a few true hyphae) with short unilateral, bilateral or multilateral branches at the septa and produces abundant refractile, thick-walled chlamydospores. Chlamydospores are often produced in triplets or in contiguous pairs attached terminally by single suspensor cells to extensively branched pseudohyphae. Produces germ tubes in fresh, pooled normal human serum following 2 h incubation at 37 °C in a waterbath. Grows well at 30 °C and at 37 °C but not at 42 °C. Biochemically, strains assimilate galactose, glucose, maltose, sucrose, mannitol, sorbitol, 2-keto-gluconate and glucosamine, and do not assimilate arabinose, xylose, ribose, sorbose, rhamnose, α-methyl-D-glucoside, cellobiose, lactose, melibiose, melezitose, raffinose, glycerol, erythritol, inositol, glucuronate, DL-lactate, levulinate, gluconate or potassium nitrate. Strains show variable assimilation of palatinose and trehalose, grow in the presence of cycloheximide and do not hydrolyse aesculin. Belongs to *C. albicans* serotype A (i.e. agglutinated with

polyclonal antisera raised against *Candida* antigenic factor No. 6).

C. dubliniensis has a unique organization of genomic DNA relative to other *Candida* species as determined by DNA fingerprint analysis of genomic DNA with the *C. albicans*-specific mid-repeat sequence probe 27A, the oligonucleotide probes (GGAT)₄, (GACA)₄, (GATA)₄, (GT)₈ and (GTG)₅, and by karyotype analysis and nucleotide sequence analysis of the V3 variable region of the large ribosomal subunit gene DNA.

The type strain is CD36 which has been lodged with the British National Collection of Pathogenic Fungi (Mycology Reference Laboratory, Bristol Public Health Laboratory, Kingsdown, Bristol BS2 8EL, UK) under the accession number NCPF 3949 (Table 1).

Latin description of *Candida dubliniensis* sp. nov.

Candida dubliniensis (de Dublinio, rei publicae Hiberniae capite).

Fermentum biforme ex ovoideis vel sphaeralibus blastosporis (3.0–7.0 µm × 3–14 µm) formatum. Coloniae flavalbidae, rotundatae et convexae nascuntur in agaro dextroso Sabouraud (Oxoid), coloniae albicae in agaro dextroso solani tuberosi (Oxoid). Culturum in agaro Tween (bioMérieux) oryzae vel Tween 80 frumenti (post dies 2–3, 25–27 °C), fert pseudohyphas et aliquot veras hyphas, quae ad septa in breves, simplices vel ancipites vel multiplices ramos dividuntur; nimis effert chlamydosporas refractiles et callosas. Haec chlamydosporae saepe trinae producuntur vel binae et adiunctae extremo cellulis singulis ad pseudohyphas multirames. In sano et recenti sero humano, post duas horas (37 °C), germina tubulata formantur. Sub 30 et 37 °C bene crescit, sed sub 42 °C non crescit. Assimilat galactosum, glucosum, maltosum, sucrosus, mannitolum, sorbitolum, 2-keto-gluconatum, glucosaminum. Non assimilant arabinosum, xylosum, ribosum, sorbosum, rhamnosum, α-methyl-D-glucosidum, cellobiosum, lactosum, melibiosum, melezitosum, rafinosum, glycerolum, erythritolum, inositolum, glucuronatum, DL-lactatum, levulinatum, gluconatum, vel nitratum kalicum. Palatinosum et trehalosum assimilantur inconstanter. Cycloheximido praesente crescit, sed aesculinum non hydrolysit. Attinet ad Candidae albicantis serotypum A (id est polyclonalibus anticiseris agglutinatum, quae provocata sunt a factore Candidam inhibente 6).

Candidae dubliniensis genomi unice ordinantur. Typus e lingua hominis HIV infecti isolatus CD36 (NCPF 3949, Tabula 1).

Description of the type strain

Strain CD36 has all the above-mentioned characteristics, including the ability to assimilate palatinose and trehalose. It was isolated from the mid-dorsum of the tongue of an Irish HIV-infected subject presenting with erythematous oral candidosis during 1988.

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