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Widespread Geographic Distribution of Oral *Candida dubliniensis* Strains in Human Immunodeficiency Virus-Infected Individuals

DEREK SULLIVAN,[1] KEN HAYNES,[2] JACQUES BILLE,[3] PATRICK BOERLIN,[3]† LAURA RODERO,[4] SIOBHÁN LLOYD,[1] MARTIN HENMAN,[5] AND DAVID COLEMAN[1]*

Department of Oral Medicine and Pathology, School of Dental Science and Dublin Dental Hospital,[1] and Department of Pharmacology, School of Pharmacy,[5] Trinity College, University of Dublin, Dublin 2, Republic of Ireland; Department of Infectious Diseases and Bacteriology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0NN, United Kingdom[2]; Institute of Microbiology, University Hospital, CH-1011 Lausanne, Switzerland[3]; and Instituto Nacional de Microbiologia Dr. Carlos G. Malbran, Buenos Aires 1281, Argentina[4]

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***Candida dubliniensis* is a recently identified chlamydospore-positive yeast species associated with oral candidiasis in human immunodeficiency virus (HIV)-infected (HIV^+) patients and is closely related to *Candida albicans*. Several recent reports have described atypical oral *Candida* isolates with phenotypic and genetic properties similar to those of *C. dubliniensis*. In this study 10 atypical chlamydospore-positive oral isolates from HIV^+ patients in Switzerland, the United Kingdom, and Argentina and 1 isolate from an HIV-negative Irish subject were compared to reference strains of *C. albicans* and *Candida stellatoidea* and reference strains of *C. dubliniensis* recovered from Irish and Australian HIV^+ individuals. All 11 isolates were phenotypically and genetically similar to and phylogenetically identical to *C. dubliniensis*. These findings demonstrate that the geographical distribution of *C. dubliniensis* is widespread, and it is likely that it is a significant constituent of the normal oral flora with the potential to cause oral candidiasis, particularly in immunocompromised patients.**

Recently several independent reports have described the recovery of atypical chlamydospore-positive oral *Candida* isolates from human immunodeficiency virus (HIV)-infected and AIDS patients in Ireland, the United Kingdom, Switzerland, and Australia which were not readily identifiable as any known *Candida* species by conventional mycological procedures (1–4, 7, 8, 11–15). In one of these reports, detailed phenotypic, molecular, and phylogenetic studies of a collection of 64 of these isolates from 55 separate Irish and Australian HIV-infected and AIDS patients and 2 isolates from separate HIV-negative Irish subjects demonstrated that they formed a homogeneous cluster that was significantly different from the other species of the genus *Candida* (13). These atypical isolates were considered to be sufficiently distinct to constitute a novel species of *Candida*, for which the name *Candida dubliniensis* has been proposed (13). Phylogenetically *Candida albicans* is the species most closely related to *C. dubliniensis* (13).

The objective of this study was to determine whether chlamydospore-positive atypical oral *Candida* isolates from individuals in widely different geographic locations which could not be identified definitively by conventional mycological methods were *C. dubliniensis*. To achieve this, selected atypical oral isolates from HIV-infected individuals in Switzerland, the United Kingdom, and Argentina and from an HIV-negative Irish subject were compared with reference strains of *C. albicans* and *Candida stellatoidea* type I and with reference isolates of *C. dubliniensis* from HIV-infected Irish and Australian subjects by the phenotypic, molecular, and phylogenetic procedures used by Sullivan et al. (13) in the original study describing the identification of *C. dubliniensis*. The atypical oral isolates and reference strains used are shown in Table 1.

All 11 of the atypical oral isolates tested were germ tube positive and produced pseudohyphae and abundant chlamydospores as described previously by Sullivan et al. for *C. dubliniensis* isolates (13). The isolates grew well at 37°C but grew poorly or not at all at 42°C, like *C. stellatoidea* ATCC 11006 and the Irish and Australian *C. dubliniensis* reference strains but unlike *C. albicans* 132A, which grew well at this temperature. These results are in complete agreement with previous studies of *C. dubliniensis* (13). Furthermore, all the atypical isolates and the reference *C. dubliniensis* strains tested yielded similar substrate assimilation profiles with the API ID 32C yeast identification system (bioMérieux) which did not correspond precisely to any known *Candida* species in the API APILAB database (Table 1), as reported previously for *C. dubliniensis* isolates (12–14). Table 2 lists the components of the API ID 32C yeast identification kit and shows the ability of *C. dubliniensis* isolates to assimilate the various substrates in comparison with the substrate assimilation profiles obtained with typical strains of *C. albicans*. The ability to assimilate sucrose was a feature common to all the atypical isolates and the reference *C. dubliniensis* strains, but unlike the reference *C. albicans* and *C. stellatoidea* strains, none produced intracellular β-glucosidase, determined by using methylumbelliferyl-β-glucoside as a substrate as described by Boerlin et al. (2). All the reference *C. dubliniensis* strains and atypical isolates belonged to *C. albicans* serotype A, as determined by agglutination reactions with antiserum raised against *Candida* antigenic factor 6 (Iatron Laboratories, Tokyo, Japan). Previous studies have shown that *C. dubliniensis* isolates belong exclusively to *C. albicans* serotype A, in contrast to type I *C. stellatoidea*, which belong exclusively to *C. albicans* serotype B (3, 13, 14). Furthermore, when cultured on the recently described chromogenic substrate-containing agar CHROMagar Candida (16), the atypical isolates and reference *C. dubliniensis* strains

* Corresponding author. Mailing address: University of Dublin, School of Dental Science, Dental School Office, Trinity College, Dublin 2, Republic of Ireland. Phone: 353 1 6081814. Fax: 353 1 6799294. E-mail address: dcoleman@mail.tcd.ie.

† Present address: Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

TABLE 1. *Candida* reference strains and clinical isolates

Strain(s) or isolate(s)	Species	Source[a]	API ID 32C profile	APILAB database			Reference(s)
				Identification[b]	Species	Predictive value (%)	
Reference strains							
132A	*C. albicans*	Irish oral isolate (HIV$^+$)	7347140015	Excellent	*C. albicans*	99.9	5
ATCC 11006	*C. stellatoidea* type I	American Type Culture Collection	3142300015	Excellent	*C. albicans* 2[c]	99.9	6
CD36[d]	*C. dubliniensis*	Irish oral isolate (HIV$^+$)	7142140015[e]	Unreliable	*C. sake*	49.3	13
					C. albicans 2[c]	48.0	
					C. albicans	2.6	
CM2, CM4	*C. dubliniensis*	Australian oral isolates (HIV$^+$)	7142140015[e]	Unreliable	*C. sake*	49.3	7, 8, 13
					*C. albicans*2[c]	48.0	
					C. albicans	2.6	
Atypical isolates[f]							
CD43	Atypical	Irish oral isolate (HIV$^-$)	7142140015	Unreliable	*C. sake*	49.3	This study
					C. albicans 2[c]	48.0	
					C. albicans	2.6	
CD70	Atypical	United Kingdom oral isolate (HIV$^+$)	7142140015	Unreliable	*C. sake*	49.3	This study
					C. albicans 2[c]	48.0	
					C. albicans	2.6	
P2, P7, P21, P27, P30	Atypical	Swiss oral isolates (HIV$^+$)	7142140015	Unreliable	*C. sake*	49.3	2
					C. albicans 2[c]	48.0	
					C. albicans	2.6	
CD71	Atypical	Argentinian oral isolate (HIV$^+$)	7142140015	Unreliable	*C. sake*	49.3	This study
					C. albicans 2[c]	48.0	
					C. albicans	2.6	
Co4, Co5, Co7	Atypical	Swiss oral isolates (HIV$^+$)	7042100011	Unreliable	*C. colliculosa*	91.0	2
					C. sake	8.5	

[a] The HIV status of the patients from whom the isolates were recovered is indicated in parentheses.
[b] Unreliable, profiles are unreliable for identification purposes and are of low discrimination in the APILAB database, corresponding to poor or unacceptable identification, in decreasing order of probability, of the species indicated.
[c] *C. stellatoidea* is listed as *C. albicans* 2 in the API APILAB database.
[d] CD36, the *C. dubliniensis* type strain, has been lodged with the British National Collection of Pathogenic Fungi (accession number NCPF 3949). Both CD36 and the Australian *C. dubliniensis* isolate CM2 have also been deposited with the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands (accession numbers CBS 7987 and CBS 7988, respectively).
[e] Many *C. dubliniensis* isolates show variable assimilation of trehalose with the ID 32C yeast identification system (13, 14). Thus, isolates which yield the profile 7142140015 on one occasion can yield the profile 7143140015 on another. However, both profiles are unreliable for identification purposes with the APILAB database.
[f] CD43 was recovered from a patient with oral candidiasis in January 1995; CD70 was recovered in 1995 (data on oral candidiasis at sampling not available); CD71 was recovered from a patient with oral candidiasis in August 1994.

yielded dark-green colonies characteristic of *C. dubliniensis*, compared to the pale-blue-green colonies typical of *C. albicans* (3). All of these results indicated that the atypical isolates were phenotypically identical to *C. dubliniensis* (13).

To confirm this, several detailed genetic tests were carried out on the reference strains and a selection of the atypical isolates. Genomic DNA was purified from each and digested with the restriction endonuclease *Hin*fI (13) with the result that the high-molecular-weight *Hin*fI fragments previously shown to be characteristic of *C. dubliniensis* (13) were evident for each of the 11 atypical isolates tested and the reference *C. dubliniensis* strains but not for the reference *C. albicans* and *C. stellatoidea* strains tested (data not shown). Further evidence was obtained by DNA fingerprinting analysis of *Eco*RI-digested genomic DNA from two of the Swiss atypical isolates (P7 and Co4) and three other atypical isolates from Ireland, the United Kingdom, and Argentina (CD43, CD70, and CD71) (Table 1) by hybridization analysis with the ^{32}P-labelled *C. albicans*-specific repeat sequence-containing DNA probe 27A (10) as described previously (13). The probe bound efficiently to the DNA from the reference *C. albicans* and *C. stellatoidea* strains tested but less efficiently to DNA from the atypical isolates in a manner characteristic of *C. dubliniensis* (13) (Fig. 1A). Following hybridization with the 27A probe, the same filter-bound DNA samples were fingerprinted separately by sequential hybridization with five synthetic oligonucleotide probes homologous to eukaryotic microsatellite sequences, $(GGAT)_4$, $(GATA)_4$, $(GACA)_4$, $(GTG)_5$, and $(GT)_8$ (13). For each oligonucleotide probe the profiles of the atypical isolates and the reference strains of *C. dubliniensis* were very similar but easily distinguishable from the corresponding profiles of the *C. albicans* and *C. stellatoidea* reference strains [a representative fingerprint generated with probe $(GTG)_5$ is shown in Fig. 1B]. Similarly, randomly amplified polymorphic DNA profiles generated with the oligonucleotide primer 5′GCGATCC CCA 3′ (13) with target genomic DNA from the same five atypical isolates examined by hybridization analysis were very similar to those of the reference *C. dubliniensis* strains used but distinctly different from the randomly amplified polymorphic DNA profiles generated with *C. albicans* 132A and *C. stellatoidea* ATCC 11006 (data not shown). The karyotype profiles of each of the five atypical isolates examined above were also examined as described previously (13), and again the reference *C. dubliniensis* strains and the atypical isolate profiles were very similar but readily distinguishable from those of the *C. albicans* and *C. stellatoidea* reference strains (Fig. 2). All of these results provided strong evidence that the atypical isolates tested were the same as *C. dubliniensis*.

To determine unequivocally the identity of the atypical isolates, the nucleotide sequences of the V3 region of the large

TABLE 2. Substrate assimilation by *C. dubliniensis* and *C. albicans* determined with the API ID 32C yeast identification system

Substrate or test	ID 32C assimilation profile code						
	C. dubliniensis[a]					*C. albicans*[b]	
	7143140015	7142140015	7143100015	7142100015	7042100011	7347140015	7347340015
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	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−
Alcohols							
Glycerol	−	−	−	−	−	−	−
Erythritol	−	−	−	−	−	−	−
Mannitol	+	+	+	+	+	+	+
Inositol	−	−	−	−	−	−	−
Sorbitol	+	+	+	+	+	+	+
Organic acids							
Glucuronate	−	−	−	−	−	−	−
DL-Lactate	−	−	−	−	−	+	+
2-Keto-gluconate	+	+	+	+	+	+	+
Levulinate	−	−	−	−	−	−	−
Gluconate	−	−	−	−	−	−	−
Amino acids							
N-Acetylglucosamine	+	+	+	+	−	+	+
Glucosamine	+	+	+	+	−	+	+
Esculin hydrolysis	−	−	−	−	−	−	−
Cycloheximide resistance	+	+	+	+	+	+	+

[a] All *C. dubliniensis* isolates reported to date yielded one of the five ID 32C substrate assimilation profile codes shown (3, 13; this study). All five codes provide unreliable identification with the APILAB database.

[b] The two *C. albicans* ID 32C substrate assimilation profile codes shown are commonly obtained with clinical isolates and correspond to excellent identification with the APILAB database (13).

ribosomal subunit genes of the five atypical isolates examined in detail by DNA fingerprinting and karyotype analysis were compared with the corresponding sequences determined previously for a variety of other *Candida* species, including *C. albicans*, type I and type II *C. stellatoidea*, *C. glabrata*, *C. kefyr*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* (13). To achieve this, PCR products spanning approximately 600 bp of the V3 region were amplified with specific primers described previously by Sullivan et al. (13), and their nucleotide sequences were determined by using the PCR primers as sequencing primers. The nucleotide sequences of the amplimers generated from each of the five atypical isolates were found to be identical to each other and to the corresponding sequences of a total of nine separate *C. dubliniensis* isolates determined previously (13). Previous phylogenetic studies based on multiple sequence alignments of this sequence (EMBL nucleotide sequence database accession number X83718) and the corresponding sequence from the other *Candida* species listed above have shown that *C. dubliniensis* forms a distinct taxon within the genus *Candida* (13).

Taken together, these data confirm that the atypical isolates described in this paper, recovered from HIV-infected patients in the United Kingdom, Switzerland, and Argentina and from an HIV-negative Irish subject, all belong to the newly de-

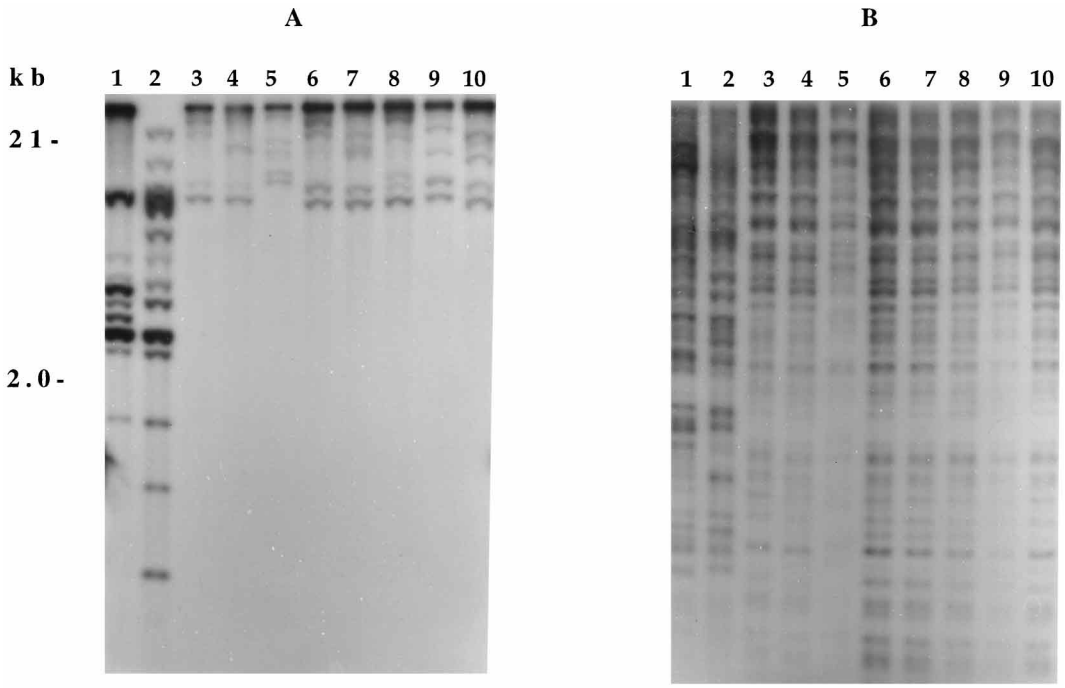

FIG. 1. Autoradiograms of *Eco*RI-digested DNA from reference *Candida* strains and atypical *Candida* isolates hybridized with the *C. albicans*-specific DNA fingerprinting probe 27A (A) and the oligonucleotide probe $(GTG)_5$ (B). Following hybridization and autoradiography, the radioactive probe was stripped from the filter used to generate the autoradiogram shown in panel A, as described previously (12), and the filter was rehybridized with ^{32}P-labelled $(GTG)_5$ probe, which, following autoradiography, generated the autoradiogram shown in panel B. The fingerprints shown correspond to *C. albicans* 132A (lane 1), *C. stellatoidea* type I strain ATCC 11006 (lane 2), the *C. dubliniensis* type strain (CD36) (lane 3), atypical isolate CD43 from an Irish HIV-negative individual (lane 4), atypical isolate CD70 from an English HIV-infected individual (lane 5), *C. dubliniensis* CM2 and CM4 from HIV-infected Australian individuals (lanes 6 and 7, respectively), atypical isolates Co4 and P7 from Swiss HIV-infected individuals (lanes 8 and 9, respectively), and atypical isolate CD71 from an Argentinian HIV-infected individual (lane 10).

scribed species *C. dubliniensis*, which previous studies have shown to be present in HIV-infected individuals in Ireland and Australia (13). Clearly *C. dubliniensis* is widespread, but because of the number of phenotypic characteristics shared between *C. dubliniensis* and *C. albicans*, it is possible that a significant proportion of *C. albicans* or *C. stellatoidea* isolates in many laboratory strain collections is *C. dubliniensis*. *C. dubliniensis* is isolated frequently but not exclusively from individuals infected with HIV (13; this study), and the majority, but not all, of the isolates studied so far have been from oral specimens (2a, 9). *C. dubliniensis* is susceptible to existing antifungal drugs, but resistance can develop rapidly (9). The evidence implicating the involvement of non-*albicans Candida* species in oral disease is growing (14). The increasing number of reports of the recovery of *C. dubliniensis* from normal healthy and HIV-infected patients suggests that as well as being a constituent of the normal oral flora, *C. dubliniensis* is likely a significant cause of oral disease.

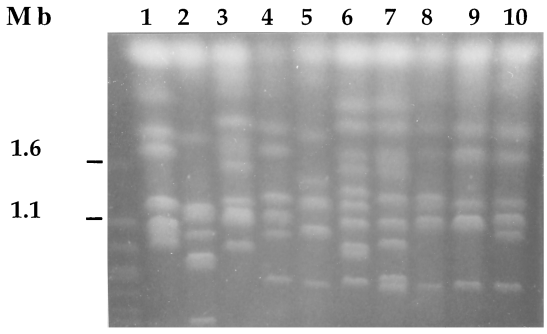

FIG. 2. Pulsed-field gel electrophoresis of DNA from reference and atypical isolates. The karyotypes shown in the lanes correspond to *Candida* isolates as for Fig. 1.

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