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,† LAURA RODERO,4 SIOBHAN LLOYD,1 MARTIN HENMAN,2 and DAVID COLEMAN1,*

Department of Oral Medicine and Pathology, School of Dental Science and Dublin Dental Hospital,1 and Department of Pharmacology, School of Pharmacy,2 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;3 Institute of Microbiology, University Hospital, CH-1011 Lausanne, Switzerland; and Instituto Nacional de Microbiología Dr. Carlos G. Malbran, Buenos Aires 1281, Argentina4

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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 phylogetic 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 API LAB 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 methylnumbelliferyl-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 antiseraum 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
Identification of decreasing order of probability of the species indicated.

C. albicans was recovered from a patient with oral candidiasis in August 1994. Filter-bound DNA samples were fingerprinted separately by 1A). Following hybridization with the 27A probe, the same 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'GCCGATCC 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 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 1. Candida reference strains and clinical isolates

| Strain(s) or isolate(s) | Species                  | Source                  | API ID 32C profile | APILAB database |
|------------------------|--------------------------|-------------------------|--------------------|-----------------|
| **Reference strains**  |                          |                         |                    |                 |
| 132A                   | C. albicans              | Irish oral isolate (HIV +) | 7347140015         | Excellent       |
| ATCC 11006             | C. stellatoidea type I   | American Type Culture Collection | 3142300015         | Excellent       |
| CD36<sup>a</sup>       | C. dubliniensis          | Irish oral isolate (HIV +) | 7142140015         | Unreliable      |
| CD71                   | Atypical                 | United Kingdom oral isolate (HIV +) | 7142140015         | Unreliable      |
| CD71                   | Atypical                 | Argentinian oral isolate (HIV +) | 7142140015         | Unreliable      |
| Co4, Co5, Co7          | Atypical                 | Swiss oral isolates (HIV +) | 7042100011         | Unreliable      |

### Notes

<sup>a</sup> The HIV status of the patients from whom the isolates were recovered is indicated in parentheses.  
<sup>b</sup> 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.  
<sup>c</sup> C. stellatoidea is listed as C. albicans 2 in the API LAB database.  
<sup>d</sup> 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).  
<sup>e</sup> 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.  
<sup>f</sup> 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.
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*, *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-
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.

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