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Simple, Inexpensive, Reliable Method for Differentiation of *Candida dubliniensis* from *Candida albicans*

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***Candida dubliniensis* is a recently described pathogenic species which shares many phenotypic features with *Candida albicans*, including the ability to form germ tubes and chlamydospores. These similarities have caused significant problems in the identification of *C. dubliniensis* by the average clinical mycology laboratory. To facilitate the differentiation of these species, we investigated the growth of 120 isolates of *C. dubliniensis* and 98 *C. albicans* isolates at 42 and 45°C on Emmons' modified Sabouraud glucose agar (SGA) and 10 isolates of each species in yeast-peptone-dextrose broth. None of the *C. dubliniensis* isolates grew on the agar or in the broth medium at 45°C, while 11 isolates were capable of growing on SGA at 42°C. In contrast, all of the *C. albicans* isolates but one grew at 45°C on or in either medium. These reproducible results clearly demonstrate that the incubation of isolates suspected to be *C. dubliniensis* or *C. albicans* at 45°C provides a simple, reliable, and inexpensive method for the differentiation of the two species.**

Candida dubliniensis is a newly described species of the most protean genus of pathogenic yeasts (9, 11). The vast majority of *C. dubliniensis* isolates identified to date have been recovered from the oral cavities of human immunodeficiency virus (HIV)-infected individuals, particularly those suffering from recurrent episodes of oral candidiasis (3, 8–11). However, a small number of isolates associated with other anatomical sites, including the vagina and the lung, have been reported (5, 9, 11). To gain a more complete understanding of the precise epidemiological role that this species plays in human infections, it is essential that rapid and reliable tests for the identification of *C. dubliniensis* in the routine clinical microbiology laboratory be available. However, the introduction of such tests has been complicated by the fact that *C. dubliniensis* shares many phenotypic characteristics with *Candida albicans* (9). The high degree of similarity between these two species has, in all likelihood, contributed to the misidentification of some *C. dubliniensis* isolates as *C. albicans* (3). The most reliable tests currently used for discriminating between the two species are based on molecular techniques such as DNA fingerprinting with repetitive-sequence-containing DNA probes, pulsed-field gel electrophoresis, and others (2, 9, 11). Although they are very effective, these techniques are not readily applicable to the identification of large numbers of isolates, nor can they presently be conducted in most standard mycology laboratories. While the use of a number of phenotypic characteristics, e.g., chlamydospore production, carbohydrate assimilation, colonial coloration on differential media such as CHROMagar Candida and methyl blue-Sabouraud agar, has been investigated, none has provided a completely reliable method for the differentiation of *C. albicans* from *C. dubliniensis* (7, 9). It has previously been suggested that the two species could be distinguished by the inhibition of the growth of *C. dubliniensis* at 42°C (2, 9, 11). However, one recent study reported that approximately 10% of *C. dubliniensis* isolates were able to grow, albeit poorly, at this elevated temperature (9). Furthermore, another recent study reported that 8 of 12 *C. dubliniensis* isolates examined grew at 42°C (7). These findings motivated us to investigate whether incubation at an even higher temperature would provide a simple, inexpensive, and reliable means of differentiating the two species.

Descriptions of the 120 *C. dubliniensis* isolates and 98 isolates of *C. albicans* used in these studies are presented in Table 1. Conventional morphological and physiological methods as well as molecular techniques were employed to identify isolates of both species (11). All yeasts studied were initially grown for 48 h at 37°C individually on 25 ml of Emmons' modified Sabouraud glucose agar (SGA) contained in 85-mm-diameter petri dishes. A small portion of a single colony of each isolate was then aseptically removed and streaked over the surface of two plates of SGA, one of which was incubated at 42°C and the second of which was placed at 45°C. Growth, if any, on both plates was visually assessed after 24 and 48 h of incubation. In addition to these experiments, 10 isolates of each species were selected for examination of their growth in yeast-peptone-dextrose (YPD) broth at 37, 42, and 45°C. The 10 *C. dubliniensis* isolates included 3 which grew poorly at 42°C on SGA medium and 7 that did not grow at all on SGA at that temperature. Nine of the *C. albicans* isolates were randomly selected, while the 10th isolate was chosen because it failed to grow at 45°C on SGA (see below). By using a hemocytometer, a standard inoculum suspension, containing 10^5 CFU in sterile distilled water, was prepared from colonies of each isolate grown on SGA for 24 h at 37°C. The inoculum was then aseptically transferred to YPD broth (to a final volume of 50 ml) in 250-ml conical flasks and incubated at the three temperatures with shaking at 150 rpm. At specific time points, aliquots of each isolate were removed to spectrophotometrically measure their optical densities at 600 nm. These values were then used to plot a growth curve for each isolate at each temperature.

No growth was found for any of the 120 *C. dubliniensis* isolates at 24 and 48 h on SGA at 45°C, although 11 isolates

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TABLE 1. *C. dubliniensis* and *C. albicans* isolates used in these studies

Species and country of isolation	No. of isolates[a]	Specimen source(s)	HIV status of subject(s) sampled[b]	Source or reference(s)
C. dubliniensis				
Argentina	1	Oral cavity	+	10
Australia	4	Oral cavity	+	4, 10, 11
Belgium	5	Oral cavity	+	This study
Canada	6	Oral cavity	+	This study
Finland	1	Oral cavity	+	This study
Germany	4	Oral cavity	+	This study
Greece	1	Oral cavity	+	This study
Ireland	52	Oral cavity	+	This study; 11
Ireland	8	Oral cavity	−	2
Ireland	5	Vaginal	−	This study; 5
Spain	9	Oral cavity	+	This study
Switzerland	6	Oral cavity	+	1, 10
United Kingdom	5	Oral cavity	+	This study; 10
United Kingdom	7	Oral cavity	−	This study
United Kingdom	6	Miscellaneous[c]	−	This study; 8
C. albicans				
Australia	1	Oral cavity	+	11
Hong Kong	6	Oral cavity	+	This study
Ireland	32	Oral cavity	+	This study
	5	Oral cavity	−	This study
	20	Vaginal	+	This study
	21	Vaginal	−	This study
	8	Blood	−	6
United Kingdom	1	Oral cavity	+	This study
United Kingdom	4	Oral cavity	−	This study

[a] Each isolate was recovered from a separate individual.
[b] +, positive; −, negative.
[c] These included two fecal isolates and one each from a blood culture, tracheal aspirate, sputum, and postmortem lung specimens.

showed limited growth after 48 h of incubation at 42°C. All 98 of the *C. albicans* isolates showed good growth at 42°C on SGA at 48 h, and all but one exhibited substantial growth after the same period of time at 45°C. The growth of more than half of the isolates was examined on at least two occasions, and identical results were obtained in these confirmatory studies. Representative growth curves for isolates of each species grown in YPD broth at 37 and 45°C are presented in Fig. 1. Although all

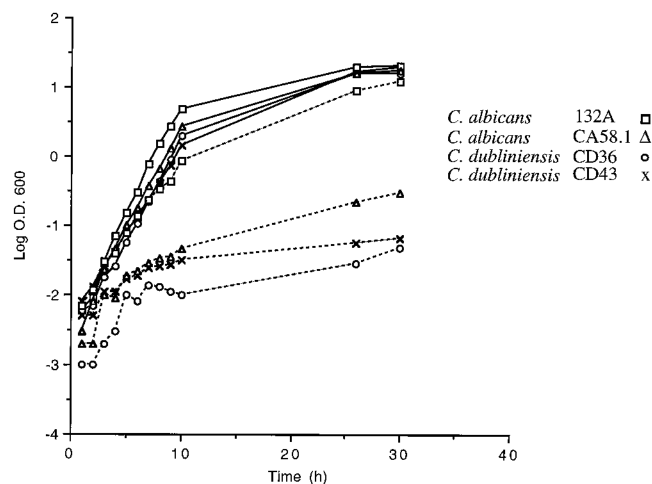

FIG. 1. Growth curves of the oral isolates *C. albicans* 132A and CA58.1 and *C. dubliniensis* CD36 and CD43 in YPD broth medium at 37°C (solid lines) and 45°C (dashed lines). O.D. 600, optical density at 600 nm.

10 selected isolates of *C. dubliniensis* grew at 37°C, 7 failed to grow at 42°C, while the 3 that showed limited growth on SGA at this temperature also showed limited growth in YPD broth. None of the 10 isolates examined showed any appreciable growth in YPD broth at 45°C. All but 1 of the 10 *C. albicans* isolates grew well in YPD broth at all three temperatures. The single isolate which failed to grow in the broth at 45°C (isolate CA58.1) also failed to grow when incubated on SGA at the same temperature. Preliminary studies with a single isolate of a closely allied taxon, *Candida stellatoidea* type I (ATCC 11006), demonstrated that it, too, like the majority of *C. dubliniensis* isolates, did not grow at 42 or 45°C. However, *C. stellatoidea* type I may be easily differentiated from *C. dubliniensis* on the basis of sucrose assimilation, production of β-glucosidase, and serotype (1, 11).

The data from these studies clearly indicate that *C. dubliniensis* can be readily distinguished from *C. albicans* by the incubation of isolates on SGA at 45°C. This test is simple, reliable, inexpensive, reproducible, and readily applicable to large numbers of isolates in either a clinical or academic mycology laboratory. This simple procedure can be employed to retrospectively evaluate the identification of stored cultures of *C. albicans* held in stock collections. In a previous retrospective analysis of the authors' collection of *C. albicans* isolates, 2 of 110 (1.8%) isolates recovered from the oral cavities of asymptomatic, normal, healthy individuals and 13 of 79 (16.5%) isolates which had been obtained from the oral cavities of HIV-infected individuals and had been identified as *C. albicans* were found to be *C. dubliniensis* (3). Accurate identification of *C. dubliniensis* isolates in archival collections and in clinical specimens should provide invaluable information concerning the epidemiology of this species and help to establish its clinical significance. In addition, data from such investigations may also help to explain the rapid emergence of *C. dubliniensis* as a potentially significant pathogen during the last decade.

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REFERENCES

1. **Boerlin, P., F. Boerlin-Petzold, C. Durussel, M. Addo, J.-L. Pagani, J.-P. Chave, and J. Bille.** 1995. Cluster of oral atypical *Candida* isolates in a group of human immunodeficiency virus-positive drug users. J. Clin. Microbiol. **33:**1129–1135.
2. **Coleman, D., D. Sullivan, K. Haynes, M. Henman, D. Shanley, D. Bennett, G. Moran, C. McCreary, L. O'Neill, and B. Harrington.** 1997. Molecular and phenotypic analysis of *Candida dubliniensis*: a recently identified species linked with oral candidosis in HIV-infected and AIDS patients. Oral Dis. **3**(Suppl. 1)**:**S96–S101.
3. **Coleman, D. C., D. J. Sullivan, D. E. Bennett, G. P. Moran, H. J. Barry, and D. B. Shanley.** 1997. Candidiasis: the emergence of a novel species, *Candida dubliniensis*. AIDS **11:**557–567.
4. **McCullough, M. J., B. C. Ross, B. D. Dwyer, and P. C. Reade.** 1994. Genotype and phenotype of oral *Candida albicans* from patients infected with the human immunodeficiency virus. Microbiology **140:**1195–1202.
5. **Moran, G. P., D. J. Sullivan, M. C. Henman, C. E. McCreary, B. J. Harrington, D. B. Shanley, and D. C. Coleman.** 1997. Antifungal drug suscep-

tibility of oral *Candida dubliniensis* isolates from human immunodeficiency virus (HIV)-infected and non-HIV-infected subjects and generation of stable fluconazole-resistant derivatives in vitro. Antimicrob. Agents Chemother. **41:**617–623.

6. **O'Connell, B., D. C. Coleman, D. Bennett, D. Sullivan, S. R. McCann, and C. T. Keane.** 1995. An epidemiological study of *Candida* species infection in cancer patients using genetic fingerprinting and morphotyping. J. Hosp. Infect. **31:**211–217.
7. **Schoofs, A., F. C. Odds, R. Colebunders, M. Ieven, and H. Goosens.** 1997. Use of specialised isolation media for recognition and identification of *Candida dubliniensis* isolates from HIV-infected patients. Eur. J. Clin. Infect. Dis. **16:**296–300.
8. **Sullivan, D., D. Bennett, M. Henman, P. Harwood, S. Flint, F. Mulcahy, D. Shanley, and D. Coleman.** 1993. Oligonucleotide fingerprinting of isolates of *Candida* species other than *C. albicans* and of atypical *Candida* species from human immunodeficiency virus-positive and AIDS patients. J. Clin. Microbiol. **31:**2124–2133.
9. **Sullivan, D., and D. Coleman.** 1998. *Candida dubliniensis*: characteristics and identification. J. Clin. Microbiol. **36:**329–334.
10. **Sullivan, D. J., K. Haynes, J. Bille, P. Boerlin, L. Rodero, S. Lloyd, M. Henman, and D. Coleman.** 1997. Widespread geographic distribution of oral *Candida dubliniensis* strains in human immunodeficiency virus-infected individuals. J. Clin. Microbiol. **35:**960–964.
11. **Sullivan, D. J., T. J. Westerneng, K. A. Haynes, D. E. Bennett, and D. C. Coleman.** 1995. *Candida dubliniensis* sp. nov.: phenotypic and molecular characterisation of a novel species associated with oral candidosis in HIV-infected individuals. Microbiology **141:**1507–1521.