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Differentiation of *Candida dubliniensis* from *Candida albicans* on Pal's Agar

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Production of a hyphal fringe around colonies grown on Pal's agar (sunflower seed agar) at 30°C for 48 to 72 h provides a simple means of discriminating between isolates of *C. dubliniensis* and *C. albicans* with 100% accuracy. Of 128 *C. dubliniensis* isolates tested on this medium, all produced a hyphal fringe. In contrast, none of the 124 *C. albicans* isolates tested produced a hyphal fringe. Pal's medium has the added advantage of being prepared from inexpensive, readily available seeds.

Candida dubliniensis is a novel *Candida* species first described by our laboratory in 1995 (20). Studies to date indicate that this species is primarily associated with oral carriage and infection in human immunodeficiency virus-infected and AIDS patients and has rarely been identified in blood cultures from patients with candidemia (3, 4, 6, 7, 10, 14, 19, 20). Routine discrimination between *C. dubliniensis* and the closely related species *Candida albicans* has been problematic (4, 13, 18). The most accurate means of identifying *C. dubliniensis* and discriminating it from *C. albicans* requires PCR-based tests; however, these are not readily applicable to the high-volume throughput of isolates in many routine diagnostic laboratories (5, 8). In contrast, while a number of phenotype-based tests (e.g., determination of colony color on CHROMagar Candida plates and lack of growth at 45°C and carbohydrate assimilation profiling) have proved to be useful for identifying *C. dubliniensis* isolates, they do not give completely reliable results (4, 12, 13, 15, 18). In a recent study, Staib and Morschhäuser suggested that Staib agar (which contains *Guizotia abyssinica* seed extract) was a good discriminatory medium (17). This finding was confirmed in a study from our laboratory in which 127 of 130 isolates of *C. dubliniensis* (97.7%) grew as rough colonies and all 166 *C. albicans* isolates tested grew as smooth colonies (1). Staib agar was originally developed for the identification of *Cryptococcus neoformans*, which produces melanin-like pigment on this agar (16). In order to develop a medium with even greater discriminatory ability, we investigated the effect of replacing *G. abyssinica* seed extract with extracts from the seeds of other plants. One such medium is Pal's agar, which contains sunflower (*Helianthus annus*) seed extract and is another medium originally developed for the identification of *C. neoformans* (16). Since both *H. annus* and *G. abyssinica* belong to the same botanical family (*Asteraceae*), and since Staib agar is a good but not an absolutely reliable medium for differentiating between *C. dubliniensis* and *C. albicans*, we investigated the use of Pal's medium for this purpose.

The yeast isolates used in this study are shown in Table 1. The identity of all isolates was reconfirmed using the ID 32C yeast identification system (bioMérieux, Marcy l'Étoile, France). In addition, the identities of *C. dubliniensis* and *C. albicans* isolates were confirmed by growth at 45°C and by PCR (5, 13). All isolates were also tested for chlamydospore formation on rice agar-Tween agar, and all the *C. dubliniensis* and *C. albicans* isolates produced chlamydospores on this medium (20). Pal's agar was prepared freshly with unsalted sunflower seeds (including kernels and shells) and used within 5 days to ensure consistent results. First, an aqueous extract of sunflower seeds was prepared by pulverizing 50 g of seeds in a domestic Moulinex (Dublin, Ireland) model B57 blender for 5 min and then adding the ground seeds to 1 liter of distilled water, followed by boiling for 30 min. Next, the seed extract was cooled and filtered, and the following ingredients were added: glucose (1 g), KH_2PO_4 (1 g), and creatinine (1 g). The pH was adjusted to 5.5, the volume was readjusted to 1 liter, and 15 g of agar (Difco) was added before the mixture was autoclaved at 110°C for 20 min. For each isolate included in the study, part of a single colony grown on potato dextrose agar (Oxoid) at 37°C for 48 h was streaked onto Pal's agar contained in 90-mm-diameter single-vent petri dishes (25 ml of agar per plate) and incubated at 30°C. Colony morphology was examined visually every 24 h for up to 10 days, and the data were recorded. At 48 to 72 h, colonies were examined microscopically for the presence or absence of chlamydospores: 10 to 20 well-separated single colonies were stained with 1 drop of 1% (wt/vol) lactophenol cotton blue stain (which stains chlamydospores preferentially), allowed to stain for 5 min, and then covered with sterile glass coverslips (22 by 22 mm) and examined microscopically under bright-field illumination with a 40× objective (9, 20). Chlamydospore-negative isolates continued to be examined at 24-h intervals for up to 10 days.

All of the yeast isolates tested grew well on Pal's agar. Following 48 to 72 h of incubation, all 124 *C. albicans* and all 128 *C. dubliniensis* isolates tested grew as smooth creamy-gray colonies (Fig. 1). However, all the *C. dubliniensis* isolates exhibited a hyphal fringe (Fig. 1B), whereas none of the *C. albicans* did (Fig. 1A). It should be noted that 36 of 124 *C. albicans* isolates (29%) were observed to produce a fringe following 10 days of incubation. Microbiological analysis re-

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TABLE 1. *Candida* isolates[a] used in the study

Yeast species	Country of isolation	No. of isolates	Reference
C. dubliniensis[b]	Argentina	1	6, 20
	Australia	2	6, 20
	Brazil	7	11, this study
	Canada	3	1, 6, 13
	France	6	1
	Germany	2	1, 6, 13
	Israel	18	1, 6, 14, this study
	Ireland	43	1, 6, 13, 20
	Malta	3	1
	New Zealand	3	This study
	Spain	2	1, 2, 6
	Switzerland	2	1, 6, 18
	The Netherlands	3	6, 10
	United Kingdom	26	1, 6, 13
	United States	7	1, 12
Total		128	
C. albicans[b]	Greece	8	This study
	Hungary	5	This study
	Ireland	61	1, 13
	United Kingdom	2	1, 13
	United States	48	1, 12
Total		124	
C. glabrata		5	This study
C. tropicalis		4	This study
C. parapsilosis		5	This study
C. krusei		1	This study

[a] All isolates were clinical isolates from the culture collection of the Microbiology Research Unit, Department of Oral Medicine and Oral Pathology, School of Dental Science, Trinity College, University of Dublin, Ireland.

[b] Ninety-five of the *C. dubliniensis* isolates and 111 of the *C. albicans* isolates investigated here were also included in a previous Staib agar study (1).

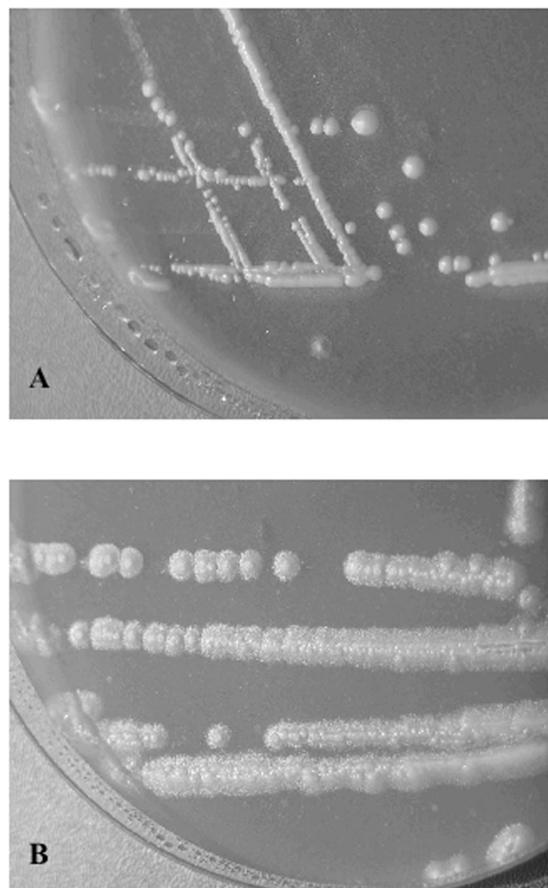

FIG. 1. Macroscopic appearance of *C. dubliniensis* and *C. albicans* colonies on Pal's medium following 72 h of incubation at 30°C. (A) Smooth colonies exhibited by *C. albicans* composed exclusively of blastospores; (B) *C. dubliniensis* colonies displaying a hyphal fringe, containing abundant hyphae, pseudohyphae, and chlamydospores.

vealed that the fringe surrounding *C. dubliniensis* colonies was found to be comprised of hyphae, pseudohyphae, and blastospores. These findings are in contrast to previous data obtained by using Staib agar, on which 127 of 130 *C. dubliniensis* isolates tested (97.7%) formed rough colonies, many (65%) with a hyphal fringe, and all 166 *C. albicans* tested grew as smooth colonies (1). In the present study, none of the *C. albicans* isolates produced chlamydospores on Pal's agar, even after 10 days of incubation, whereas 120 of 128 of the *C. dubliniensis* isolates tested (93.75%) produced chlamydospores within 48 to 72 h. The remaining 8 of 128 *C. dubliniensis* isolates (6.25%) were chlamydospore negative even after 10 days of incubation. Interestingly, in the previous study with Staib agar, none of the 166 *C. albicans* isolates tested produced chlamydospores whereas only 19 of 130 of the *C. dubliniensis* isolates examined (14.6%) were chlamydospore negative (1). Twelve of these 19 chlamydospore-negative *C. dubliniensis* isolates were included in the present study; 7 were chlamydospore positive on Pal's agar, and the remaining 5 were chlamydospore negative.

The colony morphologies of selected oral isolates of four other *Candida* species (Table 1), including five *C. glabrata*, four *C. tropicalis*, five *C. parapsilosis*, and a single *C. krusei* isolate, were also examined on Pal's medium. One *C. tropicalis* isolate, two *C. glabrata* isolates, and three *C. parapsilosis* isolates yielded smooth colonies similar to those of *C. albicans*. Three *C. tropicalis* isolates, two *C. parapsilosis* isolates, and the single *C. krusei* isolate formed rough colonies with a fringe similar to that formed by *C. dubliniensis*.

Similar results to those described above were obtained with all isolates tested on separate batches of Pal's agar prepared from the same batch of sunflower seeds, in each case with seeds purchased from two separate suppliers. However, we found that incubation of the plates at 37°C rather than 30°C resulted in poorer discrimination between the two species. We therefore propose that the formation of a hyphal fringe surrounding *C. dubliniensis* colonies on Pal's medium following incubation for 48 to 72 h at 30°C provides a definitive means of discrimination between this species and *C. albicans*. However, since colonies of some isolates belonging to other *Candida* species also produce a fringe on Pal's agar, we suggest that this medium should be used only to screen germ tube- and/or chlamydospore-positive isolates. Pal's medium has an impor-

tant advantage over Staib agar in that it permits an absolute discrimination between *C. dubliniensis* and *C. albicans*.

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REFERENCES

1. **Al Mosaid, A., D. J. Sullivan, I. F. Salkin, D. B. Shanley, and D. C. Coleman.** 2001. Differentiation of *Candida dubliniensis* from *Candida albicans* on Staib agar and caffeic acid-ferric citrate agar. J. Clin. Microbiol. **39:**323–327.
2. **Bikandi, J., R. San Millan, M. D. Moragues, G. Cebas, M. Clarke, D. C. Coleman, D. J. Sullivan, G. Quindós, and J. Pontón.** 1998. Rapid identification of *Candida dubliniensis* by indirect immunofluorescence based on differential localization of antigens on *C. dubliniensis* blastospores and *Candida albicans* germ tubes. J. Clin. Microbiol. **36:**2428–2433.
3. **Brandt, M. E., L. H. Harrison, M. Pass, A. N. Sofair, S. Huie, R.-K. Li, C. J. Morrison, D. W. Warnock, and R. H. Hajjeh.** 2000. *Candida dubliniensis* fungemia: the first four cases in North America. Emerg. Infect. Dis. **6:**46–49.
4. **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.
5. **Donnelly, S. A., D. J. Sullivan, D. B. Shanley, and D. C. Coleman.** 1999. Phylogenetic analysis and rapid identification of *Candida dubliniensis* based on analysis of *ACT1* intron and exon sequences. Microbiology **145:**1871–1882.
6. **Gee, S. F., S. Sophie, D. R. Soll, J. F. G. M. Meis, P. E. Verweij, I. P. Polacheck, D. J. Sullivan, and D. C. Coleman.** 2002. Identification of four distinct genotypes of *Candida dubliniensis* and detection of microevolution in vitro and in vivo. J. Clin. Microbiol. **40:**556–574.
7. **Kirkpatrick, W. R., S. G. Revankar, R. K. McAtee, J. L. Lopez-Ribot, A. W. Fothergill, D. I. McCarthy, S. E. Sanche, R. A. Cantu, M. G. Rinaldi, and T. F. Patterson.** 1998. Detection of *Candida dubliniensis* in oropharyngeal samples from human immunodeficiency virus-infected patients in North America by primary CHROMagar Candida screening and susceptibility testing of isolates. J. Clin. Microbiol. **36:**3007–3012.
8. **Kurzai, O., W. Heinz, D. J. Sullivan, D. J. Coleman, M. Frosch, and F. Mühlschlegel.** 1999. Rapid PCR test for discriminating between *Candida albicans* and *Candida dubliniensis* isolates using primers derived from the pH-regulated PHR1 and PHR2 genes of *C. albicans.* J. Clin. Microbiol. **37:**1587–1590.
9. **Larone, D. H.** 1993. Staining methods, p. 186–192. *In* D. H. Larone (ed.), Medically important fungi: a guide to identification, 2nd ed. American Society for Microbiology, Washington, D.C.
10. **Meis, J. F., M. Ruhnke, B. E. De Pauw, F. C. Odds, W. Siegert, and P. E. Verweij.** 1999. *Candida dubliniensis* candidemia in patients with chemotherapy-induced neutropenia and bone marrow transplantation. Emerg. Infect. Dis. **5:**150–153.
11. **Milan, E. P., P. de Laet Sant'Ana, A. S. de Azevedo Melo, D. J. Sullivan, D. C. Colleman, D. Lewi, and A. L. Colombo.** 2001. Multicenter prospective surveillance of oral *Candida dubliniensis* among adult Brazilian human immunodeficiency virus-positive and AIDS patients. Diagn. Microbiol. Infect. Dis. **41:**29–35.
12. **Pincus, D. H., D. C. Coleman, W. R. Pruitt, A. A. Padhye, I. F. Salkin, M. Geimer, A. Bassel, D. J. Sullivan, M. Clarke, and V. Hearn.** 1999. Rapid identification of *Candida dubliniensis* with commercial yeast identification systems. J. Clin. Microbiol. **37:**3533–3539.
13. **Pinjon, E., D. Sullivan, I. Salkin, D. Shanley, and D. Coleman.** 1998. Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans.* J. Clin. Microbiol. **36:**2093–2095.
14. **Polacheck, I., J. Strahilevitz, D. Sullivan, S. Donnelly, I. F. Salkin, and D. C. Coleman.** 2000. Recovery of *Candida dubliniensis* from non-human immunodeficiency virus-infected patients in Israel. J. Clin. Microbiol. **38:**170–174.
15. **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. Microbiol. Infect. Dis. **16:**296–300.
16. **Staib, F., M. Seibold, E. Antweiler, B. Frolich, S. Weber, and A. Blisse.** 1987. The brown colour effect (BCE) of *Cryptococcus neoformans* in the diagnosis, control and epidemiology of *C. neoformans* infections in AIDS patients. Zentbl. Bakteriol. Microbiol. Hyg. A **266:**167–177.
17. **Staib, P., and J. Morschhäuser.** 1999. Chlamydospore formation on Staib agar as a species-specific characteristic of *Candida dubliniensis.* Mycoses **42:**521–524.
18. **Sullivan, D., and D. Coleman.** 1998. *Candida dubliniensis*: characteristics and identification. J. Clin. Microbiol. **36:**329–334.
19. **Sullivan, D., 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.
20. **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.