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Research on *Candida dubliniensis* in a Brazilian yeast collection obtained from cardiac transplant, tuberculosis, and HIV-positive patients, and evaluation of phenotypic tests using agar screening methods

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Abstract

The aim of this study was to research *Candida dubliniensis* among isolates present in a Brazilian yeast collection and to evaluate the main phenotypic methods for discrimination between *C. albicans* and *C. dubliniensis* from oral cavity. A total of 200 isolates, presumptively identified as *C. albicans* or *C. dubliniensis* obtained from heart transplant patients under immunosuppressive therapy, tuberculosis patients under antibiotic therapy, HIV-positive patients under antiretroviral therapy, and healthy subjects, were analyzed using the following phenotypic tests: formation and structural arrangement of chlamydospores on corn meal agar, casein agar, tobacco agar, and sunflower seed agar; growth at 45 °C; and germ tube formation. All strains were analyzed by polymerase chain reaction (PCR). In a preliminary screen for *C. dubliniensis*, 48 of the 200 isolates on corn meal agar, 30 of the 200 on casein agar, 16 of the 200 on tobacco agar, and 15 of the 200 on sunflower seed agar produced chlamydoconidia; 27 of the 200 isolates showed no or poor growth at 45 °C. All isolates were positive for germ tube formation. These isolates were considered suggestive of *C. dubliniensis*. All of them were subjected to PCR analysis using *C. dubliniensis*-specific primers. *C. dubliniensis* isolates were not found. *C. dubliniensis* isolates were not recovered in this study done with immunocompromised patients. Sunflower seed agar was the medium with the smallest number of isolates of *C. albicans* suggestive of *C. dubliniensis*. None of the phenotypic methods was 100% effective for discrimination between *C. albicans* and *C. dubliniensis*.

Keywords: *C. albicans*; *C. dubliniensis*; Polymerase chain reaction; Phenotypic tests; Agar

1. Introduction

The incidence of fungal infections, as well as the variety of potential infectious fungal agents, has increased over the last 2 decades. Exogenous and endogenous predisposing factors render some individuals more vulnerable to infections by different opportunistic fungi, in particular species of the genus *Candida*. Patients with AIDS, HIV-infected patients, patients under chemotherapy, patients under treatment with broad-spectrum antibiotics and corticosteroids for a prolonged period of time, terminally ill patients with hematologic diseases, transplanted patients, and patients with diabetes mellitus are more susceptible to fungal infections (Back-Brito et al., 2009; Costa et al., 2006; Navas et al., 2009). Although *Candida albicans* is the most relevant species of the *Candida* genus associated with oral infections in both immunocompromised and immunocompetent

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subjects, the incidence of infections caused by species such as *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. dubliniensis* has been increasing (Pfaller & Diekema, 2007; Tekeli et al., 2006).

C. dubliniensis shares many phenotypic features with *C. albicans*, and although this species has been isolated and identified only about 10 years ago from AIDS and HIV-infected patients, it is probably present in the community for at least 50 years (Coleman et al., 1997). The real incidence of candidemia due to *C. dubliniensis* is still unknown mainly because of the difficulty in distinguishing this species from *C. albicans* (Jabra-Rizk et al., 2000; Milan et al., 2001; Sullivan et al., 2005).

Phenotypic tests are useful for the presumptive identification of *C. dubliniensis*, but do not provide a definitive diagnosis. Molecular methods permit a definitive identification, but require specialized pieces of equipment that are not available in all laboratories (Ellepola et al., 2003). The phenotypic tests provide relevant characteristics that, if combined, might be useful for the laboratory identification of *Candida* species. Researchers all over the world are currently investigating laboratory techniques that permit the rapid, easy, and low-cost distinction between *C. albicans* and *C. dubliniensis* (Campanha et al., 2005; Khan et al., 2005; Mosca et al., 2003; Tendolkar et al., 2003) in order to reduce the time for diagnosis and the institution of appropriate antifungal therapy. Analysis based on the colony morphology of *C. dubliniensis* and *C. albicans*, as well as determination of the capacity of isolates to produce abundant chlamydospores when grown on culture media supplemented with plant extracts, has been used in the search for a low-cost culture medium that could provide rapid results and can be used in the routine of a microbiology laboratory (Al Mosaid et al., 2003; Khan et al., 2004a; Mähnß et al., 2005).

The aim of this study was to identify *C. dubliniensis* among isolates present in a Brazilian yeast collection obtained from orthotopic cardiac transplantation patients under immunosuppressive therapy, tuberculosis patients under prolonged antibiotic therapy, HIV-positive patients under antiretroviral therapy, and healthy subjects, and to evaluate the main phenotypic methods for discrimination between *C. albicans* and *C. dubliniensis* from oral cavity.

2. Materials and methods

2.1. Clinical isolates

A total of 200 isolates from the collection of the Laboratory of Microbiology, São José dos Campos Dental School, UNESP, presumptively identified as *C. albicans* or *C. dubliniensis*, were included in this study. These strains were isolated from the oral cavity of heart transplanted patients under immunosuppressive therapy ($n = 50$), patients with tuberculosis under prolonged antibiotic therapy ($n = 50$), HIV-positive patients under antiretroviral therapy ($n = 50$), and healthy subjects ($n = 50$). None of the patients had clinical evidence of oral candidiasis or used antifungal agents for the last 6 months prior to sample collection.

The strains were plated onto Sabouraud dextrose agar, and after growth of characteristic colonies, the isolates were phenotypically and genotypically identified. The isolates were subcultured on Sabouraud dextrose agar for 24 h at 37 °C before each test. Only casein agar needed subculturing on Sabouraud dextrose agar for 48 h at 30 °C. Phenotypic criteria (Al Mosaid et al., 2003; Coleman et al., 1997; Khan et al., 2004b; Mähnß et al., 2005; Mosca et al., 2003) for presumptive discrimination between *C. albicans* and *C. dubliniensis* on corn meal–Tween 80, casein agar, tobacco agar, sunflower seed agar, and colony growth of the isolates at 45 °C are shown in Table 1. A reference strain of *C. albicans* (ATCC 18804, American Type Culture Collection, USA) and *C. dubliniensis* (NCPF 3108, National Collection of Pathogenic Fungi, United Kingdom) was used as quality control in all identification tests.

2.2. Germ tube formation

An inoculating loop of a pure 24 h culture of each isolate was transferred to 0.5 mL of sterile bovine serum. The tubes were incubated in a water bath at 37 °C for a minimum of 2 h and a maximum of 3 h. Germ tube formation was observed under a light microscope at 400× magnifications by placing a drop of suspension between a slide and a coverslip.

2.3. Formation and structural arrangement of chlamydospores on corn meal agar

Corn meal agar (Difco, Detroit, MI, USA) supplemented with 1% Tween 80 was used. For the test, 1 mL of melted agar was poured onto glass slides and the slides were placed into sterile Petri dishes. After solidification of the agar, each isolate was plated onto the agar surface and a sterile coverslip was placed in the center of the slide. After incubation for 48 to 72 h at room temperature, the presence of chlamydospores was analyzed under a light microscope at 400× magnification. *C. albicans* produces chlamydospores at the tip of or along hyphae and pseudohyphae, whereas *C. dubliniensis* produces abundant chlamydospores generally arranged in

Table 1
Basis of differentiation between *C. albicans* and *C. dubliniensis* isolates in phenotypic methods

Test method	*C. albicans*	*C. dubliniensis*
Corn meal–Tween 80	Single/pairs of chlamydospores	Pairs/triplets/bunches of chlamydospores
Casein agar	Few or no chlamydospores	Bunches of chlamydospores
Tobacco agar	White- to cream-colored colonies with no hyphal fringe or chlamydospores	Orange colonies with peripheral hyphal fringe and chlamydospores
Sunflower seed agar	No hyphal fringe or chlamydospores	Peripheral hyphal fringe and chlamydospores
Growth at 45 °C	Positive	Negative