

Short communication

Phenotypic identification of *Candida albicans* by growth on chocolate agar

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In this study, we describe a simple method for the identification of *Candida albicans* in clinical samples. A total of 383 clinical isolates of *Candida* species were streaked onto chocolate agar and incubated for 48 h at 37°C in the presence of an atmosphere of 6% CO₂. All 208 of the *C. albicans* isolates tested, developed an easy to identify filamentous colony morphology. Of 175 other *Candida* species tested, 172 (98.3%) were distinguishable from *C. albicans* by their smooth colony morphology. Three isolates (1.7%) exhibited weak filamentation after prolonged incubation. Although not a routine medium in medical mycology a significant advantage of using chocolate agar lies in its use in clinical bacteriology laboratories for the isolation of fastidious bacteria. Implementation of the proposed method is applicable across a range of specimen types, thus allowing the direct identification of *C. albicans* in clinical samples. This simple method may allow a quicker entry into directed treatment.

Keywords *Candida albicans*, differentiation, chocolate agar

Introduction

Candida albicans is routinely found as a harmless commensal in the gastrointestinal tract of humans [1]. However, this yeast is also the most common opportunistic fungal pathogen accounting for up to 60% of *Candida* species isolated from cases of infection [2]. The increase in numbers of patients in the high-risk category, i.e., those requiring long-term in-dwelling catheters, broad-spectrum antibiotic therapy, and treatment for cancer have all contributed to the escalation in prevalence of serious *Candida* infections [2]. In recent years, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis* have assumed clinical significance,

accounting for 15%, 10% and 3% respectively of cases of systemic candidiasis [3]. *Candida dubliniensis*, is primarily associated with oropharyngeal carriage and infection in HIV-infected individuals [4,5]. *C. albicans* and *C. dubliniensis* are phylogenetically closely related, sharing the capacity to produce germ tubes in response to mammalian environmental cues (i.e., pH of 7.4, an ambient temperature of 37°C, serum) [6]. These characteristics have been previously used as specific diagnostic features for *C. albicans*.

Currently, the most definitive method for differentiating between *Candida* species relies on PCR amplification, followed by sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA [7]. However, scale up of the PCR system for routine identification may prove costly and difficult to achieve, particularly when repeat sampling generates large specimen numbers. Given that *C. albicans* can be found as a harmless commensal while possibly representing a serious threat when isolated from immunocompromised patients, diagnosis may require repeat sampling.

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Species identification based on phenotypic characteristics is available (e.g., CHROMagar *Candida* differentiation plates, and carbohydrate assimilation tests) [8] and other diagnostic tests include analysis of chlamydospore formation on defined media, growth at 45°C, and germ tube formation in response to serum incubation [9,10]. However, in light of its clinical significance there continues to be a need for the simple identification of *C. albicans* from a background of non-*Candida albicans* species, including phenotypically similar species.

Methods

A range of 383 clinical isolates of *Candida* species were streaked onto chocolate agar and incubated for 48 h at 37°C in the presence of an atmosphere of 6% CO₂ (Table 1). All cultures were grown in 90 mm diameter plastic petri dishes with 25 ml of chocolate agar (Becton Dickinson limited, Oxoid limited, Health Protection Agency, UK and E&O Limited). An extended visual analysis of colony morphology of the isolates was carried out for a total of 72 h, to analyse the effects of long-term growth under increased CO₂. The identity of each of the *Candida* strains used in this study, shown in Table 1, was independently identified using a combination of PCR followed by nucleic acid sequence analysis, biochemical and phenotypic tests [7,11].

Table 1 *Candida* isolates used in this study. Superscript letters indicate the following: ^aBlood culture isolates from University Hospital Würzburg, Germany; ^bIsolates from cystic fibrosis patients University Hospital, Würzburg, Germany; ^cClinical isolates from University Hospital, Hannover, Germany; ^dClinical isolates, HPA, Bristol, UK; ^eIsolates from Department of Oral Medicine and Oral Pathology, School of Dental Science, University of Dublin, Dublin, Ireland [14]

Species	Isolates (383)
<i>C. albicans</i>	208 ^{a, b, c}
<i>C. dubliniensis</i>	52 ^{a, b, c, e}
<i>C. glabrata</i>	45 ^{a, d}
<i>C. tropicalis</i>	27 ^{a, d}
<i>C. parapsilosis</i>	22 ^{a, d}
<i>C. krusei</i>	11 ^d
<i>C. lusitaniae</i>	6 ^d
<i>C. inconspicua</i>	5 ^d
<i>C. guilliermondii</i>	2 ^d
<i>C. famata</i>	2 ^d
<i>C. kefyr</i>	1 ^a
<i>C. pelliculosa</i>	1 ^a
<i>C. lipolytica</i>	1 ^a

Results and discussion

C. albicans formed creamy-grey colonies filamenting along their outer border (Fig. 1B), while non-*C. albicans* species formed smooth, viscid, non-filamentous colonies (Fig. 1A). The differences in morphology between *C. albicans* and four other *Candida* species are highlighted in Fig. 2. The filamentous border becomes visible initially, following 24 h incubation, but is prominent following 48 h; in contrast, non-*Candida*

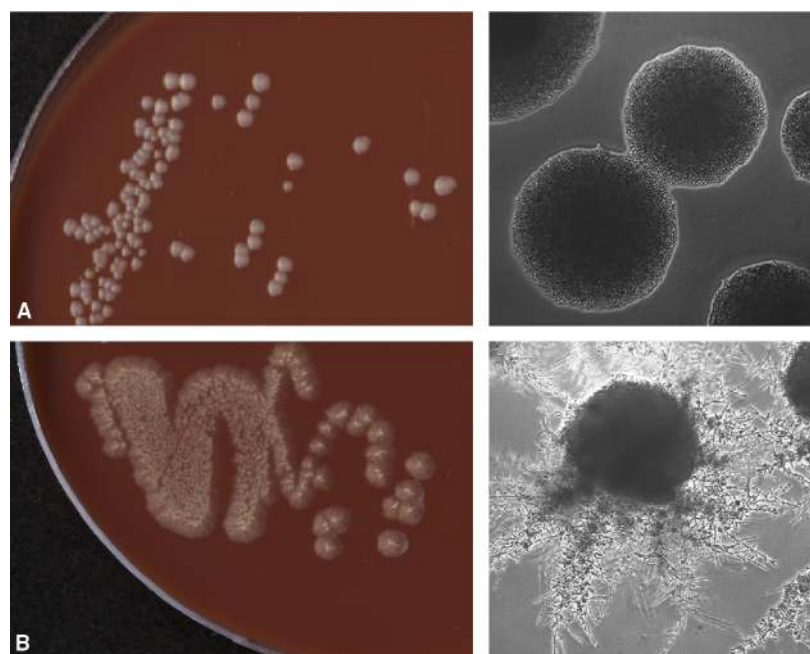


Fig. 1 Phenotypic appearance of *Candida albicans* and *Candida dubliniensis* colonies on chocolate agar following 48 h incubation at 37°C in an atmosphere of 6% CO₂. (A) *Candida dubliniensis* forms smooth-edged colonies with a viscid appearance, composed entirely of blastoconidia. (B) *Candida albicans* colonies present a rough, filamentous border, composed of hyphae and pseudohyphae. Microscopic images of *Candida dubliniensis* (above) and *Candida albicans* (below) colonies grown on minimal medium following 48 h incubation at 37°C in an atmosphere of 6% CO₂ are displayed to the right of each colony image (both images captured at ×100 magnification on a Leitz DMRB microscope using a Leica lens). Bar = 100 µm.

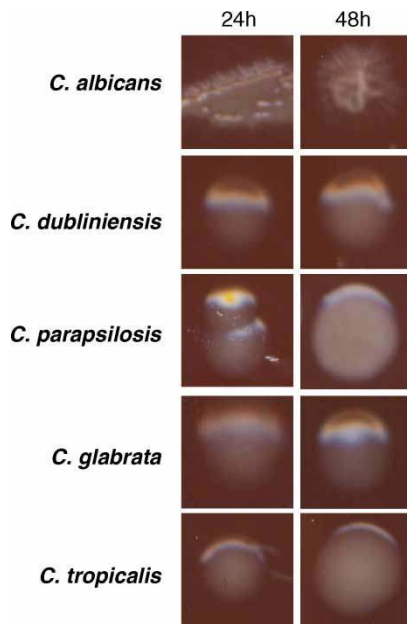


Fig. 2 Comparison of the growth of *Candida* species on chocolate agar in the presence of 6% CO₂ for 24 and 48 h. *Candida albicans* develops profuse filamentation after 24 h incubation that is maintained past 48 h. *Candida dubliniensis*, *Candida parapsilosis*, *Candida glabrata*, and *Candida tropicalis* do not produce filaments within 48 h.

albicans species colony morphology remains non-filamentous throughout. All of the *C. albicans* isolates tested exhibited the hyphal morphology, while 98.3% of non-*Candida albicans* species produced non-filamentous colonies. The differences in colony morphology were independent across the range of suppliers for chocolate agar.

Examination of the filamentous fringe surrounding *C. albicans* colonies revealed that this region contained a mixture of hyphae and pseudohyphae (Microscopy image, Fig. 1B), whereas the non-*Candida albicans* colonies consisted of a unicellular morphological phenotype (Microscopy image, Fig. 1A). Long-term growth analysis revealed that the organisms maintained their distinct morphological phenotypes at 37°C and 6% CO₂ (Fig. 2). Three (two *C. dubliniensis* and one *C. tropicalis*) out of 175 non-*C. albicans* isolates filamented weakly after prolonged growth on chocolate agar.

Typically, isolation and identification of *Candida* species in a diagnostic laboratory involves sample culture on Sabouraud's agar, followed by further analysis including germ-tube generation in a serum-containing medium. Germ-tube positive isolates are then classified as *C. albicans* or *C. dubliniensis*, while germ-tube negative organisms require further investigation. Although not routinely used in the medical

mycology laboratory, the key advantage of incubating clinical isolates on chocolate agar at 37°C in the presence of 6% CO₂ for 48 h is that it utilizes media routinely employed in clinical laboratories for the isolation and characterization of bacteria from clinical specimens [12].

Adaptation of this culture-based isolation system for use in the simple identification of *C. albicans* in samples from patients with suspected yeast infection is a novel application for the observation of filamentation upon growth in 6% CO₂. The application of this method in the identification of *C. albicans* from blood, CSF, swabs and lavage samples is practicable, and extends the diagnostic scope of this technique to a wide range of conditions and clinical sample types. However it must be taken into account that chocolate agar is not routinely used in the medical microbiology laboratory.

Although the filamentation of *C. albicans* on chocolate agar in the presence of 5–6% CO₂ has been previously documented [13], we believe that this is the first formal study to reproducibly adapt this observation in the positive identification of the yeast in clinical samples. Indeed, we have tested over three times as many samples as the most recent study with an aim to demonstrate robustness and applicability of the test in the clinical laboratory [13]. We observed that the prevalence of filamenting non-*Candida albicans* species in our study is much lower (1.7%) when compared to previously published data (10.4%) [13]. This may be explained by the comparatively larger geographic distribution of our sample population reducing the influence of clustering effects often observed within community samples. We also note that the previous study incubated samples at 35°C [13]. We have opted for incubation at 37°C reflecting physiological temperature. This may further contribute to the heightened distinction between *C. albicans* and non-*C. albicans* species.

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