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Prospective evaluation of the chromogenic medium CandiSelect 4 for differentiation and presumptive identification of non-*Candida albicans Candida* species

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ABSTRACT

Rapid identification of pathogenic yeasts is a crucial step in timely and appropriate antifungal therapy. For diagnostics in the clinical laboratory, simplified alternatives to barcoding are needed. CandiSelect 4 (CS4) medium, a chromogenic medium for isolation of clinical yeasts, allows routine recognition of *Candida albicans* and presumptive identification of *Candida tropicalis*, *Candida glabrata*, and *Candida krusei*. We evaluated an extension of this method with 46 non-*Candida albicans Candida* (NCAC) and 7 *Malassezia* species. The medium supported growth of all species tested and a wide diversity of cultural types were observed. Colony colours were in violet, turquoise (including green and blue), or white tinges. Eight NCAC species produced violet pigmentation similar to that of *C. albicans*. Most NCAC species, including *C. glabrata* and *C. tropicalis* were distributed in the turquoise group. *Malassezia* species were invariably blue.

Introduction

During the last 30 y we witness a significant increase in the incidence of fungal infections in humans, with a considerable expansion of the number and diversity of yeast species recognized to cause disease (Lass-Flörl 2009, de Hoog *et al.* 2015). Disseminated candidiasis remains one of the main clinical entities, particularly being observed in severely compromised patients. As immediate and appropriate therapy is compulsory, rapid and accurate diagnosis of the infection is essential. Of the yeasts that occur as human pathogens, *Candida albicans* is still the most prevalent species, although other species are emerging, and are increasingly recognized largely due to improvement of taxonomic and diagnostic methods. Molecular species borderlines and barcodes of the most important pathogenic yeasts are well established (Kurtzman & Robnett 2010), and a wide diversity of diagnostic tools allowing identification within hours has been developed for some

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of them. Among the most simple diagnostic tests is the use of chromogenic media, which (1) enhance recognition of mixed infections upon first isolation, and (2) allow presumptive distinction of *C. albicans* and a few other prevalent species in a single step, according to characteristic pigmentation of colonies (Freydiere *et al.* 2001; Letscher-Bru *et al.* 2002).

CandiSelect-4™ agar medium (CS4; Bio-Rad, Marnes La Coquette, France) identifies *C. albicans* on the basis of detection of hexosaminidase enzymatic activity changing the chromophore contained in the medium to pink or purple. Additionally, phosphatase enzymatic activity can be detected by using a second substrate present in the medium. This enables the presumptive identification of *Candida tropicalis*, *Candida glabrata*, and *Candida krusei*, producing cendre blue colonies associated with a typical morphological appearance for each of these species. These enzymatic reactions are taken to be species-specific, allowing identification of these important yeasts down to species level by their colour and colony characteristics (Boualem *et al.* 2007). It should be noted, however, that this statement was made on the basis of comparison with a small selection of clinical species only.

Although *C. albicans* represents on average over 80 % of isolates from all forms of human candidiasis (Calderone 2002), the proportion of non-*C. albicans Candida* (NCAC) species involved in systemic candidiasis has raised during the last decades from 10–40 % to 35%–65 % (Kauffman *et al.* 2000; Krcmery & Barnes 2002; Manzano-Gayosso *et al.* 2008; Ruan & Hsueh 2009). One of the explanations of this apparent emergence of NCAC in human candidiasis may be related to improvements in diagnostic techniques. The introduction of molecular techniques in routine diagnostics reveals species that otherwise may have remained unrecognized (Liguori *et al.* 2009). The use of chromogenic media enables separation of different yeast species derived from a single clinical sample and which are known to differ in colour, such as *Candida guilliermondii*, *Candida lusitaniae*, *Candida kefyr*, *Candida famata*, *Candida inconspicua*, *Candida rugosa*, *Candida dubliniensis*, and *Candida norvegensis* (Boualem *et al.* 2007; Papon *et al.* 2013). Only relatively few studies have examined the chromogenic characteristic of additional, clinically relevant NCAC species. The purpose of the present paper is to provide a reference for the presumptive clinical separation of additional NCAC species. We selected type and reference strains of NCAC species listed in the latest edition of the *Atlas of Clinical Fungi* (de Hoog *et al.* 2015) and recorded their chromogenic characteristics on CS4. A set of *Malassezia* species, which occasionally cause infections similar to candidiasis (Di Chiacchio *et al.* 2014; Sheila *et al.* 2014) was added to the panel.

Material and methods

Strains and medium

Type and reference strains of 47 *Candida* species including *Candida africana*, *Candida albicans*, *Candida auris*, *Candida catenulata*, *Candida* (=*Blastobotrys*) *chiropterorum*, *Candida dubliniensis*, *Candida* (=*Cyberlindnera*) *fabianii*, *Candida famata* (=*Debaryomyces hansenii*), *Candida glabrata*, *Candida* (=*Meyerozyma*) *guilliermondii*, *Candida haemuloni*, *Candida haemulonii*, *Candida intermedia*, *Candida krusei* (=*Pichia kudriavzevii*), *Candida* (=*Clavispora*) *lusitaniae*, *Candida magnoliae*, *Candida metapsilosis*, *Candida nivariensis*, *Candida* (=*Pichia*) *norvegensis*, *Candida orthopsilosis*, *Candida palmioleophila*, *Candida parapsilosis*, *Candida pseudohaemulonii*, *Candida rugosa*, *Candida tropicalis*, *Candida viswanathii*, *Candida boidinii*, *Candida colliculosa*, *Candida freyschussii*, *Candida hellenica* (=*Zygoascus meyerae*), *Candida inconspicua*, *Candida lambica* (=*Pichia fermentans*), *Candida maris*, *Candida melibiosica*, *Candida pelliculosa* (=*Wickerhamomyces anomalus*), *Candida pseudoaseri*, *Candida sake*, *Candida sphaerica*, *Candida steatolytica* (=*Zygoascus hellenicus*), *Candida stellata*, *Candida subhasii*, *Candida valida*, and *Candida zeylanoides*, *Cyberlindenera jadinii*, *Candida pseudorugosa*, *Kluyveromyces marxianus*, *Trichomonascus ciferii* and 7 *Malassezia* species including *Malassezia furfur*, *Malassezia globosa*, *Malassezia obtusa*, *Malassezia pachydermatis*, *Malassezia restricta*, *Malassezia japonica*, and *Malassezia yamatoensis* were provided by the reference collection of the CBS-KNAW Fungal Biodiversity Research Centre. Many of these species have recently been renamed according to their phylogenetic position and integration of sexual and asexual states; for cross-reference of names and synonyms is referred to de Hoog *et al.* (2015). Colours were recorded using Ridgways colour charts (Ridgway 1912).

Culture conditions

Strains were inoculated onto CS4 agar medium (CS4; Bio-Rad, Marnes La Coquette, France) and plates were incubated at 37 °C for 24–48 h. Tests were also done at 25 °C and monitored for up to 5 d. *Malassezia* isolates were inoculated after coating the CS4 plates with sterile olive oil, and were incubated at 37 °C for 48–72 h. Reading and recording of colony colour and texture was done every 24 h.

Results

The used chromogenic media supported the growth of all *Candida* and *Malassezia* species tested. Reading was done every 24 h, but most pronounced colony morphology and most intensive colour, both at obverse (pigmentation of colony) and reverse (pigments exuded into the agar), were obtained after incubation at 37 °C for 48 h and at 25 °C for 5 d. Exuded pigments eventually formed a halo around the colony, which was recorded as a separate feature. Longer incubation did not significantly alter colony characteristics and therefore results of 48 h incubation at 37 °C were taken as standard and recorded in Table 1. Examples of obtained colours (in the web version) are shown in Fig 1. Tests done at 25 °C showed a larger degree of variability than at 37 °C. Occasionally a significant difference was observed between colours obtained at the different temperatures (Fig 2).

A wide variety of colours and colony textures were obtained (Table 1, Fig 1). *Candida albicans* was consistently blue violet, with a violet pigment diffusing around the colony. Besides *C. albicans*, there are eight species that were violet at obverse or reverse, including *Candida dubliniensis*, *Candida viswanathii*, *Meyerozyma guilliermondii*, *Blastobotrys chiropterorum*, *Zygoascus hellenicus*, *Candida pseudoaaseri*, *Trichomonascus*