Evaluation of CHROM-Pal medium for the isolation and direct identification of *Candida dubliniensis* in primary cultures from the oral cavity

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*Candida albicans* is the species most frequently isolated from oral specimens, but the recovery of other *Candida* species such as *Candida dubliniensis* is increasing. Differentiation of *C. dubliniensis* from *C. albicans* requires special tests and both species are misidentified in some studies. CHROM-Pal (CH-P) is a novel chromogenic medium used in our laboratory for differentiation between *C. albicans* and *C. dubliniensis* on the basis of colony colour and morphology, and chlamydospore production. The performance of CH-P and CHROMagar Candida (CAC) was compared for primary isolation and presumptive identification of yeasts from oral specimens from human immunodeficiency virus (HIV)-infected and uninfected individuals. The identification of *Candida* species on both media was compared with two reference identification methods (API ID 32 C and multiplex PCR). A total of 137/205 oral swabs (66.8 %) plated onto CH-P and CAC media were positive by culture and resulted in the growth of 171 isolates of *Candida* species on CH-P, whilst only 159 isolates grew on CAC. *C. albicans* was the most frequently isolated species in both groups of patients, followed by *Candida parapsilosis* in the HIV-negative group, and by *C. dubliniensis* in the HIV-infected group. The other *Candida* species isolated were *Candida guilliermondii*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Candida famata*, *Candida rugosa*, *Candida kefyr*, *Candida pelliculosa* and *Candida pulcherrima*. The sensitivity and specificity for identifying *C. albicans*, *C. krusei*, *C. tropicalis* and *C. dubliniensis* on CH-P were over 98.5 %, always equal to or higher than those obtained when CAC was used. CH-P is a simple reliable medium for primary isolation and presumptive identification of yeast isolates from oral samples. The ability of CH-P to discriminate between *C. dubliniensis* and *C. albicans* was significantly higher (*P* < 0.05) than that of CAC.

INTRODUCTION

Members of the genus *Candida* are responsible for a variety of infections in humans ranging from superficial to deep-seated candidiasis. The most prevalent presentation is oral candidiasis, which includes erythematous candidiasis, pseudomembranous candidiasis, angular cheilitis, denture stomatitis, chronic mucocutaneous candidiasis and leukoplakia (Delgado & Aguirre, 1997; Samaranayake & MacFarlane, 1990; Sánchez-Vargas et al., 2005). Most patients with oral candidiasis have some degree of immunosuppression caused by human immunodeficiency virus (HIV) infection, chemotherapy for the treatment of tumours or immunosuppressors used for organ transplantation. Other factors that enhance oral candidiasis are xerostomy and a complex array of virulence factors in the fungus including adhesins that mediate the adhesion of *Candida* to the epithelial cells
and dentures, filamentation, enzyme production, phyto-
typic switching and thigmotropism (Aguirre, 2002; Quindós & Pontón, 1996).

Candida albicans is the most pathogenic species of the
genus and the most frequent cause of oral candidiasis
(Coleman et al., 1997). Recent studies have demonstrated
that the incidence of infections caused by other species of
the genus such as Candida glabrata, Candida parapsilosis,
Candida krusei, Candida tropicalis and Candida dubliniensis
is increasing (Coleman et al., 1997; Hazen, 1995; Kirkpatrick et al., 1998; Pfaller et al., 1996).

The first step in laboratory diagnosis of oral candidiasis is
usually the culture of the oral specimen. In recent years, a
number of chromogenic media including CHROMagar
Candida (CAC; CHROMagar), chromID Candida
(bioMérieux), Albicans ID2 (bioMérieux), Candida ID2
(bioMérieux) and CandiSelect 4 (Bio-Rad) have been
marketed. These media allow the isolation and presumpt-
ive identification of most clinically relevant yeasts based on
the colour of the colonies (Beighton et al., 1995; Eraso et al.,
2006; Horvath et al., 2003; Hospenthal et al., 2006; Ilkit et al.,
2007; Kirkpatrick et al., 1998; Perry & Freydière,
2007; Pfaller et al., 1996; San Millan et al., 1996; Sendid et al.,
2007). CHROM-Pal medium (CH-P) is a modifica-
tion of CHROMagar medium that is supplemented with
På’s agar. This medium has been developed in our
laboratory to differentiate C. albicans from C. dubliniensis
based on colony morphology and colour, as well as the
production of chlamydospores (Sahand et al., 2005). In this
study, we investigated, for the first time, the usefulness of
CH-P for the primary isolation and presumptive iden-
tification of yeasts from oral specimens.

METHODS

Patients. Oral swabs were processed from 151 HIV-negative patients
attending the outpatient clinic of the Department of Dentistry at the
University of Basque Country or a private dental clinic, as well as oral
swabs from 54 HIV-positive patients attending the Department of
Infectious Diseases in Cruces Hospital, Baracaldo, Spain.

Male and female patients of different age groups, ranging between 10
and 81 years (mean 47.8 years), were included in the study. Thirty-six
patients wore removable dentures. The experimental protocols were
approved by the Institutional Review Board of the School of Medicine
and Odontology at the University of the Basque Country, Leioa,
Spain, and subjects gave their informed consent.

Clinical specimens and identification of isolates. Samples were
taken from oral lesions and from buccal mucosa, tongue and dentures
in the absence of oral lesions by means of sterile swabs. Swabs were
replaced into sterile transportation medium and taken to the
mycology laboratory for identification. Each swab was first plated
on CAC and then on CH-P. The plates were incubated at 30 °C (CH-
P) or 37 °C (CAC).

CAC was prepared according to the manufacturer’s instructions. CH-
P was prepared as described by Sahand et al. (2005) by mixing equal
volumes of prepared CAC and Pal’s agar (50 g unsalted powdered
sunflower seeds were added to 1 litre of distilled water, boiled for
30 min and filtered through cheesecloth; the medium was supple-
mented with 1 g creatine, 1 g glucose and 1 g KH₂PO₄; the pH was
adjusted to 5.5 and, after the addition of 15 g agar l⁻¹, the medium
was autoclaved at 110 °C for 20 min). After stirring, the mixture
was poured into 90 mm diameter Petri dishes. Plates were inoculated
and observed daily for up to 7 days for growth, colony morphology and
pigmentation. For CH-P, plates were additionally subjected to
microscopical examination to detect chlamydospores to differentiate
C. dubliniensis from C. albicans.

Definitive identification of the yeast strains isolated was carried out by
means of their carbohydrate assimilation patterns on API ID 32 C
strips (bioMérieux). Multiplex PCR was used for further confirma-
tion of the identification of C. albicans and C. dubliniensis
simultaneously using two sets of species-specific primer pairs. One
pair was specific for C. albicans, CALB1F and CALB2R (Genotek-
Bonsai; CALB1F: 5′-TTATCACTTGTACACGCAGA-3′; CALB2R:
5′-ATCCGATTCCACATCCGG-3′), and was designed from the
internal transcribed spacer regions ITS1 and ITS2 of the rRNA gene,
amplifying a band of 273 bp (Luo & Mitchell, 2002). The other
pair was specific for C. dubliniensis, CDBF28F and CDBR110R
(Genotek-Bonsai; CDBF28F: 5′-AAATGGTTGTTGGCAAAATTA-
3′; CDBR110R: 5′-GTTGCGATGTTGGCAATAGCTTA-3′), and
amplified a region of 809 bp of the topoisomerase II gene (Kanbe et al.,
2005).

Statistical analysis. In order to establish the diagnostic value of
CAC and CH-P for identification of Candida species, the sensitivity
and specificity were calculated. The significance of differences
between both media in discriminating between C. albicans and C.
dubliniensis was assessed by the χ² method of McNemar. A value of
P <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

A total of 62 (41.1 %) out of 151 samples from non-HIV-
infected patients showed no growth on the two chromo-
genic media, whilst the remaining 89 positive specimens
(58.9 %) resulted in growth of 108 isolates of Candida
species. One hundred and six isolates grew to the same
extent on the two media, but two of the isolates were
shown in Table 1, C. albicans was the most frequently
isolated species (60.2 %), followed by C. parapsilosis
(10.2 %), Candida guilliermondii (9.3 %), C. glabrata
(6.5 %), C. krusei (3.7 %), C. dubliniensis and C. tropicalis
(both 2.8 %) and one isolate each for Candida famata,
Candida kefyr, Candida pelliculosa, Candida pulcherrima
and Candida rugosa (0.9 %). One isolate of C. parapsilosis
and another one of C. guilliermondii failed to grow on
CAC, although they grew well on CH-P (Table 2).

Six of the 54 (11.1 %) oral specimens taken from HIV-
infected patients showed no growth on either chromogenic
medium. The 48 positive specimens (88.9 %) resulted in
the growth of 63 isolates of Candida species. Twelve oral
swabs (25 %) gave rise to mixed cultures of two different
species, and in one specimen four different species were
isolated. Fifty-three isolates showed growth to the same extent on the two media, whilst ten isolates were detected only on CH-P medium (four C. parapsilosis, three C. albicans, one C. dubliniensis, one C. tropicalis and one C. famata; Table 2). As shown in Table 1, C. albicans was the most frequently isolated species (52.4%) in oral samples of HIV-infected patients, followed by C. glabrata (15.9%), C. parapsilosis (11.1%), C. dubliniensis and C. tropicalis (both 6.35%), C. famata and C. guilliermondii (both 3.2%) and C. krusei (1.6%).

The prevalence of C. dubliniensis in our study was 1.99% for the HIV-negative individuals, but rose to 18.5% in HIV-infected individuals. These data are in agreement with previously published studies (Faggi et al., 2005; Kirkpatrick et al., 1998; Milan et al., 2001; Tintelnot et al., 2000).

Finding an easy, low-cost and efficient method for identification of yeast isolates is very important in any mycological laboratory. Although identification by means of molecular methods such as PCR is more reliable, they are not affordable in many laboratories. In the present work, final identification of Candida isolates was accomplished by ID 32 C and multiplex PCR as a reference to compare the performance of the two chromogenic media under study.

CAC is one of the most widely used media in the mycology laboratory. Colony characteristics presented in Table 2 for identification of C. albicans, C. tropicalis and C. krusei using CAC were in agreement with previously published reports (Beighton et al., 1995; Hospenthal et al., 2006; Pfaller et al., 1996). The sensitivity and specificity of CAC for identifying C. krusei and C. tropicalis were over 99%. There is some debate about the ability of CAC to identify C. glabrata, as some studies claim that it can be identified by the pink/purple/violet colour of the colonies (Horvath et al., 2003; Pfaller et al., 1996). However, other Candida species including C. parapsilosis, C. guilliermondii, C. famata, C. kefyr and C. pelliculosa also produce pink/purple/violet colonies on CAC (Beighton et al., 1995; Eraso et al., 2006; San Millan et al., 1996). The data presented in Table 2 confirm the difficulty of identifying C. glabrata on CAC. In addition, nearly half of the C. albicans isolates grew as light-green colonies on CAC, whilst the remaining isolates grew as green and dark-green colonies. The latter is supposed to be the colour of C. dubliniensis colonies on CAC (Fig. 1, Table 2). Our findings are in accordance with previous reports that observed that C. dubliniensis shows different tones of green colour that are difficult to differentiate from the green colour produced by C. albicans (Eraso et al., 2006; Tintelnot et al., 2000). The diagnostic values of CAC for differentiating C. dubliniensis from C. albicans in oral swabs of HIV-infected and HIV-negative individuals were low (Table 3).

The discrimination between C. albicans and C. dubliniensis on CH-P was easier as the majority of C. albicans (94.9%) grew as light-green, smooth colonies (Fig. 1 and Table 2). Only five isolates of C. albicans (5.1%) produced bluish-green colonies, but the morphology of the colonies was smooth and they did not produce chlamydospores, making the presumptive identification very simple. In contrast, 100% of C. dubliniensis isolates produced rough, bluish-green colonies on CH-P and produced chlamydospores, as described previously by Sahand et al. (2005). The presumptive identification of C. krusei and C. tropicalis on CH-P was similar to that on CAC, as C. krusei grew as pink fuzzy colonies and C. tropicalis grew as blue colonies, so the diagnostic value of CH-P for identifying C. krusei and C. tropicalis was very close to that of CAC (over 98%).

The majority of C. glabrata isolates (10/11) grew on CH-P as smooth, white colonies and this appearance was not shared by any other Candida species in the present work. Although this is an interesting finding, further studies with more C. glabrata strains and other Candida species would be needed to validate this observation. For the rest of the Candida species, no specific characteristics were detected on CH-P.

The identification of Candida species growing on CH-P was based on the colour and morphology of the colonies and, in the case of discrimination between C. albicans and C. dubliniensis, on chlamydospore production of the isolates. The presence of at least two of these characteristics on CH-P guided the presumptive identification of the clinically most relevant Candida species to a similar extent as on CAC, but CH-P improved the ability to discriminate between C. albicans and C. dubliniensis (differences between CAC and CH-P were statistically significant, P <0.05).

In addition to these findings, CH-P was superior to CAC for the primary isolation of Candida species. Twelve isolates (C. albicans, C. dubliniensis, C. famata, C. guilliermondii, C. parapsilosis and C. tropicalis) that grew on CH-P failed to grow on CAC, although swabs were streaked first on CAC and then on CH-P. The two media

**Table 1. Distribution of Candida species isolated from oral swabs of HIV-infected and uninfected individuals**

<table>
<thead>
<tr>
<th>Yeast species</th>
<th>Uninfected patients</th>
<th>HIV-infected patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates</td>
<td>Percentage of isolates</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>65</td>
<td>60.19</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>11</td>
<td>10.19</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>10</td>
<td>9.26</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>7</td>
<td>6.48</td>
</tr>
<tr>
<td>C. krusei</td>
<td>4</td>
<td>3.70</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>3</td>
<td>2.78</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>3</td>
<td>2.78</td>
</tr>
<tr>
<td>C. famata</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td>C. pelliculosa</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td>C. pulcherrima</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td>C. rugosa</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>No. of isolates</td>
<td>Percentage of isolates</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>33</td>
<td>52.38</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>7</td>
<td>11.11</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>2</td>
<td>3.17</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>4</td>
<td>6.35</td>
</tr>
<tr>
<td>C. krusei</td>
<td>1</td>
<td>1.59</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>10</td>
<td>15.87</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>4</td>
<td>6.35</td>
</tr>
<tr>
<td>C. famata</td>
<td>2</td>
<td>3.17</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C. pelliculosa</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C. pulcherrima</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C. rugosa</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2. Comparison of the performance of the two chromogenic media with *Candida* species isolated from oral swabs of HIV-infected and uninfected individuals

<table>
<thead>
<tr>
<th>Yeast species</th>
<th>Total no. isolates</th>
<th>CAC</th>
<th>CH-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates</td>
<td>Colony colour</td>
<td>No. of isolates</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>98</td>
<td>46 Light-green 93</td>
<td>Smooth, light-green colonies and no chlamydospores</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37 Green 12</td>
<td>Smooth, bluish-green colonies and no chlamydospores</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3* Dark-green</td>
<td></td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>18</td>
<td>5 Purple 4 Violet 3 Beige 1 Pink</td>
<td>Rough, purple colonies 3 Smooth, beige colonies 1 Rough, light-violet colonies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3*</td>
<td></td>
</tr>
<tr>
<td><em>C. dubliniensis</em></td>
<td>13</td>
<td>7 Dark-green 3 Light-green 2 Green 3 Beige 1 Pink</td>
<td>Rough, bluish-green colonies and chlamydospore production 3 Smooth, beige colonies 1 Rough, light-violet colonies</td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>12</td>
<td>5 Purple 4 Violet 2 Pink 3*</td>
<td>Smooth, pink colonies 3 Smooth, light-violet colonies 1 Beige</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>11</td>
<td>9 Violet 1 Purple 1 Beige</td>
<td>Smooth, white colonies 1 Smooth, light-violet colonies 1 Beige</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>5</td>
<td>5 Pink† 6 Blue 1*</td>
<td>Fuzzy, pink colonies 3 Rough, blue colonies 4 Smooth, blue colonies</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>7</td>
<td>5 Pink 6 Blue 1*</td>
<td>5 Fuzzy, pink colonies 3 Rough, blue colonies 4 Smooth, blue colonies</td>
</tr>
<tr>
<td><em>C. famata</em></td>
<td>3</td>
<td>1 Violet 1 Beige 1*</td>
<td>Rough, beige colonies 1 Light-violet, rough colonies 1 Beige</td>
</tr>
<tr>
<td><em>C. rugosa</em></td>
<td>1</td>
<td>1 Pink/violet 1</td>
<td>Smooth, light-violet colonies 1 Beige</td>
</tr>
<tr>
<td><em>C. kefyr</em></td>
<td>1</td>
<td>1 Pink 1</td>
<td>Smooth, pink colonies 1 Beige</td>
</tr>
<tr>
<td><em>C. pelliculosa</em></td>
<td>1</td>
<td>1 Violet 1</td>
<td>Rough, pink colonies 1 Beige</td>
</tr>
<tr>
<td><em>C. pulcherrima</em></td>
<td>1</td>
<td>1 Purple 1</td>
<td>Smooth, beige colonies 1 Beige</td>
</tr>
<tr>
<td>Total</td>
<td>171</td>
<td>171</td>
<td>171</td>
</tr>
</tbody>
</table>

*No growth.
†Pink and fuzzy.

Fig. 1. Colony colour and morphology of yeasts isolated on CH-P. Cal, *C. albicans*; Cdu, *C. dubliniensis*; Cgl, *C. glabrata*; Cgu, *C. guilliermondii*; Ckr, *C. krusei*; Cpa, *C. parapsilosis*; Ctr, *C. tropicalis*.
Table 3. Diagnostic values of growth on CAC and CH-P for discrimination between *C. dubliniensis* and *C. albicans*, isolated from oral swabs of HIV-infected and HIV-negative individuals

<table>
<thead>
<tr>
<th>Statistic</th>
<th>CAC Value</th>
<th>95 % CI</th>
<th>CH-P Value</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.58</td>
<td>0.30–0.86</td>
<td>1</td>
<td>1–1</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.87</td>
<td>0.81–0.94</td>
<td>1</td>
<td>1–1</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.37</td>
<td>0.15–0.59</td>
<td>1</td>
<td>1–1</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.94</td>
<td>0.89–0.99</td>
<td>1</td>
<td>1–1</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.841</td>
<td></td>
<td>1.037</td>
<td></td>
</tr>
</tbody>
</table>

are basically the same – CHROMagar Candida – but CH-P is supplemented with a sunflower seed extract, glucose and creatinine, which may help some strains to grow. Incubation temperature could be another influencing factor, but species that failed to grow usually develop adequately at 37 °C.

In conclusion, we recommend the use of CH-P as a primary isolating medium in mycology laboratories because it is easy to prepare, is less expensive than CAC, yields more isolates, allows simple interpretation of colony characteristics and is highly efficient for the presumptive discrimination between *C. albicans* and *C. dubliniensis* (100% accuracy) and the identification of some other medically important *Candida* species. CH-P facilitates the study of oral yeast microbiota, providing information that may be useful for the treatment of oral candidiasis.

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