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Evaluation of CHROMagar Candida for Presumptive Identification of Clinically Important *Candida* Species

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CHROMagar Candida is a recently described and differential medium for the isolation and the presumptive identification of clinically important yeasts. We evaluated it with 262 yeast strains from clinical specimens, including 173 Candida albicans, *21* Candida tropicalis, *8* Candida krusei, *49* Candida glabrata, *and 12 strains of other yeast species. Strains were presumptively identified on the basis of colony color and texture. These observations were compared with conventional identification results.* Candida albicans *was identified correctly in 170 (98%) of the 173 strains. A total of 46 of the 205 specimens that were plated on CHROMagar contained mixed cultures of yeast. Thirty-seven (80%) of these mixed cultures were not detected in the original specimens. CHROMagar Candida was useful for the rapid presumptive identification of* Candida albicans *and facilitated the recognition of mixed cultures. For other yeast species, it may provide additional information to laboratories that do not regularly perform identifications beyond the germ tube test.* © *1998 Elsevier Science Inc.*

INTRODUCTION

Since the 1980s, there has been a significant increase in the number of *Candida* infections, especially in hospitalized patients (Baumgartner et al. 1996; Bernal et al. 1996). Predisposing factors include immunosuppression, prolonged administration of antimicrobial agents, surgery, burns, and indwelling catheters (Benedict and Colagreco 1994). The most frequently isolated species is *Candida albicans*, but *Candida tropicalis, Candida glabrata, Candida krusei,* and *Candida parapsilosis* are also emerging as important etiologic agents of *Candida* infection (San-Millan et al., 1996).

A major cause for concern is the frequency with which localized *Candida* infections, such as mucosal candidiasis and onychomycosis may become systemic and life-threatening in an immunocompromised host (Dupont 1995). In AIDS patients with chronic oropharyngeal candidiasis, certain *Candida* species have decreased susceptibility to fluconazole (Patterson et al. 1996). Candidal urinary infection in diabetes, or peritonitis in patients on dialysis may not respond to therapy with existing antifungal regimens. It is therefore important to develop rapid identification methods so that early diagnosis and effective empiric antifungal therapy can be started (Baumgartner et al. 1996).

CHROMagar Candida (CA) is a recently described selective and differential medium for the isolation and identification of *Candida* species. It has been suggested that contrasting colony color and texture produced by hydrolysis of chromogenic substrates in this medium allows for presumptive identification of isolated yeast species (Baumgartner et al., 1996; Odds and Bernearts 1994; San-Millan et al. 1996). The purpose of this study was to evaluate CA for presumptive identification of yeast on the basis of strongly contrasting colony color and morphology, and to evaluate its capability to serve as a routine isolation media for *Candida* species.

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MATERIALS AND METHODS

CHROMagar Candida (CA) and Phytone Yeast Extract Agar (PYE).

CA was purchased as a dehydrated medium from CHROMagar, Paris, France. Manufacturer's instructions were followed for the preparation and storage of CA and PYE (BBL, Baltimore, MD). Once inoculated, plates were incubated at 30°C for 48 to 72 h.

Confirmatory Testing

The identity of each morphotype was confirmed using the following methods; germ tube production in bovine serum incubated at 35°C for two h, API 20C AUX (BioMerieux, France), and chlamydospore production on cornmeal tween 80 agar with caffeic acid (Balow et al. 1993, Isenberg 1993).

Quality Control

Performance plates were included for CA and PYE using known species of *Candida*. Germ tubes were evaluated using *C. albicans* and *C. tropicalis.* Quality control for API 20C AUX strips included *C. albicans* ATCC 14053, *C. guilliermondii* ATCC 6260 and *C. kefyr* ATCC 4135. *C. albicans* was used as a control for chlamydospore production on Cornmeal tween 80 agar with caffeic acid.

Study Design

To establish initial chromogenic identification criteria for each species, known reference strains of *Candida* species were plated on CA. The species used were *C. albicans, C. glabrata, C. guilliermondii, C. krusei, C. tropicalis, C. parapsilosis, C. lusitaniae,* and *C. kefyr. Saccharomyces cerevisiae* and *Geotrichum candidum* were also included. For clinical evaluation, 205 patient specimens that had been shown to contain scant to many yeast on primary isolation in the diagnostic laboratory within the previous 24 h, were plated onto CA and PYE. In each case the investigator returned to the original specimen on the same day as yeasts were first discovered. Clinical specimens included bronchoalveolar lavages and washes, deep wounds, sterile body fluids, auger suctions, midstream urines, tissues, and blood cultures. Isolates on CA were presumptively identified based on colony color and morphology. The identity of all isolates was then confirmed with the API 20C AUX yeast identification system, chlamydospore production on cornmeal caffeic acid agar, and germ tube production. The number of yeast isolates that grew on both plates (CA and PYE) were then compared. To avoid reader bias in favor of CA, all PYE plates were read by a different investigator.

RESULTS

Appearance of yeast strains on CHROMagar Candida

Overall, 262 *Candida* morphotypes were observed on CA from the 205 clinical samples examined. Colonies were usually quite small and color was undefined after overnight incubation. Therefore, color readings were made at 48 h, as suggested by the manufacturer. Further incubation resulted in deepening of colors. In 11 cases, greater than 72 h of incubation was required before colonies appeared.

The distribution of colors observed in the morphotypes are listed in Table 1. Among 173 *C. albicans* isolates, 123 displayed the characteristic green color at 48 h of incubation. Blue-green colored colonies accounted for 24 of the isolates. Twenty-three isolates had a pale blue central color with white edges after 48 h, which turned green by 72 h of incubation. This blue color was never confused with the solid dark blue color of *C. tropicalis.* There were three instances where white colonies were observed that were identified by germ tube and API 20C AUX as *C. albicans.*

TABLE 1 Distribution of Colony Colors of 262 Yeast Strains on CHROMagar

Species	Green	Blue-green	Blue	Purple	Pink	White	Other
Candida albicans	123	24	23	—	—	3	—
Candida glabrata	—	—	—	24	5	11	9
Candida krusei	—	—	—	—	6	—	2
Candida tropicalis	—	—	2	11	—	1	7
Candida parapsilosis	—	—	—	1	3	1	—
Saccharomyces cerevisiae	—	—	—	3	—	—	—
Candida lusitaniae	—	—	—	—	—	1	—
Candida spp.	—	—	—	—	—	—	1
Geotrichum	—	—	—	—	—	—	1

Of the 21 isolates with confirmed identification as *C. tropicalis* 19 (90%) failed to exhibit the characteristic blue to blue-gray colony color of this species. The majority of the isolates were purple to purple-gray and some were surrounded by a purple halo in the agar. The intensity of the purple color varied from a pale purple to a dark solid purple, the latter being easy to distinguish from *C. glabrata.* In one instance, an isolate with a purple halo surrounding light gray colonies was subsequently identified as *C. tropicalis.* Color variation severely affected the presumptive identification of *C. tropicalis* on CA, even when a wider range of colors and the possible presence of a halo around the colony was considered.

C. krusei was easy to separate from all other types of yeast colonies on CA, when it displayed the following appearance; dry, flat, and rough texture, and spreading colonies with a pale pink color and white edges. This characteristic appearance was not seen with any other species, and six of the eight *C. krusei* isolates had this morphotype. Two strains of *C. krusei* were confused with *C. glabrata;* these strains had smooth purple-pink colonies with white edges.

The colony colors observed for the 49 *C. glabrata* isolates were highly variable, ranging from white to pink to the characteristic purple center with white edges. All three purple *S. cerevisiae* isolates were incorrectly presumptively identified as *C. glabrata. C. parapsilosis* isolates had white or pink tones; however, *C. lusitaniae* and other *Candida* species also had either white or pinkish-white colored isolates.

The sensitivity, specificity, positive and negative predictive values for the four most frequently isolated *Candida* species are shown in Table 2.

Recognition of Mixed Colonies, Selectivity, and Yeast Viability

Of the 205 specimens that were plated on CA a total of 262 yeast morphotypes were isolated. CA was able to support yeast growth similar to that of PYE. Mixed cultures were detected in 46 (22%) of the 205 specimens, and in all cases a clear and distinct separation of colony colors was observed that permitted easy recognition of differences.

Of the 205 CHROMagar plates, 7 grew non-yeasts; *Pseudomonas aeruginosa* grew on two plates, and five plates grew *Aspergillus* species. These were easily distinguished from the yeasts.

For identification by API 20C AUX, yeast species other than *C. albicans* were first subcultured onto Sabouraud Dextrose Agar (SDA). All colonies subcultured from CA grew on SDA, which demonstrated that the presence of the chromogenic substrate did not affect viability.

TABLE 2 Statistical Evaluation of CA for Presumptive Primary Identification of Four Commonly Isolated Yeast Species[a]

Yeast Species	Sensitivity	Specificity	PPV[b]	NPV[c]
Candida albicans	85	100	100	79
Candida tropicalis	14 (52)[c]	100	100	93 (96)[c]
Candida krusei	75	100	100	99
Candida glabrata	49	97	80	90

[a] Comparison was made to the confirmed identity of each strain by classical methods.
[b] PPV; NPV; positive and negative predictive values of a correct presumptive response.
[c] Change in sensitivity and NPV if a wider range of color and presence of a halo around the colonies were considered.

DISCUSSION

This study was undertaken to determine the value of CA as a routine isolation media for the presumptive identification of clinically important yeasts. CA was able to support the selective growth of yeasts and, at the same time, to maintain viability for further testing. CA permitted presumptive identification of *C. albicans* and demonstrated the presence of mixed cultures. Different yeast species within a mixed culture, which could not be identified directly from CA growth characteristics, could be further identified by standard methods.

CA supported the growth of yeast and some molds (*Aspergillus* spp.) while suppressing the growth of bacteria; thus, providing a high selectivity for yeast isolation.

Recognition of mixed cultures was facilitated because of clear color discrimination between yeast species. When the same specimens were planted onto PYE, this recognition was not always observed. Forty-six mixed cultures were identified using CA. In the original specimens only 9 of 205 (4%) were identified as containing mixed species.

The distinctive color recognition of *C. albicans* on CA, whether as characteristic green or blue-green color, or as premature blue-centered colonies with white edges, allowed for easy presumptive identification. At least in our laboratory the specificity was excellent (100%), suggesting that additional tests such as the germ tube, may not be required. Others have also made this observation (Odds and Bernaerts, 1994).

Our results failed to support the "unequivocably unique" morphology exhibited by *C. tropicalis* noted in other studies (Beighton et al., 1995; Bernal et al., 1996; Freydiere and Gilles, 1996). However, we did observe that solid dark purple colonies with a purple halo could be presumptively identified as *C. tropica-*

lis. Also, the halo was never seen with any other *Candida* species.

We were unable to confirm the sensitivity and specificity for identification of *C. krusei* claimed by Odds and Bernaerts (1994) because of a limited number of isolates. *C. krusei* was easy to identify when isolates showed their typical morphological appearance; other clinically important yeasts were not mistaken as *C. krusei.*

Only one study (Pfaller et al., 1996) has suggested that *C. glabrata* can be identified as accurately as *C. albicans, C. tropicalis,* and *C. krusei* on CA. In our hands, *C. glabrata* strains showed significant variability and we cannot confirm these earlier observations. *S. cerevisiae* was mistaken three times for *C. glabrata* and in many other instances colony color gave no indication of its identity. More experience with *C. glabrata* may help to resolve this problem.

CA was easy to use, relatively easy to read and interpret, and was less subjective than reading germ tubes and API 20C AUX strips. However, further testing is necessary to confirm the identity of most yeast species other than *C. albicans.* For presumptive identification of *C. krusei,* CA performed reasonably well with 75% of the strains being correctly identified on primary isolation. Early recognition of *C. krusei* is important because it is intrinsically resistant to fluconazole. Further, CA certainly proved useful in recognition of mixed cultures and may provide additional information to laboratories that do not regularly perform identifications beyond the germ tube test.

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