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Performance of CHROMAGAR candida and BIGGY agar for identification of yeast species

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Abstract

Background: The importance of identifying the pathogenic fungi rapidly has encouraged the development of differential media for the presumptive identification of yeasts. In this study two differential media, CHROMagar Candida and bismuth sulphite glucose glycine yeast agar, were evaluated for the presumptive identification of yeast species.

Methods: A total number of 270 yeast strains including 169 *Candida albicans*, 33 *C. tropicalis*, 24 *C. glabrata*, 18 *C. parapsilosis*, 12 *C. krusei*, 5 *Trichosporon spp*., 4 *C. kefyr*, 2 *C. lusitaniae*, 1 *Saccharomyces cerevisiae* and 1 *Geotrichum candidum* were included. The strains were first identified by germ tube test, morphological characteristics on cornmeal tween 80 agar and Vitek 32 and API 20 C AUX systems. In parallel, they were also streaked onto CHROMagar Candida and bismuth sulphite glucose glycine yeast agar plates. The results were read according to the color, morphology of the colonies and the existance of halo around them after 48 hours of incubation at 37°C.

Results: The sensitivity and specificity values for *C. albicans* strains were found to be 99.4, 100% for CHROMagar Candida and 87.0, 75.2% for BiGGY agar, respectively. The sensitivity of CHROMagar Candida to identify *C. tropicalis*, *C. glabrata* and *C. krusei* ranged between 90.9 and 100% while the specificity was 100%. The sensitivity rates for BiGGY agar were 66.6 and 100% while the specificity values were found to be 95.4 and 100% for *C. tropicalis* and *C. krusei*, respectively.

Conclusions: It can be concluded that the use of CHROMagar Candida is an easy and reliable method for the presumptive identification of most commonly isolated *Candida* species especially *C. albicans*, *C. tropicalis* and *C. krusei*. The lower sensitivity and specificity of BiGGY agar to identify commonly isolated *Candida* species potentially limits the clinical usefulness of this agar.

Background

The incidence of fungal infections is increasing because of a rising number of immunocompromised patients, widespread use of broad-spectrum antibiotics and invasive devices or procedures [1–3]. Although *Candida albicans* remains the most frequently isolated yeast pathogen, other *Candida* species such as *C. glabrata*, *C. krusei*, and *C. tropicalis* are emerging as opportunistic pathogens and these species are reported to be less susceptible than *C. albicans* to antifungal agents [4–6]. *C. parapsilosis* has become the second most frequently recovered Candida species from blood cultures in Europe, Canada, and Latin America and ranked third in the United States [7,8]. For

these reasons, yeast infections require rapid diagnosis and early adapted antifungal therapy.

Ideally, laboratories should be able to detect and identify the major *Candida* species in clinical specimens [9]. Most laboratories start the yeast identification process with the germ tube test and continue with more extensive testing. Reference identification procedures that use biochemical and morphological studies are not often practical for the clinical laboratory because they are labor intensive and take several days. The conventional methods of yeast identification which mainly consist of assimilation/fermentation characteristics are reported to be cumbersome and often beyond the expertise range in non-specialized clinical microbiology laboratories [10]. Packaged kit systems and automated systems are widely used, but they are expensive and are limited by the size of their databases [11].

The importance of identifying the pathogen as quickly as possible has encouraged the development of differential media for the presumptive identification of yeasts. Several chromogenic media for isolation and identification of *Candida* species are available [12]. These media are based on the formation of various colored colonies with different morphology which result from the cleavage of chromogenic substrates by species specific enzymes [13]. In this study, the performance of two differential media, CHROMagar Candida (CA) and bismuth sulphite glucose glycine yeast (BiGGY) agar were evaluated for the identification of yeast species in comparison with the standard methods.

Methods

Isolates

A total number of 270 yeast strains including 169 *Candida albicans*, 33 *C. tropicalis*, 24 *C. glabrata*, 18 *C. parapsilosis*, 12 *C. krusei*, 5 *Trichosporon spp.* 4 *C. kefyr*, 2 *C. lusitaniae*, 1 *C. guilliermondii*, 1 *Geotrichum candidum*, and 1 *Saccharomyces cerevisiae* isolated from various clinical specimens (98 urine, 82 tongue-oral swabs, 47 blood, 18 sputum and bronchoalveoler lavage, 12 wound and abscess aspirate, 5 catheter, 4 vaginal secretion, 3 peritoneal aspirate and 1 cerebrospinal fluid) were used in this study. Quality control strains were *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 90018, and *C. krusei* ATCC 6258. The strains were first identified by germ tube test, morphological characteristics on cornmeal tween 80 agar, then by Vitek 32 yeast identification (bioMérieux, France) and API 20 C AUX (bioMérieux, France) systems.

Culture Media

CA (CHROMagar Company, France) and BiGGY agar (Nickersen agar) (Oxoid Company, England) were purchased as powdered media. The plates were prepared according to the manufacturers' instructions. Briefly, 47.7 grams of dehydrated CA and 42 grams of dehydrated BiGGY agar were reconstituted in 1 litre of distilled water. They were brought to boil by repeated heatings and then cooled in a water bath at 45°C by swirling and stirring. Then 20 ml's of the media were dispensed into petri dishes. They were kept in the dark at 4°C and used within three days.

CHROMagar Candida contains peptone (10.2 g/L), chloramphenicol (0.5 g/L) and chromogenic mix (22.0 g/L), agar (15.0 g/L), pH: 6.1. The composition of BiGGY agar is yeast extract (1.0 g/L), glycine (10.0 g/L), glucose (10.0 g/L), agar (13.0 g/L), sodium sulphite (3.0 g/L), bismuth ammonium citrate (5.0 g/L), pH: 6.8 ± 0.2.

C. albicans, *C. tropicalis* and *C. krusei* were reported to be identifiable on CA by the manufacturer and Pfaller et al. [9] proposed that CA could also identify *C. glabrata* and described the characteristic appearance. *C. albicans*, *C. tropicalis*, *C. krusei* and *C. kefyr* were reported to be identifiable on BiGGY agar by the manufacturer.

Method

Isolates were subcultured twice on Sabouraud dextrose agar prior to inoculation of chromogenic media. A single yeast colony was streaked onto the plates to give isolated colonies. The plates were incubated at 37°C without CO_2 in the dark. The results were read by two different people according to the color, morphology of colonies and the existence of halo around them after 48 hours. They were identified according to the manufacturers' instructions and as described by Odds and Bernaerts [14].

Statistical Analysis

The sensitivity, specificity, positive and negative predictive values (PPV, NPV) of two media for the species, which has been reported to be identifiable, were calculated by using Epi Info version 6.0 [15].

Results

All the strains did grow and the properties of the colonies were clearly distinguished after 48 hours of incubation. The distribution of the colony colors on CA and BiGGY agar within each yeast species is listed in Table 1. The colonies of various *Candida* species on CA and BiGGY agar are shown in figure 1 and 2, respectively.

The sensitivity and specificity of CA for *C. albicans* strains were found to be 99.4 and 100%. These rates were detected as 87.0 and 75.2% for BiGGY agar, respectively. The sensitivity of CHROMagar Candida to identify *C. tropicalis*, *C. glabrata* and *C. krusei* ranged between 90.9 and 100% while the specificity was 100% (Table 2). The sensitivity and specificity of BIGGY agar obtained for *C.*

Table 1: Distribution of the colony colors on CA and BiGGY agar within each yeast species

Species	No	Number of strains										
		CHROMagar Candida						BIGGY agar				
		Light green	Dark green	Blue-purple	Pink	Dark pink	Off white-cream	Light brown	Dark brown	Yellowish-mustard	Cream	Gray
C. albicans	169	168	0	0	0	0	1	147	22	0	0	0
C. tropicalis	33	0	0	32[a]	0	1	0	13	20	0	0	0
C. glabrata	24	0	0	0	20[b]	0	4	14[c]	2[c]	0	0	8[c]
C. parapsilosis	18	0	0	0	2	1	15	8	0	0	5	5
C. krusei	12	0	0	0	12[d]	0	0	0	12[d,e]	0	0	0
Trichosporon spp.	5	0	5[a,d]	0	0	0	0	3	1	0	1	0
C. kefyr	4	0	0	0	3	0	1	1	3	0	0	0
C. lusitaniae	2	0	0	0	1	0	1	1	0	1	0	0
C. guilliermondii	1	0	0	0	0	0	1	1	0	0	0	0
G. candidum	1	0	0	0	1[d]	0	0	1	0	0	0	0
S. cerevisiae	1	0	0	0	0	1[a]	0	1	0	0	0	0

[a]: Halo which diffused into surrounding agar [b]: Pale edges [c]: Weak growth [d]: Rough, fuzzy colonies [e]: With surrounding yellow zone

tropicalis and *C. krusei* are shown in Table 3. These rates for *C. glabrata* are not calculated because no reference color and morphology is described for this species by the manufacturer.

Discussion

CA and BiGGY agar are two chromogenic media which allow the presumptive differentiation of yeasts. CA contains various substrates for the enzymes of yeast species. It has been demonstrated that β-N-acetylgalactosaminidase which was produced by *C. albicans* enables the chromogenic substrates to be incorporated into the medium and the isolates of these species were seen as green colored colonies [16]. BiGGY agar contains bismuth sulphite and the growth on this medium produces brown to black colonies because of the extracellular reduction of bismuth sulphite to bismuth sulphide.

CA is reported to give green colonies of *C. albicans* and blue colonies of *C. tropicalis*. In this study 168 of 169 *C. albicans* isolates grew as distinctive light green colonies on CA, only one isolate was seen as off white colonies. Although we didn't have any *C. dubliniensis* isolates, differentiation of this species from *C. albican*s seems to be a problem in CA. Tintelnot et al [17] and Willinger et al [18] reported that some of *C. dubliniensis* isolates yielded a dark green color. It has been suggested that dark bluish-green coloration might be taken as an indication of the presence of *C. dubliniensis* but could not be used as a criteria for identification. Jabra-Rizk et al [18] compared the results of original CA and newly reformulated CA for these two species. They concluded that the difference in colony color between *C. albicans* and *C. dubliniensis* was slightly more enchanced on the new CA. They, in accordance with Pfaller et al. [9] suggested that the dark green color was detected to be more pronounced if the plates were incubated longer than 48 h.

We obtained the sensitivity and specificity of CA for *C. albicans* as 99.4 and 100%. Our results are in parallel with various studies at which these values were found to be 97–100% and 100% [14,20–25].

Thirty two of 33 *C. tropicalis* strains produced blue-violet, smooth (S type) colonies with halo diffusing into the surrounding agar on CA. One strain was observed as dark pink colonies. It has been reported that some strains of *Pichia spp*. gave an identical appearance [14]. However; San-Millan et al. [23] emphasized that they didn't observe this appearance in their study. The same authors also found false positive results due to *S. cerevisiae* isolates. This may be true due to the fact that dark pink colonies with halo may resemble *C. tropicalis* colonies on CA. We had only one *S. cerevisiae* isolate and its color was somewhat different from blue-purple color of *C. tropicalis*. The sensitivity and specificity calculated for *C. tropicalis was* 97 and 100%. The results of this study are in agreement with those described by many authors whose sensitivity and specificity rates ranged between 66.7–99.0% and 93.8–100%, respectively [14,20,22–24].

Rapid identification of *C. krusei* and *C. glabrata* isolates with chromogenic media has a special importance because *C. glabrata* is less sensitive than other species to ketoconazole and fluconazole and *C. krusei* exhibits innate resistance to fluconazole [26]. All our *C. krusei* isolates produced rough, fuzzy, spreading, big pink colonies on CA. Cooke et al. [27] reported that the usefulness of

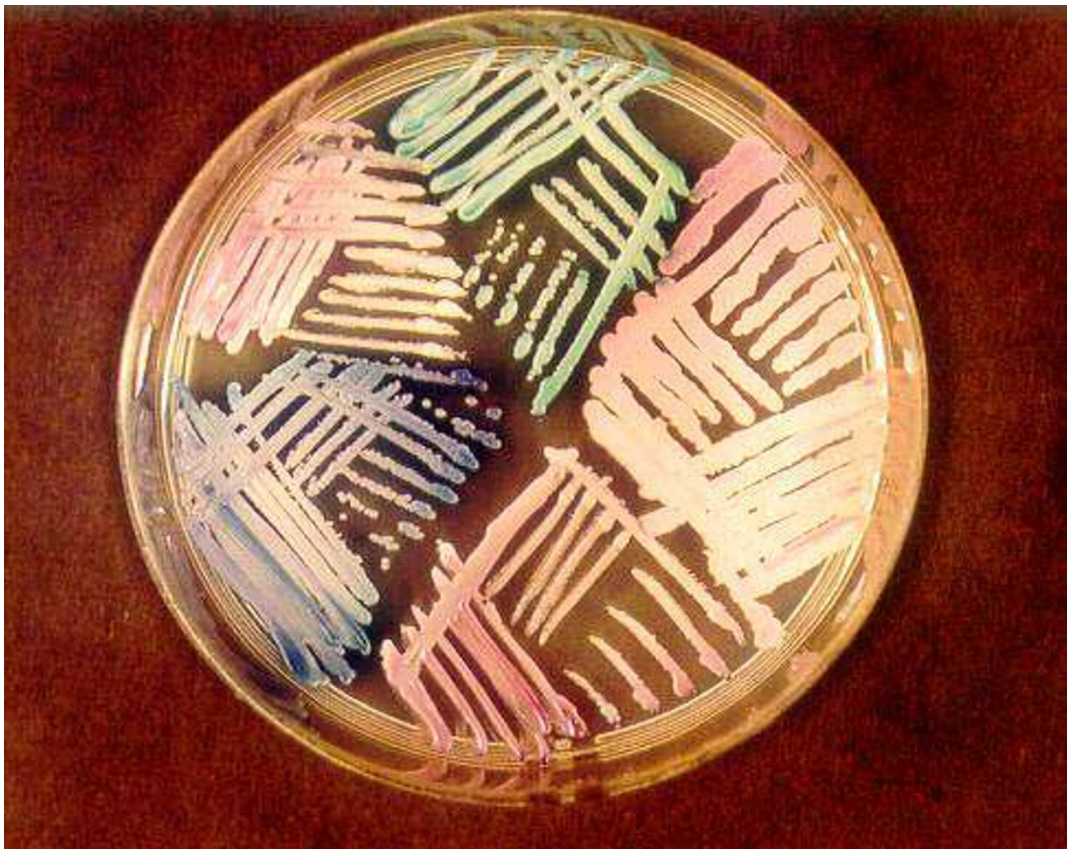

Figure 1
Different colony colors and morphologies of various *Candida* species on CHROMagar Candida. Isolates of *C. albicans* ATCC 90028 (top), *C. krusei* ATCC 6258 (right), *C. glabrata* (bottom), *C. tropicalis* (lower left), *C. parapsilosis* ATCC 90018 (upper left) on CHROMagar Candida plates

colony color in identification of *C. krusei* appeared to be limited as several other yeasts gave pink and purple colonies on this medium. Our findings are in contrast with the authors' in the fact that although there are many yeast species giving the same colony color, the morphology of the colonies of *C. krusei* are distinctly different (fuzzy, rough, large, pink). Odds and Bernaerts [14]. proposed that *C. krusei* like colonies are also formed by isolates of *C. norvegensis*. This is an important point to remember however, this species is very rarely encountered in clinical specimens. The sensitivity and specificity we obtained for this species was 100%. These values are concordant with the reults of many studies [14,22,23].

In this study, twenty of *C. glabrata* isolates produced pink, glossy colonies with pale edges but 4 of them were grown as off white-cream colonies. Pfaller et al [9] and Willinger et al [24] concluded that CA also allowed the identification of *C. glabrata* however other authors showed that many other species such as *C. kefyr*, *C. lusitaniae*, *C. guilliermondii*, *C. famata*, *C. rugosa*, *C. utilis*, *Cryptococcus neoformans*, *S. cerevisiae* produced similar colonies which might lead to a high degree of confusion [11,14,16,28]. In our study many other species also produced the same color with *C. glabrata* which is very confusing, however, it would be more helpful if special attention is given to the existence of pale edges on the pink colonies. Most of the *C. glabrata* strains showed this property in our study.

The isolates of *C. parapsilosis*, *C. guilliermondii*, *C. kefyr* and *C. lusitaniae* had various tones from off white to pink on

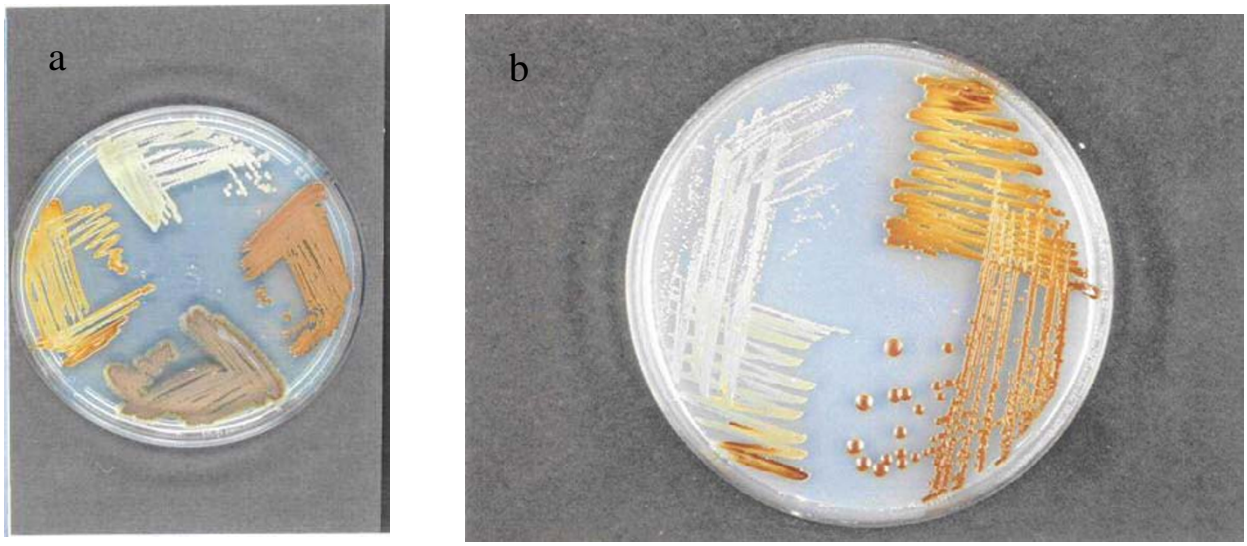

Figure 2
Different colony colors and morphologies of various *Candida* species on BIGGY agar. a) Isolates of *C. parapsilosis* (top), *C. albicans* (left), *C. krusei* (bottom)and *C. tropicalis* (right) b) Isolates of *C. glabrata* (left) *C. albicans* (right) on BIGGY agar plates

Table 2: The sensitivity, specificity, positive and negative predictive values obtained for CHROMagar Candida

Yeast species	CHROMagar Candida			
	Sensitivity **CI**	**Specificity** **CI**	**PPV** **CI**	**NPV** **CI**
C. albicans **n = 169**	99.4 96.2, 100	100 95.4, 100	100 97.2, 100	99 93.9, 99.9
C. tropicalis **n = 33**	97.0 82.5, 99.8	100 98.0, 100	100 86.7, 100	99.6 97.3, 100
C. glabrata **n = 24**	90.9 69.4, 98.4	100 98.1, 100	100 80.0, 100	99.2 96.8, 99.9
C. krusei **n = 12**	100 69.9, 100	100 98.2, 100	100 69.9, 100	100 98.2, 100

CI: Confidence interval

Table 3: The sensitivity, specificity, positive and negative predictive values of BiGGY agar

Yeast species	BIGGY agar			
	Sensitivity CI	**Specificity** CI	**PPV** CI	NPV **CI**
C. albicans	87.0	75.2	85.5	77.6
n = 169	80.7, 91.5	65.2, 83.1	79.1, 90.2	67.8, 85.1
C. tropicalis	66.6	95.4	64.5	94.6
n = 33	42.2, 76.6	91.6, 97.5	45.4, 80.2	90.7, 96.9
C. krusei	100	100	100	100
n = 12	69.9, 100	98.2, 100	69.9, 100	98.2, 100

CA. These colors were difficult to define because they were similar pastel tones and they were not typical for any species.

Most of the *C. albicans* strains showed light brown while 13 and 20 of *C. tropicalis* isolates produced light and dark brown color when grown on BiGGY agar. It was hard to differentiate the colors of these two species. In our study two species showed typical, distinctive appearance on BiGGY agar. One was *C. krusei* which produced typical large, rough, dark brown colonies with surrounding yellow zone and the other was *C. parapsilosis* which grew as light brown-geenish, gray, cream colored colonies. *C. glabrata* strains grew weakly on BiGGY agar after 48 hours. Brown color was only observed at the first streaks of the cultures, where the colonies were very crowded. Other areas especially single colonies were very small and colorless. The sensitivity and specificity were detected as 87.0 and 75.2% for *C. albicans*, 66.6 and 95.4% for *C. tropicalis* and 100% for *C. krusei*. To our knowledge, a study related to the efficacy of BiGGY agar was not reported in the literature.

Odds and Bernaerts [14] emphasized that these chromogenic media were not proposed as substitutes for thorough identification protocols. They can be used alone or with some other identification procedures for rapid identification and diagnosis in Clinical Microbiology laboratories. When the current price of both media were compared, the estimated cost of one liter of CA medium was calculated to be 7.7 times of the same amount of BiGGY agar. This difference may be offset by further identification costs for BiGGY agar.

Conclusions

As a result, it can be concluded that the use of CA is an easy and reliable method for the presumptive identification of most commonly isolated *Candida* species especially *C. albicans*, *C. tropicalis* and *C. krusei*. The lower sensitivity and specificity of BiGGY agar to identify commonly isolated *Candida* species potentially limits the clinical usefulness of this agar.

Author's Contributions

M.Y: Designed and coordinated the study, drafted the manuscript, did the laboratory work and evaluated the results with SM.

S.M: Did the laboratory work and evaluated the results with MY.

All authors read and agreed to its content. The paper has not already have been published in a journal and is not not under consideration by any other journal.

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