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Do incubation temperature, incubation time, and carbon dioxide affect the chromogenic properties of CHROMagar?

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Aim: On CHROMagar medium *Candida* species form different colors; thus, the medium enables the differentiation of these species from each other as well as from other *Candida* species. The aim of this study is to investigate the effect of incubation temperatures, incubation times, and CO_2 on the chromogenic properties of CHROMagar.

Materials and methods: A total of 112 strains of *Candida* spp. were used. A 0.5 McFarland suspension of each strain was inoculated onto CHROMagar with a calibrated loop and incubated at 26 °C and 35 °C for 24-48 h in normal atmosphere and in an atmosphere of 5% CO_2. The results were evaluated at the end of 24 and 48 h by 2 of the authors working in strict separation.

Results: The chromogenic property of the medium was best observed at an incubation temperature of 35 °C. Incubation in an atmosphere of 5% CO_2 yielded more prominent colonies at the end of 48 h. The chromogenic differentiation of *C. dubliniensis* from *C. albicans* was not easy, for *C. albicans* yielded a green color and *C. dubliniensis* a somewhat darker green color.

Conclusion: To obtain the best results with CHROMagar, the medium should be incubated at 35 °C for 48 h in an atmosphere of 5% CO_2. A control *C. albicans* strain should be inoculated on each medium plate to differentiate the color tones of *C. albicans* and *C. dubliniensis.*

Key words: *Candida* spp., CHROMagar, identification, temperature, incubation time, carbon dioxide

Introduction

The incidence of fungal infections, particularly multiple-yeast infections, is increasing due to the rising number of immunocompromised patients, the widespread use of broad spectrum antibiotics, and invasive devices or procedures (1-6). For rapid isolation and identification of the causative yeast, particularly in mixed cultures, traditional media like Sabouraud dextrose agar (SDA) are not always efficient.

CHROMagar (CHROMagar Microbiology, Paris, France) is a chromogenic medium designed for cultivation and rapid identification of *Candida* spp. within 24 to 48 h on the basis of strongly contrasting colony colors (7-11). On this medium *Candida albicans* forms green, *C. tropicalis* metallic blue, *C. krusei* dry pale pink, and other *Candida* species smooth colonies with a color ranging from white to dark pink. Thus, the medium enables the differentiation of the above mentioned species from each other as well as from other *Candida* species. The medium has now been evaluated worldwide in many laboratories and has usually been found to provide presumptive identifications of *C. albicans*,

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C. tropicalis, and *C. krusei* with high levels of sensitivity and specificity (12-17). Mahmoudi Rad et al. found that CHROMagar provides a convenient and cost-effective yet reliable method to isolate the species of *Candida*, especially in cases where more than one species is present (18). *Candida*, which is eukaryotic, forms budding cells. It multiplies principally by the production of blastoconidia and pseudohyphae. Some *Candida* species may produce true septate hyphae associated with the diminution of oxygen in the presence of 5 to 10% CO_2. The effect of CO_2 is most clearly demonstrated at 30 °C when it induces a characteristic growth form consisting of a single swollen blastoconidium giving rise to a long, unbranched mycelial tube with few secondary blastoconidia; in atmospheric concentrations of CO_2, only blastoconidial growth occurs. Growth in the blastoconidial form is more rapid in 10% CO_2 than in air (18).

The aim of this study was to evaluate the effect of different incubation temperatures, incubation times, and CO_2 on the chromogenic properties of CHROMagar.

Materials and methods

A total of 112 strains of *Candida*, isolated from clinical specimens and identified with conventional methods and stored at –20 °C (Yeast Culture Collection, Mycology Laboratory, Ege University Hospital) were used (40 *C. albicans*, 6 *C. dubliniensis*, 22 *C. tropicalis*, 20 *C. krusei*, and 24 *C. glabrata*) (19). As the first step, the yeasts were separately subcultured on SDA (Oxoid, Basingstoke, UK) at 37 °C for 48 h. A 0.5 McFarland suspension of each strain was then inoculated onto CHROMagar with a calibrated loop and incubated for 24-48 h at 2 different temperatures (26 °C and 35 °C). As the second step, upon seeing the effect of 35 °C, 2 more incubations at 35 °C were carried out, 1 at 35 °C in normal atmosphere and 1 at 35 °C in an atmosphere of 5% CO_2. The results were evaluated at the end of 24 and 48 h by 2 of the authors working in strict separation. As the third step, a mixture of 5 different *Candida* species in saline was made, inoculated on CHROMagar plates, and incubated under the different conditions stated above. In each step, *C. albicans* ATCC 90028 was inoculated onto each culture plate as a control.

Results

On CHROMagar all strains developed colonies by 24 and 48 h. All *Candida* spp. colonies were smooth and glabrous except those formed by *C. krusei*. Color development was generally poor at 26 °C and by 24 h of incubation, but good and typical at 35 °C and by 48 h of incubation, particularly in an atmosphere of 5% CO_2. Furthermore, all isolates incubated at 35 °C with 5% CO_2 formed nonspreading, well-limited colonies (Figure).

After 2 days of incubation at 26 and 35 °C in normal atmosphere and at 35 °C with 5% CO_2, all colonies of *C. albicans* isolates showed the characteristic green color. All of the *C. dubliniensis* isolates had a dark green color at all incubation times, at 35 °C, and with and without 5% CO_2 except by incubation at 26 °C. The chromogenic differentiation of *C. dubliniensis* from *C. albicans* was not easy, for *C. albicans* yielded green and *C. dubliniensis* yielded a somewhat darker green color.

After 24 h incubation at 26 °C, *C. tropicalis* isolates showed various colors, namely cream, pink, and dark blue. By incubation at 35 °C for 24 and 48 h, the yeast yielded the blue color. Only one isolate of *C. tropicalis* kept the initial cream color under all conditions of incubation.

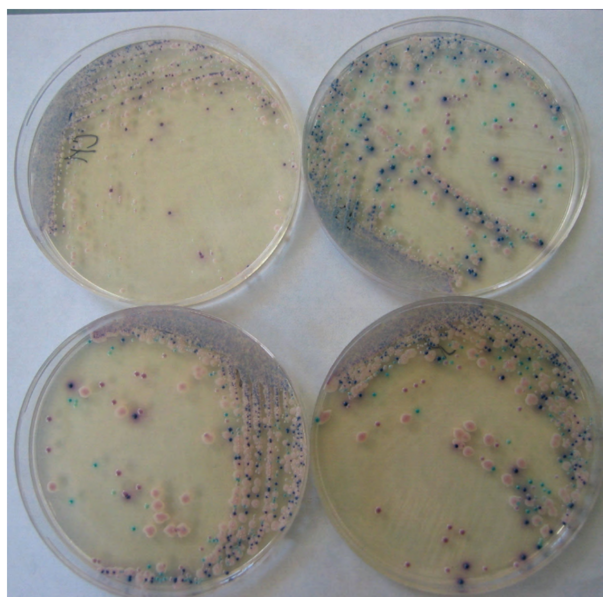

Figure. Colony colors in mixed culture with 48 h of incubation. Above left: At 26 °C. Above right: At 35 °C with CO_2. Below left and right: At 35 °C without CO_2.

All *C. krusei* isolates were cream-colored at 26 °C by 24 h of incubation, but they turned to pink at both temperatures by 48 h of incubation. With its dry, rough colonies, *C. krusei* could easily be differentiated from other pink colonies, namely those of *C. glabrata*. By 24 h of incubation, all *C. glabrata* isolates were cream-colored at 26 °C, but some isolates changed to pink at 35 °C with and without 5% CO_2. At incubation for 48 h, all strains showed a pink color under all other conditions.

Discussion

CHROMagar *Candida* is a differential culture medium for the isolation and presumptive identification of clinically important yeasts within 24 to 48 h on the basis of strongly contrasting colony colors (8,10). The medium has now been evaluated worldwide in many laboratories and has usually been found to provide presumptive identifications of *C. albicans*, *C. tropicalis*, and *C. krusei* with high levels of sensitivity and specificity (8,9,11-14). Liguori et al. reported that the specificity for *C. albicans* identification by CHROMagar is around 75% (20). In the same study, sensitivity was found to be in the range of 81.9% to 87.7% by phenotypic tests.

For the medium as originally supplied by CHROMagar Microbiology, the color of colonies on the medium was intended to be read after 48 h of incubation at 37 °C (15). Some investigators found that an incubation temperature of 30 °C gave results similar to those at 37 °C, though with less intense color hues. The recommendations from some distributors of the chromogenic medium are for incubation at 30-37 °C for 48 h (15). While these are the conditions most often used by investigators, there are isolated reports of incubation at 25 °C and for periods longer than 48 h. Odds and Davidson (12) examined the formation of distinctive colors by *Candida* species incubated at 3 temperatures, 25, 30, and 37 °C, and found that the color formation of colonies were generally slower at 25 °C. In this study, among 5 different species of *Candida* tested, *C. albicans* was found to show color stability; it formed a green color under all test conditions. CHROMagar seems to be a suitable medium for the rapid diagnosis of these species under various conditions of incubation. A drawback is that *C. dubliniensis*, which is difficult to differentiate from *C. albicans* by phenotypic tests, is also often recognizable at the time of first isolation by its formation of colonies with a green color, albeit with a darker green hue than is normal for *C. albicans* (12,21-23). As shown in the present study, incubation not at 26 °C but at 35 °C would be necessary for the formation of the typical dark green hue, which could help the objective differentiation from *C. albicans*. Inclusion of a control strain of *C. albicans* in the test can also be of help in differentiating color hues of the 2 distinct species.

Candida tropicalis could be easily recognized with its distinct blue color formed under all incubation conditions except incubation at 26 °C and for 24 h. In the latter conditions the yeast showed varying colors, namely cream, pinkish cream, and light blue. It seems that for the formation of the typical blue color, it is not the temperature or CO_2, but rather a longer incubation such as 48 h that is necessary. Out of 22 *C. tropicalis* isolates only 1 isolate (the identification of which was checked twice) formed cream-colored colonies under all incubation conditions, but this rare finding should not be seen as a disadvantage of the medium in the identification of *C. tropicalis*.

Some publications stated that *C. glabrata* could be differentiated from other *Candida* species on CHROMagar in view of its pink colonies, but other papers studying many more species of *Candida* reported that *C. parapsilosis* and *C. lusitaniae* also form a pink color (7-9,24). In the present study, it was observed that at both temperatures at 24 h, *C. glabrata* formed uniform colors varying from cream to pink, but at 48 h of incubation the yeast uniformly developed a pink color. Since this study did not include other species previously reported to form pink colonies, like *C. parapsilosis* or *C. lusitaniae*, we are not definite about the relationship between *C. glabrata* and CHROMagar (7,9,25,26)

Among *Candida* species, *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. glabrata* are the most frequent causes of candidoses. CHROMagar can be used in the rapid identification of these species. In general, incubation of the inoculated medium at 35 °C and for 48 h leads to formation of uniform color for each species. Incubation at 26 °C and for 24 h seems not to be optimal for the function of the medium. Color formation seems not to be dependent on CO_2 in the

atmosphere, but the colonies formed in the presence of 5% CO_2 were more compact, which can be an advantage in evaluations of a mixed culture. Both *C. albicans*, the most frequent cause of candidoses, and *C. dubliniensis* form green colonies. They can be differentiated from each other by the difference in color tone using a control *C. albicans* strain, which should be inoculated on each batch of cultures.

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