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Persistence of Pigment Production by Yeast Isolates Grown on CHROMagar Candida Medium

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We evaluated the persistence of pigmentation in yeast isolates grown on the chromogenic medium CHROMagar Candida over 7 days. *Candida*, *Cryptococcus*, and *Trichosporon* isolates were inoculated alone or mixed onto duplicate sets of plates and incubated at 30 and 35°C. *Candida albicans* and *Candida krusei* were readily identified throughout the reading period, but *Candida glabrata* was difficult to differentiate from other species until the 3- or 4-day time point. *Candida tropicalis* produced colonies similar to those of rare *Cryptococcus* and *Trichosporon* species, and mixed cultures were often difficult to identify as such.

In this era of major cutbacks in laboratory funding and increasing use of referral facilities to perform standard clinical testing, microbiology laboratories do not always have the resources to read each incubating culture on a daily basis. In many facilities, mycological cultures may be read only two to three times weekly; thus, cultures may be left unevaluated for periods of more than 72 h. The use of the chromogenic medium CHROMagar Candida (CHROMagar Microbiology, Paris, France) has been claimed to be effective for the rapid and accurate identification of *Candida albicans*, *C. krusei*, and *C. tropicalis*. The company that produces this medium claims "rapid and reliable species detection and differentiation." Multiple studies indicate that when grown on CHROMagar Candida at 30 to 37°C for 24 to 72 h, *C. albicans*, *C. krusei*, and *C. tropicalis* can be identified by their colonial colors and morphologies (11, 12, 14, 18). This also appears to be true for *C. dubliniensis* (9, 10, 15), *Trichosporon beigelii* (8, 11, 12), and perhaps *C. glabrata* (5, 8, 14). We evaluated growth of single and mixed (two or three) isolates on CHROMagar Candida, in a blinded fashion, daily for seven consecutive days. Identification of yeast species remained possible for up to 7 days and in some cases improved over time.

A total of 83 yeast isolates recovered from specimens submitted to our laboratory and identified by standard methods were used in the study. Isolates included *C. albicans* (32 isolates), *C. parapsilosis* (10 isolates), *C. tropicalis* (10 isolates), *C. krusei* (5 isolates), *C. glabrata* (4 isolates), *C. lusitaniae* (4 isolates), and *C. guilliermondii* (2 isolates) and one each of *C. firmetaria*, *C. kefyr*, and *C. rugosa*. Noncandidal isolates included *Cryptococcus neoformans*, 4 isolates; *C. albidus*, 4 isolates; *C. humicolus*, 1 isolate; *C. uniguttulatus*, 1 isolate; *T. beigelii*, 2 isolates; and *T. mucoides*, 1 isolate. A sample of each frozen isolate was grown on plates of Trypticase soy agar with 5% sheep blood (Fisher Scientific, Houston, Tex.) at 30°C. A single colony selected from each plate was subcultured onto a second plate. An inoculum was prepared from this second plate by the transfer of individual colonies into 0.45% saline. Inoculum density was adjusted to a McFarland standard of 2.0 by using a Vitek Colorimeter (HACH Company, Loveland, Colo.), and 1-μl aliquots of various inocula (one species or mixtures of 2 or 3 species) were streaked for isolation onto duplicate sets of 100 CHROMagar Candida plates. CHROMagar Candida (lot 392-c) was obtained from DRG International (Mountainside, N.J.) and prepared according to the manufacturer's instructions. Plates were coded by number to blind readers to their contents. Duplicate plates were then incubated at either 30 or 35°C and read by four independent, blinded readers daily for 7 days. Readers recorded colony color and morphology as observed. Readers were also asked to record the number (from one to three) of distinguishable colony types.

Yeast isolates grown on CHROMagar Candida produced similar pigmentation patterns over the 7 days of study (Table 1). With the exceptions noted below (*Cryptococcus* species), the yeast isolates grew well and produced similar colors at 30 and 35°C. *C. albicans* and *C. krusei* were identifiable after 24 h of incubation at either temperature. *C. albicans* produced smooth, convex, green colonies. *C. krusei* produced rough, dry-appearing, flat, pink colonies with pale borders. *C. glabrata* produced small, convex, dark pink to violet colonies, often with faint pale borders or diffusion of pigment into the medium. *C. glabrata* colonies deepened in hue over the first 4 days of observation and were only reliably differentiated from other pink-lavender yeast colonies after 3 days and, in one isolate, 4 days. *C. tropicalis* grew in colonies that ranged in color from blue-gray to steel blue, some with a purple hue, most producing a purple diffusible pigment into the surrounding medium. *C. humicolus* and *T. mucoides* also grew as steel-blue colonies, similar to that of *C. tropicalis*, although neither produced the purple diffusible pigment. *T. beigelii* isolates grew in a lighter-blue shade (becoming powdery in texture with time) with a dark-blue reverse. *C. rugosa* grew with characteristics similar to those of *C. krusei* (rough, dry, flat colonies with a pale border) but in a distinct light blue-green color. *C. firmetaria* also grew similarly to *C. krusei* but could be differentiated by a waxy

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TABLE 1. Colony characteristics of yeast isolates grown on CHROMagar Candida

Species	No. of isolates	Colony characteristic(s)[a]
Candida albicans	32	Green
Candida firmetaria	1	Pink-lavender, flat, waxy
Candida glabrata	4	Dark violet[b]
Candida guilliermondii	2	Pink-lavender, mucoid
Candida kefyr	1	Pink-lavender
Candida krusei	5	Pink, flat, rough, pale border
Candida lusitaniae	4	Pink, mucoid
Candida parapsilosis	10	Pink-lavender, sometimes mucoid
Candida rugosa	1	Light blue-green, flat, rough, pale border
Candida tropicalis	10	Steel blue to blue-gray, purple diffusion
Cryptococcus albidus	4	Tan, sometimes mucoid
Cryptococcus humicolus	1	Steel blue
Cryptococcus neoformans	4	Pink-lavender or tan, sometimes mucoid
Cryptococcus uniguttulatus	1	Tan
Trichosporon beigelii	2	Blue to powder blue, dark blue-green reverse
Trichosporon mucoides	1	Steel blue

[a] Includes morphology if not that of typical yeast colonies.
[b] After 3 to 4 days of incubation.

texture and lack of a pale border. Of the cryptococcal isolates, only *C. neoformans*, *C. humicolus*, and one of the four *C. albidus* isolates grew at 35°C. *C. albidus* and *C. uniguttulatus* grew as small tan colonies, *C. humicolus* (as previously described) produced steel-blue colonies, and *C. neoformans* grew as pink, pink-lavender, or tan colonies.

Nineteen mixed sets were prepared, nine with two isolates each and ten with three isolates each (Table 2). Nine of the 19 sets were correctly identified as mixed and as containing the correct number of inoculated yeast isolates at each of the two temperatures by 48 h of incubation. Seven of nine mixed cultures that contained two isolates (mixtures A to C, E to G, and I) were readily discerned as mixed. Of the ten plates with three species, only two (mixtures J and M) were read as containing three differing colony types on both plates at 48 h. Plates that included combinations of two or more of *C. lusitaniae*, *C. parapsilosis*, *C. albidus*, and *C. neoformans* proved difficult to differentiate. None of the seven mixed sets (mixtures H and N to S) that contained two or more of these yeast isolates was correctly identified at either temperature after 48 h.

With the availability of newer antifungal drugs, physicians can no longer make therapeutic decisions based on the broad identification of fungi as either yeasts or molds. Identification of candidal yeast isolates to the species level is now required to aid therapeutic agent selection. Identification procedures presently take several days and require highly skilled personnel and are not always able to detect whether clinical isolates contain more than one species of yeast. Candidal yeasts grow in various colors on the CHROMagar Candida based on the presence of species-specific enzymes. Routine use of this medium may lead to faster, more economical identification of candidal yeasts and allow a more accurate examination of the role of mixed cultures. Although parameters of cost effectiveness and rapidity of diagnosis have been explored with the use of CHROMagar Candida (1, 2, 11), persistence or durability of pigmentation with delayed reading of this medium has not been examined. With the present economic pressure on clinical microbiology laboratories, evaluation of all culture specimens in the mycology sections on a daily basis may not be possible in all laboratories.

Our study found that colonial pigmentation and typical morphology persisted throughout a seven-day period. Color and colony descriptions used by others and the manufacturer of CHROMagar Candida can readily be applied to identify colonies after the recommended 48 h of incubation. This held true for cultures incubated at either 30 or 35°C. *C. albicans* and *C. krusei* were easily identifiable by 24 h. *C. albicans* grew in various shades of green without overlap with the coloration of any other species studied. *C. krusei* produced typical colonies as previously described. *C. firmetaria* produced similar color characteristics but could be differentiated by its waxy, raised morphology. *C. humicolus* and *T. mucoides* produced steel-blue pigmentation like that produced by *C. tropicalis*. Neither produced the typical purple diffusion or "halo" typically seen with *C. tropicalis*, but *C. tropicalis* did not always produce this feature either. Some isolates did not produce it at all, and others produced it at various times during this prolonged incubation. Other researchers have reported similar problems with the differentiation from *C. tropicalis* of some of the rarely isolated yeast organisms. These include reports of *Candida catenulata* (3), *C. guilliermondii* (5), *C. humicolus* (5), and *Saccharomyces cerevisiae* (18). *T. beigelii* also produced colonies which were blue. These were readily differentiated from those of *C. tropicalis* and had characteristics similar to those reported

TABLE 2. Identification of mixed cultures of yeasts on CHROMagar Candida

Mixture	Species inoculated onto plates	No. of distinct colony types identified
A	*C. albicans-C. glabrata*	2
B	*C. albicans-C. krusei*	2
C	*C. albicans-C. parapsilosis*	2
D	*C. albicans-C. rugosa*	1[a]
E	*C. albicans-C. tropicalis*	2
F	*C. glabrata-C. krusei*	2
G	*C. glabrata-T. beigelii*	2
H	*C. lusitaniae-C. parapsilosis*	1
I	*C. tropicalis-T. beigelii*	2
J	*C. albicans-C. glabrata-C. krusei*	3
K	*C. albicans-C. glabrata-C. tropicalis*	2[b]
L	*C. albicans-C. guilliermondii-T. beigelii*	2[c]
M	*C. albicans-C. krusei-C. tropicalis*	3
N	*C. albicans-C. parapsilosis-C. neoformans*	3[d]
O	*C. krusei-C. lusitaniae-C. neoformans*	2
P	*C. lusitaniae-C. parapsilosis-C. neoformans*	2[e]
Q	*C. parapsilosis-C. tropicalis-C. neoformans*	2
R	*C. parapsilosis-C. tropicalis-C. albidus*	2
S	*C. tropicalis-C. albidus-C. neoformans*	2

[a] No colonies typical of *C. albicans*.
[b] No colonies typical of *C. glabrata*.
[c] No colonies typical of *C. albicans*.
[d] Third isolate (presumed *C. neoformans*) noted only on 30°C plate after 3 days of incubation.
[e] Second isolate (presumed *C. neoformans*) noted only after 4 days of incubation.

by others (8, 11, 12). Controversy exists in the literature as to whether *C. glabrata* isolates can be readily distinguished from other yeast isolates that produce pink colonies. While some researchers report reliable identification (5, 8, 14), many large studies report unreliable identification (7, 11, 12, 13, 15; A. Freydiere, Letter, J. Clin. Microbiol. **34:**2048, 1996). *C. glabrata* could be clearly differentiated in our study after prolonged incubation, but the study only included four *C. glabrata* isolates.

Our investigations of mixed cultures were somewhat disappointing. Previous studies of the use of CHROMagar Candida to identify the presence of mixed yeast cultures from clinical isolates have documented the efficacy of this method (3–7, 12, 14–18). In our hands, we found that combinations of two species were readily apparent to the reader, with the exception of two mixtures, *C. lusitaniae* with *C. parapsilosis* and *C. albicans* with *C. rugosa*. In the latter mixture, no colonies consistent with *C. albicans* were ever noted, and we suspect that this isolate failed to grow. Mixtures of three isolates were even harder to distinguish. Most of these contained yeast isolates that produced similar, difficult-to-differentiate colonies in shades of pink and pink-lavender or contained slow-growing *Cryptococcus*. Other than a brief mention by Odds and Bernaerts (12), this study is the first to describe spiked mixed cultures on CHROMagar Candida. Previous studies reported only those mixed cultures that happened to be observed when clinical isolate submissions were plated on CHROMagar Candida. We suspect that mixtures of similar-appearing yeast isolates could have been missed in those studies as well.

CHROMagar Candida appears to produce reliable results even when reading is delayed for up to 7 days. *C. albicans* and *C. krusei* can clearly be differentiated from other yeast isolates on this medium. Mixed cultures can be distinguished on this medium, although this can be hampered in mixtures of yeast isolates (other than *C. krusei*) that produce pink colonies.

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