NOTES

Supplementation of CHROMagar Candida Medium with Pal's Medium for Rapid Identification of *Candida dubliniensis*

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CHROMagar Candida medium is used for the isolation and identification of *Candida* species, but it does not differentiate *Candida albicans* from *Candida dubliniensis*. This differentiation can be achieved by using Pal's agar, which cannot be used in primary isolation. We have combined both media to obtain a new medium that can be used for the isolation and identification of *C. dubliniensis* in primary cultures.

Candida dubliniensis and *Candida albicans* are two fungal species that show very close phenotypic characteristics that make the discrimination between them difficult. Differentiation of both species can be achieved by a limited number of both phenotypic and genotypic tests (17, 18). While molecular techniques remain the most reliable methods for identification of *C. dubliniensis* (17) and its described genotypes (3, 5), they are not available in many mycology laboratories. Therefore, the search for less-sophisticated, rapid, and accurate techniques for differentiating *C. dubliniensis* from *C. albicans* still continues. A number of phenotypic tests to differentiate both *Candida* species, including growth at 42 to 45°C (14), chlamyospore production in different media (1, 7, 16), colony color development in differential media such as CHROMagar Candida medium (6, 17) or Candida ID medium (15), differential carbohydrate assimilation (4, 13), and an indirect immunofluorescence reaction with a specific anti-*C. dubliniensis* serum (2), have been described previously.

CHROMagar Candida medium is a widely used medium for the identification of *Candida* spp. which develop colonies with distinguishable colors. This medium allows the presumptive identification of *C. albicans*, *Candida tropicalis*, and *Candida krusei* (8, 12). Some authors (6, 9, 17) have observed that *C. albicans* and *C. dubliniensis* may show colonies with different shades of green; however, the color intensity is not restrictive for each individual species, and differentiation of both species based on the colony color is difficult (9, 19).

Pal's agar was originally developed for the identification of *Cryptococcus neoformans* (10, 11) and has been used for differentiation between *C. dubliniensis* and *C. albicans*. In this medium, *C. dubliniensis* forms rough colonies and chlamydo-spores, while *C. albicans* shows smooth colonies and does not

produce chlamydo-spores (1). However, other species of *Candida* share the colony morphology characteristics of both species. In the present study, we supplemented CHROMagar Candida agar with Pal's agar to improve the differentiation of *C. dubliniensis* from *C. albicans* achieved with each culture medium individually.

Eleven reference strains were used as controls in this study. They were obtained from the National Collection of Pathogenic Fungi (NCPF), Bristol, United Kingdom, the Centraal-bureau voor Schimmelcultures (CBS), Baarn, The Netherlands, the American Type Culture Collection (ATCC), Manassas, Va., and the Colección Española de Cultivos Tipo (CECT), Valencia, Spain, and included *C. dubliniensis* NCPF 3949, CBS 2747, CBS 8500, CBS 8501 and CECT 11473; *C. albicans* NCPF 3153; *C. tropicalis* NCPF 3111; *Candida glabrata* NCPF 3203; *C. krusei* ATCC 6258; *Candida guilliermondii* NCPF 3099; and *Candida parapsilosis* ATCC 22019. The remaining 131 yeast strains were clinical isolates of oral, vaginal, sputum, or blood origin previously isolated at our laboratory and included 95 *C. dubliniensis* strains, 29 *C. albicans* strains, 2 *C. krusei* strains, 2 *C. glabrata* strains, 2 *C. guilliermondii* strains, and 1 *C. parapsilosis* strain. Clinical isolates were identified by their carbohydrate assimilation patterns on ID 32C strips (bioMérieux, Marcy-l'Etoile, France), growth at 42 to 45°C, chlamydo-spore production in casein agar (7), and indirect immunofluorescence with a specific anti-*C. dubliniensis* serum (2). Genotypic identification of *C. dubliniensis* and *C. albicans* isolates was performed by PCR with specific primers. The four genotypes of *C. dubliniensis* were determined according to methods described previously by Brena et al. and Gee et al. (3, 5). Some *C. dubliniensis* and *C. albicans* isolates were sequenced to verify their identity. In all cases, the identity was the same as that obtained by other tests. For some experiments, 10 fresh oral swabs were directly plated onto the different culture media tested.

Pal's agar was prepared by adding 50 g unsalted powdered sunflower seeds to 1 liter of distilled water, boiling for 30 min, and filtering through cheesecloth. This extract was supple-

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TABLE 1. Colony aspects of and chlamyospore formation by *C. albicans* and *C. dubliniensis* isolates on different culture media

Characteristic and medium	No. of isolates (%)	
	<i>C. albicans</i> (n = 30)	<i>C. dubliniensis</i> (n = 100)
Colony color		
CHROMagar Candida medium		
Light green	13 (43.3)	2 (2)
Green	11 (36.6)	40 (40)
Dark green	6 (20)	58 (58)
CHROMagar Candida medium supplemented with Pal's agar		
Light green	30 (100)	0 (0)
Bluish green	0 (0)	96 (96)
Light bluish green	0 (0)	4 ^a (4)
Colony morphology		
Pal's agar		
Rough	0 (0)	96 (96)
Smooth	30 (100)	4 (4)
CHROMagar Candida medium supplemented with Pal's agar		
Rough	0 (0)	96 (96)
Smooth	30 (100)	4 ^b (4)
Chlamyospore production		
CHROMagar Candida medium	0 (0)	0 (0)
Pal's agar	0 (0)	93 (93)
CHROMagar Candida medium supplemented with Pal's agar	0 (0)	93 (93)

^a These isolates show rough colonies in CHROMagar Candida medium supplemented with Pal's agar. These isolates were sequenced to verify their *C. dubliniensis* identity.

^b These isolates show bluish green colonies in CHROMagar Candida medium supplemented with Pal's agar. These isolates were sequenced to verify their *C. dubliniensis* identity.

mented with creatinine (1 g), glucose (1 g), and KH_2PO_4 (1 g). After pH adjustment to 5.5, agar (15 g/liter) was added, and the mixture was autoclaved at 110°C for 20 min. The cooled medium (45 to 55°C) was poured into 90-mm-diameter petri dishes (25 ml/plate). CHROMagar (Paris, France) Candida medium was prepared according to the manufacturer's instructions as follows: 47.7 g of the powder medium was slowly dispersed in 1 liter of purified water and brought to a boil by repeated heating until complete fusion of agar grains. Medium was poured into petri dishes as described above. CHROMagar Candida medium supplemented with Pal's medium was prepared by mixing equal volumes of prepared CHROMagar Candida medium and Pal's agar. After stirring well, the medium was poured into 90-mm-diameter petri dishes. Several concentrations of each medium were tested, and the best results for discrimination of *C. albicans* and *C. dubliniensis* isolates were obtained by mixing equal volumes of both media at their normal strength. Strains were grown simultaneously on Pal's agar, CHROMagar Candida medium, and CHROMagar Candida medium supplemented with Pal's agar. Inoculum of collection strains was obtained from cells grown on Sabouraud agar for 48 h at 37°C. Plates were incubated at 30°C (Pal's agar and CHROMagar Candida medium supplemented with Pal's agar) or 37°C (CHROMagar Candida medium), and colony characteristics were recorded after 24, 48, and/or 72 h of incubation by two different observers. Chlamyospore formation was de-

tected microscopically using lactophenol cotton blue (Merck, Darmstadt, Germany) as a stain.

Table 1 shows colony color and morphology as well as chlamyospore formation by *C. albicans* and *C. dubliniensis* isolates when isolates were grown on different media. Ninety-six out of 100 *C. dubliniensis* isolates formed rough colonies on Pal's agar, 75% showed hyphal fringe, and 93% produced chlamyospores. On the contrary, all *C. albicans* strains formed smooth colonies without hyphal fringe and failed to produce chlamyospores. All three *C. krusei* isolates and one *C. parapsilosis* isolate formed rough colonies. The remaining *Candida* species formed smooth colonies, and none of them produced chlamyospores. Fifty-eight out of 100 *C. dubliniensis* strains grew as dark green colonies on CHROMagar Candida medium, and the remaining 42% grew as lighter green colonies. Thirteen out of 30 (43.3%) *C. albicans* isolates formed light green colonies, and the remaining 56.6% formed green and dark green colonies. The other *Candida* species developed different colors. Ninety-six out of 100 *C. dubliniensis* isolates grew as rough colonies on CHROMagar Candida medium supplemented with Pal's agar (Fig. 1), 75 isolates formed hyphal fringe, and 93 produced chlamyospores. Ninety-six isolates grew as bluish green colonies that were easy to distinguish from *C. albicans* colonies. On the contrary, *C. albicans* isolates formed smooth light green colonies (Fig. 1) and failed to produce either hyphal fringe or chlamyospores. Other *Candida* species formed smooth or rough colonies but with a distinguishable color, and none of them was able to form chlamyospores in this medium.

The colony morphology (rough/smooth) and chlamyospore production results for *C. dubliniensis* and *C. albicans* isolates were the same for Pal's agar and CHROMagar Candida medium supplemented with Pal's agar. On the contrary, colony color was not definitive in CHROMagar Candida medium, as *C. albicans* and *C. dubliniensis* isolates showed the three intensities of green. However, when this medium was supplemented

FIG. 1. Colonies of different species of *Candida* after growing for 48 h at 37°C in CHROMagar Candida medium supplemented with Pal's agar.

with Pal's agar, *C. albicans* isolates appeared as light green, smooth colonies, while *C. dubliniensis* showed a distinguishable bluish green color which was accompanied in most cases (96%) by the production of rough colonies, making differentiation of both species much easier. Other species of *Candida* that were assayed in this study showed rough or smooth colonies in CHROMagar Candida medium supplemented with Pal's agar; however, they never appeared as green or bluish green colonies, and in addition, they never developed chlamydospores, making their misidentification as *C. albicans* or *C. dubliniensis* highly unlikely. Interestingly, *C. tropicalis* and *C. krusei* still retained a distinguishable blue and pink-and-rough colony aspect, respectively, that allowed their identification on CHROMagar Candida medium supplemented with Pal's agar, even when used as a primary isolation medium for the fresh oral specimens assayed.

The differentiation between *C. dubliniensis* and *C. albicans* could be achieved by the observation of their colony aspects and color in CHROMagar Candida medium supplemented with Pal's agar with 92% sensitivity and 100% specificity. The remaining *C. dubliniensis* strains (8%) could presumptively be identified either by the rough aspect or the bluish green color of their colonies.

The *C. dubliniensis* reference strains from culture collections gave the expected results. The four different genotypes of *C. dubliniensis* were all positive in CHROMagar Candida medium supplemented with Pal's agar, growing as typical *C. dubliniensis* colonies.

In conclusion, CHROMagar Candida medium supplemented with Pal's agar allows the rapid and easy differentiation between *C. albicans* and *C. dubliniensis* on the basis of their colony characteristics. When used as a primary isolation medium, CHROMagar Candida medium supplemented with Pal's agar allows the identification of *C. dubliniensis* even in mixed cultures containing *C. albicans*, *C. tropicalis*, *C. krusei*, and/or *C. glabrata*.

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