JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2008, p. 3498–3500 Vol. 46, No. 10
0095-1137/08/$08.00+0 doi:10.1128/JCM.00982-08
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Production of White Colonies on CHROMagar *Candida* Medium by Members of the *Candida glabrata* Clade and Other Species with Overlapping Phenotypic Traits▽

Justin A. Bishop,[1] Nancy Chase,[1]† Richard Lee,[1] Cletus P. Kurtzman,[2] and William G. Merz[1]*

Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, Maryland,[1] and Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois[2]

Received 21 May 2008/Returned for modification 7 July 2008/Accepted 25 July 2008

We hypothesized that species of the *Candida glabrata* clade and species with phenotypic traits that overlap those of *C. glabrata* would produce white colonies on CHROMagar *Candida* medium. Of 154 isolates (seven species) tested, *C. bracarensis*, *C. nivariensis*, *C. norvegensis*, *C. glabrata*, and *C. inconspicua* produced white colonies; the *Pichia fermentans* group and *C. krusei* did not. Many of these species are difficult to identify phenotypically; white colonies may signal the need for the use of molecular approaches.

Chromogenic media have been formulated to discern common *Candida* species on the basis of the color of the colonies (7). Beginning in Europe in the 1990s, investigators published reports of the development of several chromogenic media; many of the media are now commercially available products in the United States. These publications include several on the performance of CHROMagar *Candida* medium (CAC; Becton Dickinson, Sparks, MD, and Hardy Diagnostics, Santa Maria, CA). These data support the conclusions that there are relatively specific colony colors for *Candida albicans*, *Candida tropicalis*, and *Candida krusei* (2, 8, 11, 12). In addition, *Candida glabrata* and *Candida dubliniensis* can often be distinguished by their specific colony colors (5, 9). A major advantage of these media is that they provide identifications (presumptive or definitive) in times shorter than those required for identification by many standard mycologic phenotypic assays (11). In addition, these media permit the detection of the growth of multiple yeast species from a single clinical specimen due to the colony color differences (8, 11). However, the lack of a wide range of colors limits the expansion to more yeast genera or species, and the increased cost (higher than that for standard mycologic media) has prevented some laboratories from implementing these media.

Since its original descriptions, there have been reports of yeast species that produce white colonies on CAC (8). However, white colony-producing yeasts are not frequently encountered among yeasts recovered from clinical specimens (7, 8, 12). We recently observed white colonies while investigating *Candida bracarensis* and *Candida nivariensis* (3), two species within the *C. glabrata* clade recently defined molecularly (1, 4). These species are difficult to separate by the use of phenotyping due to their significantly overlapping traits and also due to the relatively few positive reactions for these species (e.g., by a carbohydrate assimilation assay). From these observations, we hypothesized that white colonies would be produced on CAC by other strains or members of the *C. glabrata* clade and also by *Candida* spp. that are not in the clade but that have few positive reactions and that share many phenotypic traits.

A total of 154 isolates was tested in this study, including 13 *C. krusei* and 127 *C. glabrata* recent clinical isolates identified by phenotypic characteristics at the Clinical Mycology Laboratory of The Johns Hopkins Hospital. Phenotypic identification of *C. glabrata* and *C. krusei* was made by the germ tube assay; the urease test; determination of microscopic morphology on cornmeal agar with Tween 80; the phenoloxidase enzyme assay; and fermentation patterns on glucose, maltose, sucrose, lactose, galactose, trehalose, and cellobiose. Fourteen reference type strains and clinical laboratory reference strains of seven other *Candida* species were also tested (Table 1). Each isolate was coded to blind the reader and streaked onto the surfaces of three CAC plates; one set of plates was incubated at 25°C, one at 30°C, and one at 37°C. The plates were read independently by at least two readers after 24 and 48 h of incubation. An isolate was designated positive if it produced white colonies on CAC at two or three of the temperatures. All isolates that produced white colonies were reidentified by molecular assays, D1 and D2 sequencing (6), or peptide nucleic acid fluorescence in situ hybridization (3) and phenotypically by using the carbohydrate patterns obtained with the API 20C system (BioMerieux, Hazelwood, MO).

Numerous white colonies were noted after 24 h of incubation, but many of these colonies developed into shades of pink and occasionally purple after an additional 24 h of incubation. Therefore, all subsequent results are from data collected after 48 h of incubation. The color distinction was the most pronounced at 37°C and was the least pronounced at 25°C. Similarly, although white colonies were noted at all three temperatures, more white colonies were observed at 37°C than at 25°C and 30°C. In addition, all "positives" (i.e., isolates that grew as white colonies at two of the three temperatures) were white at both 30°C and 37°C.

* Corresponding author. Mailing address: Department of Pathology, The Johns Hopkins Medical Institutions, Meyer B1-193, 600 N. Wolfe St., Baltimore, MD 21112. Phone: (410) 955-5077. Fax: (410) 614-8087. E-mail: wmerz@jhmi.edu.

† Present address: Walter Reed Army Medical Center, 6900 Georgia Ave. NW, Washington, DC 20307.

▽ Published ahead of print on 6 August 2008.

TABLE 1. Type strains and reference strains of *Candida glabrata* and related species

Yeast species	Class culture[a]	Strain designation
C. glabrata	JHH lab control	CGL-C
C. krusei	IVST control	ATCC 6258
C. bracarensis	Type	NRRL Y-48270
C. bracarensis	Reference	NRRL Y-27794
C. nivariensis	Type	NRRL Y-48269
C. (*Pichia*) *norvegensis*	Reference	NRRL YB-3899
C. (*Pichia*) *norvegensis*	Reference	NRRL Y-27195
C. (*Pichia*) *norvegensis*	Reference	NRRL Y-27202
C. (*Pichia*) *norvegensis*	Reference	NRRL Y-12804
C. (*Pichia*) *norvegensis*	Type	NRRL YB-2029
C. (*Pichia*) *norvegensis*	Reference	NRRL YB-3924
C. monosa (*Pichia fermentans*)	Type	NRRL Y-7180
C. lambica (*Pichia fermentans*)	Type	NRRL Y-7181
Pichia fermentans	Type	NRRL Y-1619

[a] JHH lab, The Johns Hopkins Hospital Clinical Mycology Laboratory; IVST, in vitro susceptibility testing.

White colonies were produced by a total of 18 isolates representing five of the seven species tested (Table 2). The five species were *C. glabrata*, *C. bracarensis*, *C. nivariensis*, *Candida norvegensis*, and *Candida inconspicua.* The first three species are members of the *C. glabrata* clade, whereas *C. inconspicua* and *C. norvegensis* share phenotypic traits with *C. glabrata* but, along with *C. krusei* and *C. inconspicua*, are members of the *Pichia membranifaciens*/*P. fermentans* clade (6). The two species studied that did not produce any white colonies were *C. krusei* and the *P. fermentans* group, two species that also share phenotypic traits with members of the *C. glabrata* clade. These data support our hypothesis that members of the *C. glabrata* clade as well as distantly related species with overlapping phenotypic traits would produce white colonies on CAC.

TABLE 2. Production of white colonies on CAC

Type of isolate and yeast species[a]	No. of isolates positive/total no. tested	Species reidentified[b]	CHO assimilated[c]
Recent clinical *C. glabrata* isolates	13/127	*C. glabrata* (8/8)	− − − +
		C. norvegensis (1/0)	−/+ − − −
		C. bracarensis (2/0)	+ − − +
		C. bracarensis (1/0)	− − − +
		C. bracarensis (1/0)	+ − − −
Reference or type strains			
C. bracarensis	2/2	*C. bracarensis* (1/0)	+ − − −
		C. bracarensis (1/0)	+ − − +
C. nivariensis	1/1	*C. nivariensis* (1/0)	+ − − +
C. inconspicua	2/2	*C. inconspicua* (2/0)	−/+ − − −

[a] The species that did not produce white colonies were *C. glabrata* (1 control isolate and 119 recent clinical isolates), *C. krusei* (1 control isolate and 13 recent clinical isolates), *P. fermentans* (3 reference strains), and *C. norvegensis* (4 reference strains).

[b] The data in parentheses indicate the number of isolates identified by molecular methods/number identified by phenotypic methods.

[c] The assimilation results are for the five carbohydrates (CHO) glycerol, xylose, *N*-acetylglucosamine, and trehalose, respectively.

TABLE 3. Clinical significance of 11 of the 13 isolates of *Candida* species that produced white colonies on CAC

Yeast species (no. of isolates)	No. of isolates					
	Colonizations			Infections		
	Urine	Stool	Throat	Urine	Blood	Abscess
C. glabrata (6)[a]	2	1	1	0	1	1
C. bracarensis (4)	0	2	1	0	0	1
C. norvegenesis (1)	0	0	0	1	0	0
Total (11)	2	3	2	1	1	2

[a] For two additional isolates of *C. glabrata*, insufficient clinical information was available to determine whether a positive urine culture represented infection or colonization.

Confirmations of the identifications of the 18 isolates by an additional phenotypic assay (carbohydrate assimilation patterns with the API 20C system) and by sequencing of the large subunits D1 and D2 yielded interesting results (Table 2). The sequencing data identified all 18 isolates to the species level, and the identifications of 13 isolates were reconfirmed. Five isolates whose identifications were not confirmed by sequencing were among the eight recent clinical isolates of *C. glabrata.* Molecularly, four were *C. bracarensis* and one was *C. norvegensis*. The use of standard mycologic phenotyping, including the assimilation patterns, correctly identified only 4 of the 18 white colony-producing isolates; these 4 isolates were the same 4 *C. glabrata* clinical isolates whose identifications were also reconfirmed by sequencing. Unfortunately, the assimilation patterns were not helpful for the identification of the other isolates since *C. bracarensis* and *C. nivariensis* are not in the API database and since this group of species assimilates only a few carbohydrates (0 to 2 carbohydrates, not including glucose), yielding multiple species with the same biocode and low probabilities of correct identifications. This low level of reactivity not only limits the discriminatory patterns for identification but also may contribute to the lack of color production by these species.

The 13 isolates that produced white colonies were recovered from specimens representing five anatomic sites (Table 3). Ten isolates were recovered from three nondeep sites (urine, stool, and throat) and included all three species. For seven of these isolates, a review of the patients' charts did not reveal evidence of infection; therefore, the positive cultures likely represented colonizations. For two of the *C. glabrata* isolates recovered from urine, there was insufficient clinical information to determine whether the positive cultures represented infection or colonization. The remaining four isolates (two *C. glabrata* isolates, one *C. bracarensis* isolate, and one *C. norvegenesis* isolate) were recovered from patients with clinical evidence of infections caused by these organisms. The *C. bracarensis* isolate was recovered operatively from an intra-abdominal abscess in an immunocompetent 67-year-old woman with a history of perforated diverticulitis (3). *C. norvegenesis* was recovered via straight catheterization from the urine of a 43-year-old woman with a history of end-stage renal disease and kidney transplantation who had recurrent urinary tract infections caused by a yeast. One isolate of *C. glabrata* was recovered in a sterile fashion from a peripancreatic fluid collection recovered from a 67-year-old man with end-stage renal disease and chronic pan-

creatitis with infected pseudocysts. Finally, another isolate of *C. glabrata* was recovered from the blood of a 49-year-old man undergoing chemotherapy for relapsed chronic myelogenous leukemia.

In conclusion, we have generated data that supported our hypothesis. Five of seven selected species produced white colonies on CAC. These included *C. glabrata*; two other members of the *C. glabrata* clade, *C. bracarensis* and *C. nivariensis*; and two of four of the species with overlapping phenotypic traits, *C. norvegensis* and *C. inconspicua.* Four of the five species that produced white colonies were not identifiable by the commonly used phenotypic assays. These data may contribute to the use of chromogenic media in the clinical mycology laboratory. Isolates with white colonies might indicate that molecular assays might be required for identification.

We thank the staff of The Johns Hopkins Hospital Clinical Mycology Laboratory for collecting the panel of clinical isolates used in this study.

REFERENCES

1. **Alcoba-Florez, J., S. Mendez-Alvarez, J. Cano, J. Guarro, E. Perez-Roth, and M. del Pilar Arevalo.** 2005. Phenotypic and molecular characterization of *Candida nivariensis* sp. nov., a possible new opportunistic fungus. J. Clin. Microbiol. **43:**4107–4111.
2. **Baumgartner, C., A.-M. Freydiere, and Y. Gille.** 1996. Direct identification and recognition of yeast species from clinical material by using Albicans ID and CHROMagar *Candida* plates. J. Clin. Microbiol. **34:**454–456.
3. **Bishop, J. A., N. Chase, S. S. Magill, C. P. Kurtzman, M. J. Fiandaca, and W. G. Merz.** 2008. *Candida bracarensis* detected among isolates of *Candida glabrata* by peptide nucleic acid fluorescence in situ hybridization: susceptibility data and documentation of presumed infection. J. Clin. Microbiol. **46:**443–446.
4. **Correia, A., P. Sampaio, S. James, and C. Pais.** 2006. *Candida bracarensis*, sp. nov., a novel anamorphic yeast species phenotypically similar to *Candida glabrata*. Int. J. Syst. Evol. Microbiol. **56:**313–317.
5. **Jabra-Rizk, M. A., T. M. Brenner, M. Romagnoli, A. A. M. A. Baqui, W. G. Merz, W. A. Falkler, Jr., and T. F. Meiller.** 2001. Evaluation of reformulated CHROMagar *Candida*. J. Clin. Microbiol. **39:**2015–2016.
6. **Kurtzman, C. P., and C. J. Robnett.** 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek **73:**331–371.
7. **Murray, C. K., M. L. Beckius, J. A. Green, and D. R. Hospenthal.** 2005. Use of chromogenic medium in the isolation of yeasts from clinical specimens. J. Med. Microbiol. **54:**981–985.
8. **Odds, F. C., and R. Bernaerts.** 1994. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. **32:**1923–1929.
9. **Odds, F. C., and A. Davidson.** 2000. "Room temperature" use of CHROMagar Candida. Diagn. Microbiol. Infect. Dis. **38:**147–150.
10. Reference deleted.
11. **Pfaller, M. A., A. Houston, and S. Coffman.** 1996. Application of CHROMagar Candida for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida* (*Torulopsis*) *glabrata*. J. Clin. Microbiol. **34:**58–61.
12. **Powell, H. L., C. A. Sand, and R. P. Rennie.** 1998. Evaluation of CHROMagar Candida for presumptive identification of clinically important *Candida* species. Diagn. Microbiol. Infect. Dis. **32:**201–204.