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Appearance of Colonies of *Prototheca* on CHROMagar Candida medium

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Abstract

The microorganisms capable of producing opportunist infections include the yeast-like organisms of the genus *Candida*, and the unicellular algae of the genus *Prototheca*, which share common features and can, therefore, lead to confusion. Their colonies are almost identical and they grow in the same culture media used routinely in mycology.

CHROMagar Candida is a new chromogenic differential isolation medium that facilitates the presumptive differentiation of some of the most clinically important yeast-like organisms. To our knowledge, the use of CHROMagar Candida with *Prototheca* spp. has not been reported in the literature.

This report describes the growth of 151 strains of *Prototheca* on CHROMagar Candida compared to the growth of a total of 326 well-characterized yeast organisms of the genera *Candida, Cryptococcus, Trichosporon, Geotrichum,* and *Saccharomyces*.

It is clinically relevant to note that algae of the genus *Prototheca (P. wickerhamii, P. zopfii*, and *P. stagnora*) and of the genus *Candida parapsilosis* produced similar cream-colored colonies on CHROMagar Candida medium.

Based on their growth on CHROMagar, a new species of *Candida* is described, *C. zeylanoides*, which has blue-green colonies. The colonies of two species of *Trichosporon* are also differentiated: the blue-green colonies of *T. beigelii* and the pink colonies of *T. capitatum*.

Introduction

Algae of the genus *Prototheca*, first described by Kruger in 1894, have recently become important because of the growing number of opportunist human pathogens that have been identified in the group. The disease entity known as protothecosis occurs sporadically and is characterized by cutaneous and, less frequently, systemic manifestations [1]

Prototheca species have been isolated from tree sap, potato peels, sea water, lakes, marshes, streams and pond mud, as well as from rivers and waste waters [2]. *Prototheca* have also been isolated from several animal species, in both healthy and diseased specimens, including dogs, cats, deer, salmon, and cow, and from animal products such as excrement and milk from cows with mastitis [3–5]. In healthy humans, *Prototheca* algae have sometimes been found under the fingernails, in feces, sputum, skin and blood. They have also been isolated from specific skin lesions and bursitis [6].

Until now, the only species associated with human disease have been *Prototheca wickerhamii* and *Prototheca zopfii*, not *Prototheca stagnora*. However, laboratory differentiation of these species may be difficult. Separation of species is usually based on macroscopic and microscopic study, temperature of growth, and sugar and alcohol assimilation patterns [7]. Immunofluorescence may be used to confirm species identification [8].

A new chromogenic medium, CHROMagar Candida (CHROMagar Company, Paris, France) has been proposed for the simultaneous identification of *Candida albicans* and *Candida tropicalis*, which produce distinctive colony colors in this medium [9]. CHROMagar Candida also serves to differentiate other species on the basis of colony color. We undertook this study to evaluate the performance of CHROMagar Candida in the genus *Prototheca*.

Material and methods

The isolates tested included a total of 326 yeast organisms and 151 *Prototheca* species strains isolated from various sources: 89 *P. wickerhamii*, 49 *P. zopfii* and 13 *P. stagnora*. The cultures came from the collections of L. Ajello, Centers for Disease Control, Atlanta, GA; E.H. Ball, University of Glasgow, Scotland; M. Feo, Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Venezuela; P. Hocquet, Service des Maladies Parasitaires et Exotiques, Centre Hospitalier Regional, France; H. Koeing, Institut de Parasitologie et Patologie Tropical, Université L. Pasteur, Strasbourg, France; C.P. Kurtzman, Agricultural Research Service Culture Collection, Peoria, IL; R.S. Pore, Department of Microbiology, West Virginia University, Morgantown, WV; and our own laboratory [2, 6].

Three hundred and twenty-six well-characterized yeast strains were tested: *C. albicans* (115 isolates), *Candida parapsilosis* (68 isolates), *Candida glabrata* (16 isolates), *C. tropicalis* (15 isolates), *Candida guilliermondii* (14 isolates), *Candida krusei* (11 isolates), *Candida famata* (8 isolates), *Candida humicola* (6 isolates), *Candida zeylanoides* (2 isolates), *Cryptococcus neoformans* (12 isolates), *Trichosporon beigelii* (8 isolates), *Trichosporon capitatum* (4 isolates), *Geotrichum candidum* (5 isolates), *Saccharomyces cerevisiae* (2 isolates). Of these, 285 strains recently isolated from clinical specimens in our diagnostic laboratory were also tested. The remaining yeasts were selected from our laboratory's stock collection. The following characterized strains were used as controls for the experiments: *C. albicans* ATCC 64550, *C. albicans* ATCC 64548 and *C. glabrata* ATCC 2238.

All strains were subcultured on Sabouraud glucose agar, then streaked onto CHROMagar Candida plates. The plates were incubated at 30 °C and examined after 48 h for colony growth and morphology.

The identification of *Prototheca* spp. isolates was confirmed by macroscopic and microscopic examination, determination of their ability to use sucrose, trehalose, lactose, inositol, *n*-propanol and xylose as carbon sources for growth, API 20C, and Antimicrobial Susceptibility Disk (ATMSD) [10–12, 1, 7]. Species identification was confirmed by W. Kaplan, Division of Mycotic Disease, Centers for Disease Control, using immunofluorescence [8].

Yeast strains were identified using conventional methods, including tests for carbohydrate fermentation and assimilation, cycloheximide resistance, nitrate assimilation, growth at 37 °C, and urease production of Christensen urea agar slant.

The 153 fresh *C. albicans* isolates were identified by germ tube induction in foal serum at 37 °C for 2–4 h, chlamydospore formation on diluted milk medium [13], and, if necessary, assimilation pattern with API 20C (BioMerieux).

CHROMagar Candida medium [9] is designed for direct identification on the primary plate by means of colony color for *C. albicans* (green) and *C. tropicalis* (blue). Other species may be distinguishable on CHROMagar medium by means of colony color and other morphologic characteristics.

Results

All of the yeast isolates and *Prototheca* species tested grew on CHROMgar Candida (Table 1).

Every /*C. albicans* strain produced green colonies (sensitivity 100%); none of the other yeast colonies produced green colonies (specificity 100%). *C. tropicalis* produced characteristic blue colonies with a pink halo. *C. zeylanoides* gave blue-green colonies that were intermediate in appearance between *C. albicans* and *C. tropicalis*.

The *T. beigelii* strains gave blue-green colonies that differed from *C. albicans*, *C. tropicalis* and *C. zeylanoides* colonies in their rough, crenated appearance. *T. capitatum* gave pink colonies.

Forty-eight yeast strains belonging to 6 different species produced pink colonies; the 11 *C. krusei* were distinguished by their downy appearance. Some 68 isolates of *C. parapsilosis* produced cream-colored colonies. All the *Prototheca* species (n = 151) developed cream-colored colonies similar to those of *C. parapsilosis*.

Discussion

Protothecosis has attracted increasing interest the world over. To date, more than 50 cases have been reported from a variety of regions and countries, including Sierra Leone, Transvaal, the United States, China, Vietnam, Hong Kong, Iran, New Zealand, France, Germany, Thailand, Japan, Taiwan, Panama, Canada, Brazil, Australia, Scotland, and Spain [2, 6].

Prototheca spp. is a rare but well-defined cause of human infection. However, we now know that pathological cases caused by algae of the genus *Prototheca*

Table 1. Identity and colony color of the 477 tested strains grown for 48 h at 30 °C on CHROMagar Candida.

Strains	No.	Colony color after 48 h of incubation at 30 °C						
		Green	Blue	Blue/green	Pink	Cream	Purple	Gray/pink
Candidia albicans	155	155						
Candida parapsilosis	68					68		
Candida glabrata	16						16	
Candida tropicalis	15		15					
Candida guilliermondii	14				14			
Candida krusei	11				11			
Candida famata	8				8			
Candida humicola	6				6			
Candida zeylanoides	2			2				
Cryptococcus neoformans	12							12
Trichosporon beigelii	8			8				
Trichosporon capitatum	4				4			
Geotrichum candidum	5				5			
Saccharmyces cerevisiae	2						2	
Prototheca wickerhamii	89					89		
Prototheca zopfii	49					49		
Protetheca stagnora	13					13		
Total	477	155	15	10	48	219	18	12

occur in immunocompromised hosts. Most commonly the infections are cutaneous [14]; however, disseminated infections have been reported [15–17]. Because *Prototheca* infection is uncommon, it can be overlooked or wrongly diagnosed as a yeast organism if a precise laboratory diagnosis is not made; such a diagnosis is sometimes time-consuming and not always possible in hospital laboratories [18].

The differential diagnosis between protothecosis and candidiasis is important, not only scientifically, because of the knowledge of new cases that it gives us, but also from a practical point of view because the therapies for these illnesses may differ [16].

The most important finding of this paper is that the macroscopic morphology of colonies of *C. parapsilosis* and *Prototheca* spp. growing on CHROMagar Candida medium was similar in color (cream) and texture after 48–72 h of growth, although *Prototheca* colonies were a little smaller than *C. parapsilosis*. There are many species of *Candida* that are indistinguishable from *C. parapsilosis* (and presumably *Prototheca*) on CHROMagar after 24–48 h of growth. In many instances it is difficult to distinguish the pink colonies from the cream and/or gray/pink colonies.

Given the increasing incidence of *C. parapsilosis* in clinical processes and the possible occurrence of *Prototheca* in clinical samples, it is important to report this similarity in their growth on the new medium, CHROMagar Candida. CHROMagar Candida cannot be used to differentiate among the *Prototheca* species *P. wickerhamii, P. zopfii* and *P. stagnora*, which all produce similar colonies.

Growth on CHROMagar Candida medium has been reported for organisms such as *C. glabrata, C. guilliermondii, C. krusei, Candida famata, Candida humicola, Saccharomyces*, and *Geotrichum* [13]. However, until now the growth of *C. zeylanoides* on CHROMagar Candida medium has not been reported. The color of *C. zeylanoides* colonies is intermediate between *C. albicans* and *C. tropicalis* and similar to *T. beigelii*, which can be distinguished by colony morphology.

Another important finding of this paper was the differentiation between species of *T. beigelii*, which had blue-green colonies, and *T. capitatum*, with pink colonies. In this respect, our study differs from that of Odds [13], since he makes no reference to correct differentiation of these *Trichosporon* species using the same medium.

Nonetheless, the use of the new culture medium CHROMagar Candida permitted a precise definition of associations of some species of *Candida* (*C. albicans, C. tropicalis, C. krusei* and *C. glabrata*) and *Prototheca* found in clinical samples. Moreover, *C. albicans* and *C. tropicalis* could be identified directly after culture. Differentiation of these species is based on specific colony colors produced by the reaction of species-specific enzymes with chromogenic substrates.

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