JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1979, p. 551-553
0095-1137/79/04-0551/03$02.00/0 Vol. 9, No. 4

New Medium for Differentiation of *Candida albicans* from *Candida stellatoidea*

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Received for publication 6 February 1979

A simple medium effectively differentiated *Candida stellatoidea* from *Candida albicans* on the basis of a new criterion, relative sensitivity to cycloheximide.

Candida stellatoidea is morphologically and physiologically similar to *Candida albicans* (9). Although the etiological significance of the latter has been well established, questions persist as to the frequency and extent of association of *C. stellatoidea* with human infections. Although Jones and Martin (3) noted in their original description that it "may be a harmless parasite in the human vagina," subsequent studies have demonstrated an association with human vaginitis (5, 8), as well as occasional involvement in systemic infection (2, 4, 6, 7).

To properly assess the clinical significance of *C. stellatoidea* isolates and the specific types of candidiasis with which they may be involved, this species should be routinely identified and differentiated from *C. albicans*. To differentiate them, most clinical laboratories use only one method, sucrose assimilation. However, the identification of any fungus—and especially one such as *C. stellatoidea*, which is so similar to another member of the genus—should be based upon more than a single character. To provide an additional criterion for distinguishing between these two species, we describe a new diagnostic medium. (Some of these results were previously presented; I. F. Salkin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, F47, p. 320.)

Species of *Candida* differ in their sensitivity to cycloheximide (1), but most investigations have considered only a single concentration of the drug: 0.5 mg/ml or 1.8 mM, the amount used in Difco's Mycobiotic agar or BBL's Mycosel. Few studies have evaluated the response of *C. stellatoidea* to this antibiotic.

In preliminary screening experiments we incorporated from 0.5 (1.8 mM) to 5.0 mg/ml (18.0 mM) mg of cycloheximide per ml into 50 ml of modified Sabouraud dextrose broth (2% dextrose, 1% neopeptone) in 125-ml flasks. The medium was inoculated with 1 ml of a saline suspension containing 10^7 yeast cells per ml, prepared by washing the surface of a 48-h-old culture tube. The flasks were then incubated on a gyratory shaker (170 rpm) at 27°C, and cells were harvested daily to obtain dry-weight estimates of growth.

The dry weight of each species was constant at concentrations of cycloheximide ranging from 1.0 to 5.0 mg/ml. Four days after inoculation, the growth of *C. albicans* was strikingly less inhibited (35 to 60% of the control) than that of *C. stellatoidea* (5 to 10% of the control). For this reason we decided to incorporate 1 mg of cycloheximide per ml (double the concentration used in commercial media) into the experimental diagnostic medium.

This difference in sensitivity could be exploited as a means of differentiating the two species. We considered a solid medium easier to handle in a clinical setting, so modified Sabouraud dextrose agar (2% agar) was chosen as the nutrient base.

Since most yeasts acidify a medium as they grow, we reasoned that the difference in sensitivity could be demonstrated by incorporating a pH indicator along with 1.0 mg of cycloheximide per ml into modified Sabouraud dextrose agar. The greater growth of *C. albicans* in the presence of the antibiotic should produce a more significant change in pH. Since the medium would have to remain unbuffered, the indicator would have to be within its effective range even after the drop in pH that normally accompanies the autoclaving of such a medium. Bromocresol green (28 ml of an 0.08% solution per liter of medium) was found to meet our requirements.

Since a Wickerham (10) 1+ suspension is the inoculum in commercially available yeast identification kits, a similar suspension was prepared in 5 ml of sterile saline or filtered water (Millipore membrane filter) to inoculate the experimental medium. Two drops of the suspension were added to each plate with a sterile Pasteur pipette and streaked over the surface of the medium with a wire loop. The plates were then incubated in the dark at 27°C and observed daily for color change.

Consistent results were obtained with 28 strains of *C. albicans* and 18 strains of *C. stel-*

latoidea (Table 1), one of the most extensive studies of the latter species in the literature. At 48 h of incubation the growth of *C. albicans* induced an almost complete change from blue to yellow (Fig. 1). In contrast, the plates inoculated with *C. stellatoidea* showed at this time only a partial, although readily distinguishable, color conversion.

Similar studies with five other *Candida* species (Table 1) revealed that the medium is effective principally in differentiating *C. stellatoidea* from *C. albicans*. *C. pseudotropicalis* was unique: it induced complete conversion within 12 to 24 h of inoculation. The change by *C. guilliermondii*, however, was identical to that of *C. albicans*. Some strains of *C. tropicalis* produced a color change similar to that observed with *C. stellatoidea*; other strains produced no such change as late as 7 days after inoculation. However, morphological and physiological factors, i.e., hyphae and blastospore morphology, chlamydospore development, germ tube formation, and sucrose assimilation, would preclude confusing *C. guilliermondii* with *C. albicans* or *C. tropicalis* with *C. stellatoidea*. As expected (1), *C. krusei* and *C. parapsilosis* did not grow on the experimental medium.

On the basis of color change in the medium, one could differentiate between *C. albicans* and

TABLE 1. *Candida species used in evaluation of experimental medium*[a]

Species	No. of strains	Strain no.[b]
C. albicans	28	M73, M73A, M73B, M74, M75, M76, M76A, M77,. M78, M79, M80, M81, M82, M82A, M83, M84, M84A, M84B, M85, M85A, M85B, M86, M87, M88, M89, M89A, M90, M90A
C. stellatoidea	18	M114, M114B, M114C, M115, M117, M118, M119, M121, M124, B395, B459, B460, B1017, B1018, B2213, CS-1, CS-2, MS-1899.33
C. guilliermondii	5	M91, M92, M93, M94, M95
C. tropicalis	8	M125, M126, M127, M128, M131, M132, M133, M137
C. pseudotropicalis	3	M110, M112, M113
C. krusei	5	M96, M97, M98, M98A, M99
C. parapsilosis	5	M106, M107, M109, M109A, M109B

[a] The identity of all strains was established by standard morphological and physiological procedures prior to their use in the study.

[b] Culture numbers. The sources were: M, Laboratories for Mycology and Mycobacteriology, Division of Laboratories and Research, New York State Department of Health, Albany, N.Y.; B, Rocky Mountain Laboratory, National Institutes of Health, Hamilton, Mont.; CS, Department of Biology, Georgia State University, Atlanta, Ga.; MS, Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York, N.Y.

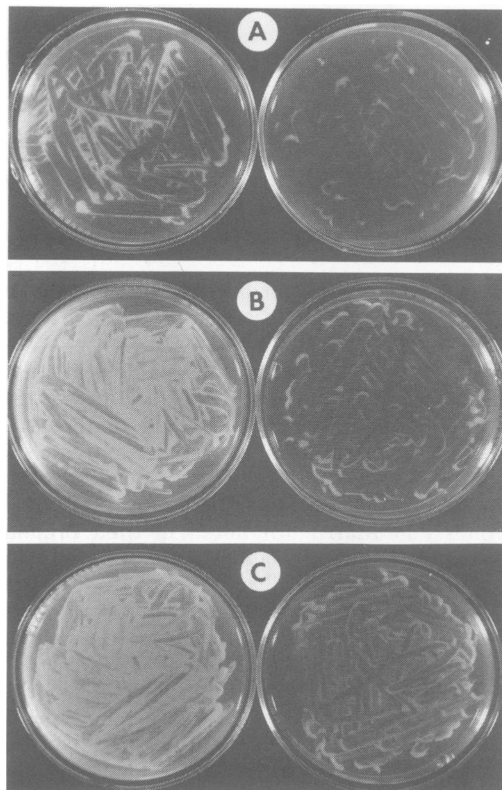

FIG. 1. *Growth of C. albicans (left) and C. stellatoidea (right) on the new diagnostic medium. The yeasts induce a change in the color of the medium from blue (dark) to yellow (light). (A) 24 h, (B) 48 h, (C) 72 h after inoculation.*

C. stellatoidea 48 h after inoculation. The medium is simple to prepare, as all components (2% dextrose, 1% neopeptone, 2% agar, 0.1% cycloheximide, and 28 ml of an 0.08% solution of bromocresol green per liter, adjusted to pH 6.5 before autoclaving) can be autoclaved together. Further, since a 1+ inoculum suspension is used, the medium can be an adjunct to the commercially available yeast identification kits.

To identify and differentiate *C. albicans* and *C. stellatoidea* isolates from clinical specimens, we suggest the following tentative protocol: (i) Streak yeasts to test purity on standard antimicrobial media. (ii) Assess hyphae and blastospore morphology, as well as chlamydospore development, on corn meal plus Tween 80 (Fisher Scientific) or similar medium. (iii) Concomitantly determine germ tube formation in sterile normal saline or an equivalent commercial product. (iv) If the isolate is germ tube positive and the morphology of hyphae and blastospores is typical of *C. albicans*–*C. stella-*

toidea, regardless of whether chlamydospores are formed promptly and abundantly, assess sucrose assimilation. (v) Determine growth and pH and color change on the new diagnostic medium described in this report.

Further studies with additional isolates may be desirable to assess the value of these procedures.

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