A SIMPLE MEDIUM FOR ISOLATION AND IDENTIFICATION OF CANDIDA ALBICANS DIRECTLY FROM CLINICAL SPECIMENS*

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Abstract

A simple and specific medium consisting of chitosan, trypticase, Tween-80 and agar is devised to isolate the organisms directly from the clinical specimens and to produce germ tubes and chlamydospores for rapid differentiation and identification of *Candida albicans* from other closely related *Candida* species. By manipulating the incubating conditions, the specific phase of the organism can be produced in liquid or on solid medium at different time intervals to study the physiology of the organism.

Many methods and media have been proposed in the past for identification of *Candida albicans* and to differentiate this from the closely related species of *Candida* (5-8, 15). Taschdjian, Burchall & Kozinn (15) showed that *C. albicans* produces germ tube within an hour or two when it is grown in human or animal serum or serum substitutes. The specificity of this germ tube test was later confirmed by various workers by using different media (3-5). The distinctive feature that differentiates *C. albicans* from other species is the production of chlamydospores (14). However, in all these studies three types of media were required to isolate the organisms from clinical specimens and to produce germ tubes and chlamydospores for identification. Recently studies have shown that a single medium can be employed to produce both structural components of the organism from the primary isolation medium but the preparation of the medium is more exhaustive (1) and time consuming (13) than the medium to be described here. The present investigation was therefore undertaken to develop a simple and specific medium to isolate the organism directly from the clinical specimens and to produce various morphological phases of *Candida albicans* to differentiate from other closely related *Candida* species for clinical diagnosis and to provide a medium to study the physiology and metabolism of the organism under *in vitro* conditions.

Materials and methods

Organism and cultural conditions

One hundred fifty clinical isolates of different *Candida* species along with two strains of *Candida albicans* (# 10231 and # 10261) from American Type Culture Collection (ATCC) Rockville, Maryland were used for the study. In order to evaluate the specificity of the medium in demonstrating diagnostic morphological characteristics, other *Candida* species, *C. guilliermondii* (ATCC # 6260), *C. krusei* (ATCC # 6258), *C. parapsilosis* (ATCC # 14054), *C. pseudotropicalis* (ATCC # 4135) and *C. tropicalis* (ATCC # 750) were also used. Stock cultures were maintained on Sabouraud's dextrose agar at room temperature and transferred to fresh medium at weekly intervals. All the cultures were tested and confirmed by sugar fermentation (10).

Inoculum was prepared by adding a loopful of stock culture to 5 ml of sterile distilled water. A known concentration of inoculum (10^7/ml) was used to inoculate the plate by the Dalmau technique (16). A cover slip was placed over the slit at the inoculation site and the plates were incubated under different conditions. The cultures were examined under the microscope at various time intervals to determine the different morphological phases of the organism.

Basal medium and modifications

Chitosan (N-deacetylated glucosaminoglycan) was obtained from Calbiochem, California. A stock solution (2 mg/ml) of chitosan was prepared by dissolving 200 mg in about 90 ml of 1N hydrochloric acid after adjusting the pH to 6.5, the final volume was brought to 100 ml with distilled water. * Supported in part by Grant CA 20917, National Cancer Institute, National Institutes of Health and ALSAC.
Stock solution was stored in the refrigerator until ready to use. The basal medium consists of chitosan 30 mg; trypti-case 5 g; Tween-80, 10 ml; agar 15 g (CTTA) and distilled water 1000 ml.

The effect of various amino sugars or their derivatives on germ tube and chlamydospore formation was compared with chitosan by substituting these for chitosan in CTTA medium. To study the influence of temperature, cultures with the CTTA medium were incubated at 37 °C for 4 hours and later transferred to room temperature and vice versa. In order to study the effect of surfactants and peptones on both structural formations, they were added to the basal medium individually and observations were made at various time intervals.

**Clinical evaluation of the medium**

The specificity of CTTA medium on both structural components was determined with one hundred fifty clinical isolates of different Candida species of various origin obtained from the clinical laboratory. The isolates that produced germ tubes and chlamydospores were inoculated on rice extract agar or corn meal agar medium for comparison. The other isolates were identified on the basis of sugar fermentation tests and other morphological structures (10). To prevent the bacterial growth and to isolate C. albicans from clinical specimens (throat, skin rectal etc.) on CTTA medium, 0.5 mg of acti-dione and 0.05 mg chloramphenicol/liter were added. One hundred eightyfive clinical specimens were directly inoculated on antibiotic amended plates and incubated for 4 hours at 37 °C. At the end of the incubation period, the entire plate, including the area under cover slip, was scanned under the microscope to observe the germ tube formation. After the observation, plates were re-incubated at room temperature and observed again under the microscope, at different time intervals for chlamydospore formation. Same clinical specimens were also cultured independently in clinical laboratory by conventional method which includes the primary isolation medium (Sabouraud's dextrose agar with antibiotics) and identification medium (Corn meal agar) for comparison.

**Results**

Most of the amino sugars and their derivatives except mucin, used in this study supported the chlamydospore formation of C. albicans (Table 1). Germ tubes normally observed at 37 °C within 4 hours after inoculation. Chitosan was found to be the best source followed by chitin and N-acetyl glucosamine for yeast, germ tube, pseudohyphae, blastospore and chlamydospore formation at various time intervals under different incubating conditions. Among the peptones, trypcase induced the germ tubes and chlamydospores (Table 2) followed by polypeptone and bactopeptone. Only yeast cells were observed in cultures that are incubated at room temperature and later re-incubated at 37 °C. Cultures that are incubated at 37 °C produced germ tubes after 3 hours, later changed into pseudohyphae and blastospores. On the other hand, the cultures that are incubated at 37 °C and later moved to room temperature produced large number of germ tubes and chlamydospores on CTTA medium in 3 and 24 hours respectively.

Of the five surfactants tested on chlamydospore formation, Tween-80 was found to be the best (Table 3) followed by Triton X-100. Others had very little or no effect on chlamydospore formation. Optimum concentration for components of the medium for both germ tube and chlamydospore formation was determined (Table 4) and the results indicate that CTTA medium described above was found to be optimum. Increase in concentration of the components in the medium up to twice (2X) that of the standard (1X) medium, decreased the germ tube and chlamydospore formation. Organisms were cultured repeatedly on CTTA medium to test the role of endogenous metabolism on the formation of both structural components. There was no significant difference in number of germ tubes and chlamydospores between the subcultures. The study on the specificity of CTTA medium on growth of different Candida species (ATCC) and clinical isolates at various time intervals indicated that chlamydospores were formed only with C. albicans (Table 5) except in one isolate of C. stellatoidea (Table 6) even after 72 hours of inoculation. Since CTTA medium does not differentiate C. albicans from the chlamydospore forming C. stellatoidea, this could be achieved simply either by sucrose assimilation test (10) or by cultural method based on profuse filamentation of C. stellatoidea on corn meal agar at 37 °C (9).

Among the organisms isolated from the clinical specimens on CTTA medium (Table 7) the majority of them were identified as C. albicans based on the germ tube formation after 4 hours at 37 °C (Fig. 1) and chlamydospore formation at room temperature (Fig. 2) after 24-48 hours of inoculation. Though there is no significant difference in isolation of the organisms between CTTA and conventional method, the identification of C. albicans can be achieved with CTTA much earlier than the conventional method in addition to its simplicity. Although we found extensive pseudohyphae in a few cases, we did not make any