Modified Medium Containing Phosphomolybdic Acid Useful in the Identification of Specific Yeast-Like Fungi

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A medium containing phosphomolybdic acid is described which permits growth and differentiation between closely related yeast-like fungi. When properly formulated and packaged, the vials of medium can be stored at room temperature for 6 months or more without loss of stability or effectiveness.

CANDIDA ALBICANS is the most frequent isolate from mycotic vaginitis infections and is considered by many clinicians to be the only pathogenic *Candida* species. However, studies by Haley (1), Kearns and Gray (2), Jillson and Lyle (3), Hurley and Morris (4), and De Sousa and Van Uden (5) have shown that yeast species other than *C. albicans* can initiate or complicate this infection. Taubert and Smith (6) have reported a significant number of cases where a clinical diagnosis of vaginal candidiasis was made and from which *C. albicans* was not cultured.

Various laboratory media have been developed to aid in the identification of *C. albicans*. However, these media are less precise in their ability to distinguish between *Candida* species other than *C. albicans*. Biological methods described by Lodder and Kreger-Van Rij (7), Wickerham (8, 9), and Martin *et al.* (10) are definitive but time consuming. It would appear that a selective medium able to rapidly differentiate between closely related yeast-like fungi would be of value in the diagnosis of mycotic vaginitis.

The use of a medium containing phosphomolybdic acid for *C. albicans* differentiation was first described by MacLaren and Armen (11) in 1958. The cultures were identified by their macrocolony pigmentation and an extracellular reaction seen in the medium.

This original medium had deficiencies which were reported by Holland and Kunz (12), who found a variation in the colony pigmentation obtained within *C. albicans* strains. In unpublished studies in this laboratory it was found that the medium often showed closely related *Candida* species with similar colony pigmentation, a result which hindered positive identification.

The modification described in this report results in a formulation that significantly improves the differentiation of yeast-like fungi encountered in cases of mycotic vaginitis. The distinct colony pigmentation or growth characteristics obtained with the various species, on this medium, makes possible a relatively rapid identification.

PROCEDURE

Essentially the medium consists of two portions: the basal medium and the stock solutions. These are prepared and sterilized before use.

Materials.—Agar (Difco), sucrose (Difco), proteose peptone (Difco), phosphomolybdic acid (20 $MoO_3 \cdot 2\ H_3PO_4 \cdot 48\ H_2O$, Merck), polysorbate 21 (polyoxyethylene sorbitan monolaurate)[1] yeast nitrogen base (Difco), and neomycin sulfate.

Basal Medium.—Distilled water is added to 1.5 Gm. of agar to give a total volume of 75 ml. in a 300-ml. flask. This size flask allows the medium to be properly agitated during formulation. The agar is dissolved by heating in water then auto-

TABLE I.—STOCK SOLUTIONS

Ingredient	Concn. w/v	Amt./75 ml. Basal Medium
Sucrose	40%	10 ml.
Proteose peptone	10%	10 ml.
Phosphomolybdic acid	1.66%	5 ml.
Polyoxyethylene sorbitan monolaurate	11.4%	1 ml.
Yeast nitrogen base	6.7%	3 ml.
Neomycin sulfate	7 mg. neomycin base/ml.	3.8 ml.
	Total vol.	107.8 ml.

TABLE II.—COLONY COLOR AND GROWTH CHARACTERISTICS

Organism	Results
C. albicans	A distinctive olive colony—the reverse of the colony is also olive.
C. tropicalis	A deep blue or blue-green colony—the reverse is also blue. Blue pigment is usually present in the medium.
C. guilliermondi	A brown or dark gray colony with a definite white border.
C. krusei	A small dull white colony.
Torulopsis glabrata	A small, flat, white shiny colony that may be hard to distinguish from *C. krusei*.
C. albicans var. stell.	A small light tan colony.
C. parakrusei	A dull brown slow growing colony, larger and more irregular than the smooth *C. albicans* colony.
Saccharomyces cerevisiae	A bright blue colony, lighter in color and larger than *C. tropicalis*.
Cryptococcus neoformans	A white raised moist colony, larger than *T. glabrata*. Later may show mucoid growth with a tan cast.
Rhodotorula sp.	A pink to pinkish - brown yeast-like colony.

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[1] Marketed as Tween 21 by the Atlas Powder Co., Wilmington, Del.

TABLE III.—MOST FREQUENTLY ISOLATED SPECIES FROM MYCOTIC VAGINITIS INFECTIONS, RESULTS OF CULTURE ON TEST MEDIUM

Organism	Strains Tested, Total No.	Typical Reactions, No.	Atypical Reactions, No.	Explanation
C. albicans	171	162	9	2 = Olive green colony 3 = Light tan colony 1 = Poor growth 3 = No growth
T. glabrata	17	16	1	= No growth
C. tropicalis	8	8		
C. krusei	7	5	2	= No growth
C. guilliermondi	5	3	2	= No growth
C. parakrusei	2	1	1	= Olive white colony
Totals	210	195	15	

claved at 120° for 15 min. The flask is cooled to 50° in a water bath; if necessary the volume is readjusted to 75 ml. with sterile distilled water. The requisite amounts of stock solution are then added.

Stock Solutions.—All solutions are prepared using distilled water and are sterilized by Millipore filtration using type HA filter pads.

The stock solutions must be added to the basal medium in the order shown (Table I). After the addition of the phosphomolybdic acid, the flask is shaken vigorously to insure homogeneity of the heavy precipitate that results. The addition of polyoxyethylene sorbitan monolaurate reduces foam formation and permits the medium to be tubed satisfactorily. It is not necessary to adjust the pH of the basal medium before sterilizing. The pH of the complete medium is about 5.3.

Packaging.—Sufficient medium is slanted in clear glass vials to permit visual examination of colony growth. A screw cap with a moisture proof liner is necessary for storage. The vials can then be stored at room temperature for periods of 6 months or more without loss of sensitivity.

Inoculation.—The medium can be inoculated with a sterile cotton swab or a sterile loop. Better results are obtained with a light inoculum. Mixed yeast infections are more readily detected when discrete colonies develop. Often the presence of excess body exudate may hinder colony growth. If necessary, streak plates on Sabouraud's dextrose agar can be made, then discrete colonies can be transferred to the medium for growth and identification.

RESULTS AND DISCUSSION

Table II shows the colony pigmentation and growth characteristics for those yeast or yeast-like fungi most frequently isolated from mycotic vaginitis infections. These species samples were individually identified by the biological methods mentioned previously (7–10). In addition, some species are listed that appear infrequently in vaginitis infections: *Saccharomyces cerevisiae, Cryptococcus Neoformans, and Rhodotorula species* (2, 5, 13). For purposes of comparison, duplicate cultures representing the various species were obtained from the American Type Culture Collection.

Table III lists the clinical isolates, the number of each species studied, and the results obtained on the test medium. The majority of cultures showed characteristics similar to those described in Table II for the particular species. Of the 15 cultures considered atypical, eight failed to grow on the test medium while showing good growth on Sabouraud's medium.

In a separate experiment, a number of strains were inoculated on freshly prepared media, and media stored at room temperature for 6 months. There was no difference between the two lots of media.

Although the medium described in this report is concerned with the differentiation of fungi associated with mycotic vaginitis, it has also been tested for possible use in the isolation and identification of dermatophytic organisms. With the exception of *Trichophyton rubrum*, most dermatophytes grow rapidly on the medium, but there is no color differential as seen with the yeast-like fungi.

The concentration of phosphomolybdic acid is critical for the production of specific pigments among the closely related *Candida* species. Neomycin sulfate is not necessary for the development of the differential characteristics but does inhibit the utilization of phosphomolybdic acid by bacterial contaminants often found in clinical samples. Those bacteria that do succeed in growing usually show a low, moist blue, confluent streak of growth easily distinguished from that of *C. tropicalis*. To date, no bacterial species that grows similar to *C. albicans* on the medium has been encountered in the clinical samples from cases of mycotic vaginitis.

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