Dermatophyte Test Medium: Evaluation with Nondermatophytic Pathogens

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The growth and color change produced on dermatophyte test medium (DTM) by 25 strains of zoopathogenic fungi associated with human skin lesions were evaluated quantitatively. DTM only partially suppressed the development of nondermatophytes, with total growth in most instances comparable with that observed with dermatophytes. Whereas all dermatophytes induced a rapid color change on DTM, several nondermatophytic pathogens induced an equally intense and almost as rapid conversion. The rapidity of this color change and the colonial morphology of these forms on DTM could cause their misidentification as dermatophytes under clinical conditions.

The antimycotic agent cycloheximide has been widely employed in the selective isolation of zoopathogenic fungi. Georg et al. (4, 5), Fuentes et al. (3), and Shapiro et al. (13) found that the addition of cycloheximide to Sabouraud dextrose agar (SDA), either as the sole antibiotic agent or in conjunction with streptomycin and penicillin, greatly facilitated the isolation of zoopathogens by suppressing the development of contaminating saprophytes or bacteria, or both. Rosenthal and Furnari (10) and McDonough et al. (6, 7) reported that SDA fortified with cycloheximide and the broad-spectrum antibacterial agent chloramphenicol was important in the isolation of dermatophytes and dimorphic zoopathogens. The latter formulation has been adopted by many clinical laboratories as the medium of choice in routine diagnostic mycology and is commercially produced under the trade names Mycosel agar (BBL) and Mycobiotic agar (Difco).

Recently, Taplin et al. (15) described a new cycloheximide-containing medium, dermatophyte test medium (DTM), for the isolation of dermatophytes. In addition to the selectivity provided by cycloheximide, chlortetracycline and gentamicin were incorporated to inhibit the growth of bacteria. A pH indicator (phenol red), which converts from straw-yellow to vivid red under the alkaline conditions associated with the growth of dermatophytes, was used to provide a differentiating quality to the medium. These authors found that, of 1,400 cultures obtained from private and clinic patients, 97% could be properly evaluated for the presence of dermatophytes on the basis of color change alone. Since this initial report, several clinical evaluations (1, 2, 8, 11, 14) have provided further information about the effectiveness of DTM in the isolation of dermatophytes. However, few reports have dealt with the development on DTM of subcutaneous or systemic zoopathogens which might be confused with dermatophytic forms. The use of DTM by relatively untrained technicians, together with its increasing acceptance as a definitive diagnostic test by many laboratories, necessitated a comparison of the growth and color change produced on DTM by dermatophytes and by nondermatophytic pathogens which may be associated with human skin lesions.

MATERIALS AND METHODS

Test organisms. Axenic cultures of Trichophyton rubrum (strains 379, 387, 392, and 689), Trichophyton mentagrophytes (strains 360-A, 361, and 369), Sporothrix schenckii (strains 335 and 338), Allescheria boydii (strains 1, 17-A, 20-A, and 21), Phialophora jeanselmei (strains 302-E and 303-A), Cephalosporium falciforme (strains 139 and 139-B), Phialophora verrucosa (strain 303), Fonsecaea pedrosoi (strain 194-A), Histoplasma capsulatum (strains 209-C, 211, 212, and 219-A), and Blastomyces dermatitidis (strains 54 and 56), obtained from the collections of the Laboratories for Mycology in our institution, were maintained at 27 C on SDA (2% dextrose, 1% Neopeptone, and 2% agar).

Media. DTM used in this study was prepared in our laboratory by the protocol outlined by Rebell and Taplin (9). Ingredients were obtained from the commercial sources listed by these authors. The develop-
ment of the test organisms on DTM was compared with that on SDA plus cycloheximide (SDA+; 0.5 mg of antibiotic per ml of medium, as in DTM). In the preparation of SDA+, the antibiotic was dissolved in a 0.067 M solution of KH₂PO₄, sterilized by passage through a Nalgene filter unit (0.45 μm), and then added to molten, sterile SDA. The DTM and SDA+ media were dispensed by means of sterile Salvarsan tubes in 30-ml portions into glass petri dishes (100 mm). Two plates of each medium were prepared for each strain. Two plates of SDA without antibiotics served as controls for each strain. Experiments were run three times for a total of six determinations at each combination of strain and medium.

Inoculation. By using procedures described previously (12), plugs (4 mm) were removed with a stainless steel cork borer from the edge of 2-week-old stock cultures of each strain and placed in precut 4-mm wells in the centers of the experimental plates. All test cultures were incubated at 27°C.

Colonial growth. At 2-day intervals, the diameter of each mycelial mat was measured under an 8-power dissecting microscope along two mutually perpendicular axes marked on the bottom of each plate. The average diameter for each strain was computed from the six observations made on each medium. Deviation from the average was found to vary with the strain and medium, ranging from 2.5 to 3.2 mm. For purposes of comparison, all experiments were terminated 17 days after inoculation.

RESULTS

Whereas the development of dermatophytes caused the rapid conversion of DTM from straw-yellow to deep red, an equally intense and almost as rapid change was associated with the growth of several nondermatophytic pathogens.

A red ring surrounded the inoculation wells of all dermatophyte DTM cultures within 24 h of inoculation. The extent of this color conversion exceeded the colonial diameter; whereas the colonies had attained a total growth of about 40 to 70 mm (Table 1) by the completion of the experiment, the entire medium, 100 mm in diameter, had converted to vivid red. A color change was also evident in cultures of all strains of *H. capsulatum* and *B. dermatitidis* after 3 days of incubation on DTM. A red zone consistently extending 10 mm beyond the edge of the mycelial mat surrounded the colonies throughout the experimental period. The growth of *A. boydii* strains 17-A and 21 initiated a color change 9 to 11 days after inoculation. Because the colonies were well established on DTM at this point, complete conversion of the medium rapidly ensued. A red zone, initially noted in DTM cultures of *C. falciforme* strain 139 after 11 days of incubation, completely covered the reverse of the mycelial mat by the completion of the experiment. A color change first observed with cultures of *S. schenckii* strain 335 on the

| Table 1. Colonial diameters (mm) of selected zoopathogenic fungi on experimental media
| Fungus | Diameters (mm) in medium* |
|--------|-------------------------|
|        | DTM | SDA+ | SDA  |
| Trichophyton rubrum 379 | 38.75 | 43.87 | 46.94 |
| 387 | 53.25 | 51.50 | 60.25 |
| 392 | 52.50 | 48.00 | 60.15 |
| 689 | 53.37 | 42.25 | 58.62 |
| Trichophyton mentagrophytes 360-A 361 | 34.50 | 45.00 | 48.00 |
| 369 | 62.50 | 79.50 | 81.00 |
| Sideris schenckii 335 | 37.87 | 37.87 | 39.00 |
| 338 | 38.63 | 36.50 | 36.60 |
| Allescheria boydii 17-A 17-A | 56.50 | 78.13 | 78.25 |
| 20-A | 60.37 | 73.00 | 76.25 |
| 21 | 47.00 | 74.85 | 75.25 |
| 21 | 82.40 | 79.75 | 86.25 |
| Phialophora jeaneselmei 302-E 303-A | 26.30 | 38.75 | 44.75 |
| 27.25 | 30.00 | 45.75 |
| Cephalosporium falciforme 139-B 139 | 21.25 | 23.50 | 100.00 |
| 35.45 | 40.37 | 48.12 |
| Phialophora verrucosa 303 | 35.75 | 33.87 | 33.50 |
| Fonsecaea pedrosoi 194-A | 25.25 | 19.45 | 22.75 |
| Histoplasma capsulatum 209-C 211 | 46.25 | 48.80 | 52.50 |
| 212 | 36.37 | 31.87 | 43.87 |
| 219-A | 36.50 | 33.35 | 40.80 |
| Blastomyces dermatitidis 54 56 | 51.20 | 69.25 | 79.40 |
| 41.00 | 51.60 | 64.70 |

* Each figure represents the average diameter of the mycelial mat of six cultures of each organism 17 days after inoculation.

DTM, dermatophyte test medium; SDA+, SDA plus cycloheximide (0.5 mg/ml); SDA, Sabouraud dextrose agar.

13th day after inoculation had progressed by the 17th day to a red zone extending 15 to 20 mm beyond the colonial margin. Initial conversion of DTM cultures of both strains of *P. jeaneselmei*
and of *S. schenckii* strain 338 was noted only at the termination of the experiment. *P. verrucosa*, *F. pedrosoi*, *C. falciforme* strain 139-B, and *A. boydii* strains 1 and 20-A never induced a color change during the test period.

Although considerable quantitative differences in growth were noted (Table 1) among strains of several of the test species, the development of nondermatophytic pathogens on DTM was in general comparable to that observed with the two dermatophyte species. The total growth of most strains of *T. rubrum*, *S. schenckii*, *P. jeanselmei*, *C. falciforme*, *P. verrucosa*, *F. pedrosoi*, and *H. capsulatum* on DTM was roughly equivalent to that on SDA+. In contrast, whereas all strains of *T. mentagrophytes*, *B. dermatitidis*, and *A. boydii* grew well on DTM, their total growth (with one exception) was consistently greater on SDA+. With few exceptions, the total growth of all test organisms on SDA was greater than that observed on either antibiotic-containing medium.

**DISCUSSION**

Of the antibiotics used in DTM, cycloheximide is the sole antymycotic agent. Previous investigations of the action spectrum of the drug (5, 12, 16) have shown that the resistance of dermatophytes to cycloheximide is equaled or exceeded by several other pathogens and by some saprophytes. This is confirmed by our results (compare, for example, the growth of *H. capsulatum* on DTM versus SDA with that of *T. rubrum*, or the growth of *A. boydii* with that of *T. mentagrophytes*).

Qualitative clinical evaluations of the efficacy of DTM have shown that this medium compares favorably with other commonly used formulations in supporting the growth of dermatophytes. A quantitative investigation by Sinski et al. (14) revealed essentially equivalent growth and sporulation of *T. mentagrophytes* on a variety of media, including DTM. Our more extensive quantitative studies clearly demonstrate that DTM will support the development of nondermatophytic pathogens as well.

Taplin et al. (15) noted that DTM is not absolutely specific for dermatophytes, because several species of saprophytic fungi are capable of inducing a color change. Our investigation indicates that many nondermatophytic pathogens may also cause the conversion of DTM. Of 18 strains tested, 13 induced a color change. The delayed conversion induced by *P. jeanselmei* and *S. schenckii* might prevent their confusion with dermatophytes, but the relatively rapid color change observed with *H. capsulatum*, *B. dermatitidis*, *A. boydii*, and *C. falciforme* and/or their gross appearance on DTM would necessitate an evaluation by microscopy to differentiate them from dermatophytic forms.

DTM was introduced as a simplified method by which relatively untrained technicians could definitively evaluate dermatophytoses. As Taplin and co-workers (15) state: “Although microscopic features are required for positive botanical identification, the nonmycologist can now recognize a griseofulvin-sensitive infection quite well by noting the change of the color of the agar from yellow to red.” They thus advocate that nonmycologists, lacking the training for a proper botanical identification, use DTM as a definitive test medium for dermatophytes, i.e., “griseofulvin-sensitive infections.” This interpretation is supported by the authors’ statement in their conclusion that “DTM, the indicator culture medium reported here, enables unskilled personnel to determine the presence of dermatophytic fungi from skin and hair under most environmental conditions. Cultures from soil, from heavily soil-contaminated feet, and from nails require additional interpretation.” Whereas cultures from soil require evaluation by microscopy, apparently those obtained from skin and hair can be judged solely on the basis of color change.

Even allowing for shades of difference in the interpretation of these statements, the fact remains that DTM is used by many diagnostic laboratories as a definitive medium. On several occasions our laboratory has been asked to evaluate cultures which have been recognized by technicians trained in mycology as nondermatophytes but which have caused a color change on DTM. These people found DTM a source of confusion. Wider acceptance of DTM as a definitive medium in the hands of untrained technicians will inevitably lead to further confusion in the diagnosis of dermatophytoses until its limitations have been properly described.

We have conducted this evaluation of DTM with nondermatophytic pathogens as a step toward properly delineating its role in the diagnosis of dermatophytoses. Although results obtained with axenic cultures are not entirely applicable to a clinical setting, our studies indicate that (i) the growth of nondermatophytes is comparable to that of dermatophytes on DTM, (ii) many of the former induce a color change, and (iii) several may readily be misidentified as dermatophytes if identification is based solely upon the gross appearance of the colony and of the medium.

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LITERATURE CITED

1. Allen, A. M., R. A. Drewry, and R. E. Weaver. 1970. 
   Evaluation of two new color indicator media for 
   diagnosis of dermatophytosis. Arch. Dermatol. 102:68-70.

2. Allen, A. M., D. Taplin, J. A. Lowy, and L. Twigg. 1972. 
   Skin infections in Vietnam. Mil. Med. 137:296-301.

3. Fuentes, C. A., F. Tresepalacios, G. F. Baquero, and R. 
   Aboulafia. 1952. Effect of actidione on mold contami- 
   nants and on human pathogens. Mycologia 54:170-175.

4. Georg, L. K., L. Ajello, and M. A. Gordon. 1951. 
   A selective medium for the isolation of Coccidioides 
   immitis. Science 114:387-389.

5. Georg, L. K., L. Ajello, and C. Papageorge. 1954. 
   Use of cycloheximide in the selective isolation of fungi patho- 
   genic to man. J. Lab. Clin. Med. 44:422-428.

6. McDonough, E. S., L. Ajello, L. K. Georg, and S. 
   Brinkman. 1960. In vitro effects of antibiotics on yeast 
   phase of Blastomyces dermatitidis and other fungi. J. 
   Lab. Clin. Med. 55:116-119.

7. McDonough, E. S., L. K. Georg, L. Ajello, and S. 
   Brinkman. 1960. Growth of dimorphic human patho- 
   genic fungi on media containing cycloheximide and 
   chloramphenicol. Mycopathol. Mycol. Appl. 
   8:113-120.

8. Merz, W. G., C. L. Berger, and M. Silva-Hutner. 1970. 
   Media with pH indicators for the isolation of dermato- 
   phytes. Arch. Dermatol 102:545-547.

9. Rebell, G., and D. Taplin. 1970. Dermatophytes: their 
   recognition and identification. University of Miami 
   Press, Coral Gables.

10. Rosenthal, S. A., and D. Furnari. 1957. The use of a 
    cycloheximide-chloramphenicol medium in routine 
    culture for fungi. J. Invest. Dermatol. 28:367-371.

11. Rosenthal, S.A., and D. Furnari. 1971. Efficacy of 
    dermatophyte test medium. Arch. Dermatol. 
    104:468-489.

12. Salkin, I. F., and N. Hurd. 1972. Quantitative evaluation 
    of the antifungal properties of cycloheximide. Antimi- 
    crob. Ag. Chemother. 1:177-184.

13. Shapiro, E. M., J. F. Mullins, and M. E. Pinkerton. 1956. 
    Comparison of Littman and cycloheximide mediums 
    for primary isolation of dermatophytes. Amer. J. Clin. 
    Pathol. 26:131-135.

14. Sinski, J. T., J. R. Swanson, and L. M. Kelley. 1972. 
    Dermatophyte test medium: clinical and quantitative 
    appraisal. J. Invest. Dermatol. 58:405-411.

15. Taplin, D., N. Zaias, G. Rebell, and H. Blank. 1969. 
    Isolation and recognition of dermatophytes on a new 
    medium (DTM). Arch. Dermatol. 99:203-209.

16. Whiffen, A. J. 1950. The activity in vitro of cycloheximide 
    (Acti-dione) against fungi pathogenic to plants. Myco- 
    logia 42:253-258.