JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1997, p. 2660–2662 Vol. 35, No. 10
0095-1137/97/$04.00+0

A New Medium for the Presumptive Identification of Dermatophytes

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Received 5 May 1997/Returned for modification 17 June 1997/Accepted 25 July 1997

A new medium, Dermatophyte Identification Medium (DIM) (trade mark pending), was specifically developed to eliminate problems of false-positive results associated with commercially marketed media, such as dermatophyte test medium (DTM). Previous investigations had demonstrated that DTM only partially suppressed growth of nondermatophytes and that several of these nondermatophytic fungi that were morphologically similar to dermatophytes caused false-positive results. Presumptive identification of an unknown isolate as a dermatophyte required only the transfer of a portion of the suspected colony recovered from the specimen to DIM. Positive results, evidenced by a change in the color of the medium, were observed within 24 to 48 h. In studies of over 500 isolates of dermatophytes and common nondermatophyte molds, as well as close to 600 yeast isolates, false-positive results were always associated with bacterial contamination of the mold isolates while false negatives were only observed with occasional isolates of *Trichophyton verrucosum*. DIM culture was an inexpensive, rapid, and accurate method for the presumptive identification of dermatophytes in the clinical mycology laboratory.

In 1969, Taplin and coworkers (1, 9, 10) introduced dermatophyte test medium (DTM) to provide a simple and rapid method for medics in Vietnam to isolate and recognize dermatophytes from soldiers with ringworm infections. The incorporation of antibacterial (gentamicin sulfate and chlorotetracycline HCl) and antifungal (cycloheximide) antibiotics in a nutrient agar base provided a selective substratum for the isolation of dermatophytes by suppressing the growth of most fungal and bacterial contaminants. A pH indicator (phenol red solution), which converted the color of the medium from straw yellow to bright red under the alkaline conditions associated with growth of dermatophytes, was used to provide the medium with a differentiating quality. Consequently, the authors noted, the isolation of a dermatophyte on DTM could be easily recognized by simply "noting the change of the color of the agar" (9). Since its original description, DTM has gained wide acceptance in clinical mycology and physician office laboratories as a simple and cost-effective method for the isolation and/or identification of dermatophytes (4, 6–8).

However, subsequent studies have demonstrated that the growth of nondermatophytic fungi, both saprobes and pathogens, was not inhibited by the antifungal antibiotic in DTM at the concentration used (2, 5). In addition, the growth of some of the nondermatophytic fungi caused a color change in the medium as intense and rapid as that caused by dermatophytes. Several such nondermatophytes are routinely associated with the same types of clinical specimens as dermatophytes and/or have similar colony morphologies.

In this report, we describe a new medium, Dermatophyte Identification Medium (DIM) (trade mark pending), which was specifically developed to eliminate the problems associated with the use of DTM. In studies of over 572 primary, reference, and culture collection isolates encompassing 42 genera and 71 species of dermatophytes and nondermatophytic molds, we found that DIM provided a simple, rapid, and specific means of presumptively identifying dermatophytes.

Test organisms. All primary and reference specimens received by the Mycology Reference Laboratory, Wadsworth Center, New York State Department of Health, Albany, over a 3-year period were inoculated and subcultured, respectively, onto DIM. Primary clinical specimens were initially processed as appropriate for the type of clinical material received by the laboratory, e.g., centrifugation of cerebrospinal fluid. Portions of the specimens were then aseptically inoculated onto slants (7.0 ml of nutrient medium/20- by 100-mm culture tube) of each of the following media: (i) Emmons' modified Sabouraud glucose agar (MSGA; Difco, Detroit, Mich.), (ii) MSGA fortified with 40 μg of gentamicin and 25 μg of chloramphenicol per ml (MSGA+), and (iii) BBL Mycosel agar (Becton Dickinson, Cockeysville, Md.). All cultures were incubated at 30°C and checked for growth on alternate days for 2 weeks. Portions of growth from reference specimens were subcultured to the same three nutrient media and incubated at 30°C.

The identification of all molds was established through studies of their morphological features by using teased mounts and slide cultures. Physiologic characteristics (e.g., temperature optimum, nitrate assimilation, and nutritional requirements) were examined by the use of appropriate supplemental tests (3). The morphologies of all yeasts on cornmeal plus Tween 80 agar in 100-mm-diameter petri plates and slide cultures, inoculated by the Dalmau and streak-cut methods, respectively, were studied. Carbohydrate assimilation characteristics were investigated through the use of API 20C (bioMerieux, Hazelwood, Mo.) and other commercial identification kits. Supplemental physiologic features (e.g., urease activity and temperature optimum) were studied with appropriate test media (3).

DIM studies. Once fungal growth was noted on an MSGA+ culture inoculated with a primary specimen or as a subculture of a reference culture, a portion of the colony was aseptically transferred to DIM and MSGA+ (growth control) slants, which were incubated at 37°C. Both media were examined

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TABLE 1. Results of growth on and color change of DIM for *Arthroderma*, *Epidermophyton*, *Microsporum*, and *Trichophyton* spp.

Organism	No. of strains	Growth[a]	Color change of medium[b]
Arthroderma ciferrii	1	+	+
A. cookiellum	5	+	+
A. fulvum	1	+	+
A. gypseum	2	+	+
A. incurvatum	2	+	+
A. uncinatum	2	+	+
Epidermophyton floccosum	8	+	+
Microsporum audouinii	3	+	+
M. canis	17	+	+
M. canis var. *distortum*	3	+	+
M. racemosum	2	+	+
M. vanbreuseghemii	1	+	+
Trichophyton concentricum	2	+	+
T. gertleri	1	+	+
T. gloriae	2	+	+
T. mentagrophytes	43	+	+
T. rubrum	76	+	+
T. tonsurans	44	+	+
T. verrucosum	5	4(+)/1(−)[c]	4(+)/1(−)[d]
T. violaceum	1	+	+
T. vaoundei	2	+	+

[a] +, growth; −, no growth.
[b] Presence (+) or absence (−) of a color change in DIM as detected, on average, within 48 h after inoculation.
[c] Number of isolates which grew/number of isolates which did not grow on DIM.
[d] Number of isolates which caused a color change/number of isolates which did not cause a color change on DIM.

daily for 1 week for growth, and DIM color changes were noted.

Cultures of dermatophytes, related keratinophilic fungi, and other filamentous molds often isolated from skin, hair, or nails were selected from the mycology culture collection of the Centers for Disease Control and Prevention for this study. All isolates were subcultured on MSGA+ slants, which were incubated for 10 days at 30°C. A pinhead portion of growth from each developing colony was then aseptically transferred to slants of DIM and MSGA+, which were incubated at 37°C. Both media were examined daily for 1 week for growth, and DIM color changes were noted. Positive- and negative-control isolates were included with each group of 10 isolates tested on DIM, and all tests were repeated at least twice.

All components of DIM (dextrose, neopeptone, cycloheximide, penicillin, streptomycin, and bromcresol purple), except the antibiotics, were prepared as a single agar-containing solution which was autoclaved for 30 min at 15 lb/in^2. The antibiotic solutions were separately filter sterilized and then added to the molten agar solution prior to the dispensing of the medium in 7-ml aliquots into 20- by 150-mm culture tubes for solidification at a slant. DIM contains a pH indicator which alters the color of the medium when there is a change in pH such as that caused by the growth of dermatophytes. *Trichophyton mentagrophytes* was used as the positive control (purple on DIM), and *Aspergillus flavus* was used as the negative control (greenish blue on DIM).

The results of growth on and the consequent color change of DIM medium for a total of 223 isolates belonging to the genera *Arthroderma*, *Epidermophyton*, *Microsporum*, and *Trichophyton* are summarized in Table 1. The results for 349 nondermatophytic isolates of filamentous fungi grown on DIM are summarized in Table 2. The 223 dermatophyte isolates either were recovered directly from clinical specimens or were from the culture collection. Two hundred twenty-two of the 223 isolates grew on DIM and caused the color conversion of DIM from greenish blue to deep purple, on average within 48 h of inoc-

TABLE 2. Results of growth on and color change of DIM for nondermatophytic filamentous fungi

Organism	No. of strains	Growth[a]	Color change of medium[b]
Absidia sp.	8	−	−
Acremonium kiliense	2	−	−
Acremonium spp.	12	8(+)/4(−)[c]	−
Acrodontium sp.	3	−	−
Alternaria sp.	6	+	+
Aspergillus clavatus	5	−	−
Aspergillus flavus	20	−	−
Aspergillus fumigatus	43	30(+)/13(−)[c]	−
Aspergillus glaucus	6	−	−
Aspergillus niger	14	−	−
Aspergillus terreus	4	−	−
Aspergillus ustus	1	−	−
Aspergillus versicolor	8	3(+)/5(−)[c]	−
Aureobasidium pullulans	4	−	−
Blastomyces dermatitidis	3	−	−
Chaetomium sp.	3	−	−
Chrysosporium sp.	8	−	−
Chrysosporium keratinophilum	1	−	−
Chrysosporium tropicum	2	−	−
Cladosporium sp.	17	−	−
Coccidioides immitis	2	−	−
Cunninghamella sp.	5	−	−
Curvularia sp.	3	−	−
Exophiala jeanselmei	7	3(+)/4(−)[c]	3(+)/4(−)[d]
Fonsecaea pedrosoi	8	−	−
Fusarium spp.	13	−	−
Histoplasma capsulatum	5	−	−
Madurella mycetomatis	1	−	−
Madurella grisea	1	−	−
Malbranchea sp.	6	−	−
Mortierella sp.	7	5(+)/2(−)[c]	−
Mucor sp.	5	−	−
Myrothecium sp.	1	−	−
Onychocola canadensis	1	−	−
Paecilomyces sp.	18	10(+)/8(−)[c]	−
Penicillium sp.	23	−	−
Phialophora parasitica	3	−	−
Phialophora verrucosa	5	3(+)/2(−)[c]	3(+)/2(−)[d]
Phoma sp.	11	−	−
Rhinocladiella aquaspersa	2	−	−
Rhizopus sp.	10	−	−
Scedosporium apiospermum	5	−	−
Scedosporium prolificans	4	−	−
Scopulariopsis brevicaulis	6	−	−
Scytalidium dimitiatum	3	−	−
Sporothrix schenckii	5	−	−
Stachybotrys sp.	2	−	−
Ulocladium sp.	2	−	−
Uncinocarpus reesii	2	−	−
Wangiella dermatitidis	4	2(+)/2(−)[c]	2(+)/2(−)[d]

[a] +, growth; −, no growth.
[b] Presence (+) or absence (−) of a color change in DIM as detected, on average, 48 h after inoculation.
[c] Number of isolates which grew/number of isolates which did not grow on DIM.
[d] Number of isolates which caused a color change/number of isolates which did not cause a color change on DIM.

ulation. Only one isolate of *Trichophyton verrucosum* failed to grow on DIM and MSDA+ at 37°C. The morphology and physiology of this isolate were reinvestigated, and it was found to have been correctly identified.

While 71 isolates belonging to eight genera of nondermatophytic molds were able to grow on DIM, only 15 isolates belonging to four genera (*Alternaria*, *Exophiala*, *Phialophora*, and *Wangiella*) caused a color change in DIM. The purple color induced by growth of six *Alternaria* sp. isolates was less intense than that produced by various species of dermatophytes. Although a color change in DIM could be observed within 48 h of the inoculation of three isolates each of *Exophiala jeanselmei* and *Phialophora verrucosa*, as well as two *Wangiella dermatitidis* isolates, color conversion comparable to that seen with dermatophytes was not evident until 72 to 96 h after inoculation.

Portions of growth of yeast isolates recovered from primary and reference specimens were also tested on DIM slants, incubated at 37°C, to obtain a more complete evaluation of the performance of the medium. All isolates of *Blastoschizomyces capitatus* (14 studied), *Candida glabrata* (36 isolates), *Candida guilliermondii* (8 isolates), *Candida krusei* (12 isolates), *Candida parapsilosis* (22 isolates), *Candida rugosa* (5 isolates), *Saccharomyces cerevisiae* (18 isolates), and *Rhodotorula* (16 isolates) sp. were unable to grow on DIM. In contrast, 307 isolates of *Candida albicans* and 15 isolates of *Candida kefyr* were able to grow but did not induce a color change in the medium. All possible combinations of results were found with the 44 isolates of *Candida tropicalis*; i.e., approximately 35% of the isolates could not grow on DIM, about 45% did grow but did not cause a color shift to purple, and approximately 20% of the isolates grew and induced a color change in the medium.

The results of these studies clearly indicate that the use of DIM is an inexpensive, rapid, and accurate means of presumptively identifying dermatophytes recovered from clinical specimens. The sensitivity of the medium in indicating the development of dermatophytes through the shift in its color from greenish blue to purple exceeded 99%; that is, only one false-negative response was noted, for an isolate of *T. verrucosum*. Although 71 nondermatophytic fungi grew on DIM, only 15 of these isolates caused a color change in the medium similar to that found with dermatophytes. The specificity of the medium would therefore be 95.7% if the change and intensity of color of DIM are taken into consideration in evaluating its use in identifying dermatophytes. However, all 15 isolates that gave false-positive results on DIM were dematiaceous fungi with distinctive, phaeoid colonies which even on DIM could be readably distinguished from the colonial morphology of dermatophytes. When clinical source, colony morphology, and color change of DIM were considered together as criteria in the identification process, the specificity of DIM rose to 100%.

The high specificity and sensitivity of DIM for the presumptive identification of dermatophytes are most likely due to the combination of the use of high concentrations of antifungal antibiotics and the incubation of cultures at elevated temperatures. Molds that could tolerate the high level of antifungal antibiotics could not grow at the elevated incubation temperature used for DIM. Those that were thermotolerant were inhibited by the higher-than-routine concentration of antifungal antibiotics.

The lack of growth on DIM of those yeasts isolates which are known to develop at elevated incubation temperatures must be due to the high concentration of antifungal drugs. The lack of a color change in DIM associated with the development of *C. albicans* and *C. keyfr* isolates is attributable to their acidification of the growth medium.

In an earlier study of DTM (5), several nondermatophytic pathogens [*Blastomyces dermatitidis*, *Acremonium* (*Cephalosporium*) *falciforme*, *Histoplasma capsulatum*, *Scedosporium apiospermum*, and *Sporothrix schenckii*] were found to be readily misidentified as dermatophytes if the identification was based on their colony morphologies and their ability to induce the appropriate color change in the medium. In another study (2), several saprobic nondermatophytes (*Alternaria* sp., *Aspergillus* sp., *Chrysosporium* sp., *Penicillium* sp., and *Scopulariopsis* sp.) that were commonly associated with skin, hair, and nails were reported to cause a color change in DTM. In the present study (Table 2), only isolates of *Alternaria* sp. were able to grow on DIM, and none induced as intense a color shift of the medium as dermatophytes. Thus, DIM virtually eliminated the problems caused by false-positive results associated with the use of DTM in the presumptive identification of dermatophytes.

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