Rapid Method for Differentiation of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and Related Dermatophyte Species

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Bromocresol purple-milk solids-glucose medium, proposed by Fischer and Kane in 1971 (Mycopathol. Mycol. Appl. 43:169-180, 1971) as an aid in the rapid determination of *Trichophyton rubrum* and *Trichophyton mentagrophytes*, was evaluated across a wide range of isolates to determine its accuracy and efficacy in the clinical laboratory. Results showed that it facilitated accurate determination of typical and atypical isolates of both species and also permitted the rapid identification of other closely related and similar species. Identification of all dermatophyte species tested was possible within 7 to 10 days. Occult contamination of isolates by antibiotic-resistant bacteria did not hinder identification.

**MATERIALS AND METHODS**

*T. rubrum* and *T. mentagrophytes* isolates were obtained during routine clinical practice at the New York University Medical Center in New York and at the Ontario Ministry of Health Laboratory Services Branch, Toronto, Ontario, Canada. Reference isolates of these species, as well as *T. georgiae* and *Microsporum persicolor*, were obtained from the collection of the Ontario Ministry of Health. Isolates of *T. mentagrophytes* var. erinacei Smith & Marples were obtained from A. A. Padhye, Centers for Disease Control, Atlanta, Ga., and mating types of *Arthroderma benhamiae* Ajello & Cheng (European and African isolates) and *A. vanbreuseghemii* Takashio were obtained courtesy of I. Weitzman, Department of Health, New York, N.Y.

Isolates were maintained on peptone-glucose agar (per liter of distilled water: 40 g of high-glucose corn syrup [Hi-dex; St. Lawrence Starch Co., Toronto, Ontario, Canada], 10 g of Bacto-Peptone [Difco Laboratories, Detroit, Mich.], 15 g of Difco Bacto-Agar, adjusted to pH 7.0) supplemented with cycloheximide (50 mg liter⁻¹), chloramphenicol (50 mg liter⁻¹), and gentamicin (20 mg liter⁻¹). This was also the medium on which clinical isolates were cultured from samples of skin, hair, and nails. For identification, isolates were subcultured on BCP-MS-G slants (autoclaved separately and then mixed and dispensed: [a] 1,000 ml of distilled water, 80.0 g of skim milk powder [Carnation Co., Toronto, Ontario, Canada], 2 ml of a 1.6% alcohol solution of bromocresol purple [BDH Chemicals, Toronto, Ontario, Canada]; [b] 200 ml of distilled water, 40 g of glucose; and [c] 800 ml of distilled water, 30 g of Bacto-Agar; final pH adjusted to 6.8) and incubated at 25°C for 7 days. In addition, isolates were inoculated in Christensen urea broth (11) ([a] 900 ml of distilled water, 1 g of Bacto-Peptone, 5 g of sodium chloride, 2 g of monopotassium phosphate [KH₂PO₄], 8 ml of phenol red [Sigma Chemical Co., St. Louis, Mo.]) 0.2% solution in 95% ethanol, dissolved, adjusted to pH 6.9, and autoclaved; and [b] 100 ml of distilled water, 20 g of urea [Sigma], 1 g of glucose, sterilized by filtration and added aseptically to autoclaved, cooled solution a) for detection of urease activity, and onto vitamin-free Casamino Acids agar (7) (920 ml of distilled water, 2.5 g of Difco vitamin-free Casamino Acids, 0.1 g of magnesium sulfate [MgSO₄ ∙ 7H₂O], 1.8 g of monopotassium phosphate [KH₂PO₄], 15 g of Bacto-Agar; autoclaved at 10 lb/in² for 10 min and then cooled to 50°C)
cooled, and 80 ml of a sterile stock solution of 50% glucose added) for elicitation of microconidia in *T. rubrum* and demonstration of typical morphology and vitamin independence in *T. mentagrophytes*.

At the end of the 7-day incubation period, BCP-MS-G slants were examined for pH change and growth characteristics of the subcultured isolates. A color change from the original pale blue ("glaucous sky blue" [19]) to violet-purple ("greyish flax blue" to "greyish flax blue" [19]) indicated a positive alkaline reaction. Urea broth was examined for a color change from orange to pink or purple indicating catalysis of urea.

In vitro hair perforation tests were done by the method of Ajello and Georg (1) for 25 of the routine isolates (10 presumptively identified as *T. mentagrophytes*, 15 as *T. rubrum*) plus 5 additional isolates representing the slow-growing, yellow-orange-pigmented "nodular" variant of *T. mentagrophytes* (8). All in vitro hair tests were examined after 4 weeks at 25°C; most were given preliminary examinations at 2 and 3 weeks.

### RESULTS

The results of BCP-MS-G and urea broth trials are shown in Table 1. All isolates of *T. rubrum* tested were urease negative and produced no alkalinity on BCP-MS-G at 7 days. In addition, they showed restricted growth on BCP-MS-G, as described in previous studies (4, 15). This restricted growth form contrasted greatly with the much more diffuse, spreading growth of *T. mentagrophytes*.

The results of 7-day BCP-MS-G trials for *T. rubrum* were entirely in agreement with the results of the in vitro hair perforation trials evaluated after 2 to 4 weeks. These trials all gave negative results.

The *T. rubrum* isolates used in BCP-MS-G, urea broth, and in vitro hair trials included representatives of the melanoid (6, 16), flavous (23), and granular or velutinous variants, as well as the typical cottony or floccose variant. Melanoid variants secreted a weak pinkish brown pigment on BCP-MS-G, but this could not be mistaken for a positive alkaline reaction. Typical *T. rubrum* isolates, as well as representatives of the velutinous variant, also produced the typical red colony reverse pigment of the species within 4 to 6 days on BCP-MS-G. This pigmentation was seen through the colony from above or from the side, since the underside of the colony was partially or completely obscured by the opaque milk solids of the medium. On peptone-glucose agar, the same isolates often required up to 14 days to produce this pigment. Melanoid isolates also tended to produce at least some visible red pigment on BCP-MS-G. Flavous isolates did not produce red pigment; however, identification of these isolates was partially based on their not developing red pigments on any media, including Casamino Acids-erythritol-albumin (5), potato dextrose, cornmeal dextrose, and modified Borelli lactritmel agars (9).

Recently described segregate species closely related to *T. rubrum* including *T. fischeri* Kane, *T. rautitschekii* Kane, Weitzman, Salkin, and Smirka, and *T. kanei* Summerbell all showed a pattern of restricted growth, rapid development of red colony reverse pigments, and an absence of catalysis yielding alkaline products on BCP-MS-G. *T. rautitschekii* differed from *T. rubrum* and the other segregates by being positive in the urea broth test after 7 days. This reaction is typical for this species (13). *T. fischeri*, *T. rautitschekii*, and *T. kanei* were not included in hair perforation trials, as all have previously been rigorously tested and shown not to perforate hair in vitro (10, 13, 22). Many of the isolates used in the present study were the same as had been tested previously.

It should be noted that when test media were kept for periods exceeding 14 days, most isolates of *T. rubrum* and closely related species began to produce an alkaline reaction on BCP-MS-G. In general, results of the urea broth and in vitro hair tests did not alter after extended periods exceeding 8 weeks. *T. kanei*, however, showed a weak and latent catalysis of urea in broth medium after 7 to 10 days (22).

*T. mentagrophytes var. mentagrophytes* (including isolates representing the cottony, velvety, and granular growth forms as defined by Kane and Fischer [12]) generally differed from *T. rubrum* in all three of the tests used here. If perforated hair, produced a strong positive urease reaction, and showed rapid, diffuse growth with a concomitant moderate-to-strong alkaline reaction visible within 7 days on BCP-MS-G. Identical reactions were shown within both intersterile groups of *T. mentagrophytes* isolates, including those corresponding to the teleomorphic species *A. benhamiae* and those corresponding to *A. vanbreuseghemii. T. mentagrophytes var. erinacei*, which has been shown through mating to correspond to *A. benhamiae*, reacted

### Table 1. Reactions of *T. rubrum*, *T. mentagrophytes*, and other selected taxa in BCP-MS-G and Christensen’s urea broth tests

<table>
<thead>
<tr>
<th>Species</th>
<th>BCP-MS-G medium (7 days)</th>
<th>Christensen’s urea broth (7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates tested</td>
<td>No. of isolates producing alkalinity</td>
</tr>
<tr>
<td><em>Arthroderma benhamiae</em></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Arthroderma vanbreuseghemii</em></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Microsporum persicolor</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Trichophyton fischeri</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><em>Trichophyton georgiae</em></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Trichophyton kanei</em></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes var. erinacei</em></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes var. mentagrophytes</em></td>
<td>59</td>
<td>58</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes nodular variant</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><em>Trichophyton rautitschekii</em></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>90</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Three weak positive isolates and one strong isolate.

<sup>b</sup> Weakly positive, becoming strongly positive at 10 days.

The growth of nodular isolates is slow, but it is not restricted in comparison with controls on media other than BCP-MS-G and hence is not referred to as restricted.
similarly to members of *T. mentagrophytes* var. *mentagrophytes* on BCP-MS-G. It was, however, negative in the urea broth test after 7 days. Slow-growing nodular isolates of *T. mentagrophytes* were detectably but only slightly positive in the BCP-MS-G and urea broth tests after 7 days. They became fully positive within 10 days. Highly similar yellow slow-growing *T. rubrum* isolates were persistently negative in both tests.

Only a single *T. mentagrophytes* isolate had an anomalous reaction. This isolate, OMH-FR 2443.86, grew rapidly on BCP-MS-G but did not produce detectable alkalinity. After the possibility of contamination was excluded, the same result was obtained. The possibility that the isolate was a misidentified representative of *Microsporum persicolor* was ruled out by demonstration of smooth macroconidia on salt-amended Sabouraud agar and good growth at 37°C (14).

As the taxonomic status of the isolate was in doubt, we conducted mating trials. The isolate mated as *A. benhamiae*. Finally, on a third subculture to BCP-MS-G, the isolate produced a typical alkaline reaction. Since the use of typical *T. mentagrophytes* control isolates in all trials excluded the possibility of medium quality control problems, we concluded that the anomalous isolate had for unknown reasons failed to express its alkaline-producing properties when first isolated. No similar isolates have been discovered in our routine practice, despite the screening of almost 7,000 *T. mentagrophytes* isolates on BCP-MS-G by one of us (J.K.) between 1975 and 1988.

*Microsporum persicolor* isolates catalyzed the breakdown of urea in the broth test, but invariably produced no alkalinity in the BCP-MS-G test after 7 days or more extended periods. They grew rapidly and diffusely. These results are identical to those obtained by Kane et al. (14). In vitro hair tests were not done because this species is well known to penetrate hair in vitro (20). *T. georgiae* grew rapidly on BCP-MS-G and produced a weakly alkaline reaction. It was urease positive. Since it also perforates hair (20), it cannot readily be distinguished from the superficially similar *T. mentagrophytes* by means of the tests used in the present study. It can be distinguished by its small conidia borne on extended, stalklike branches, red-brown colony pigmentation, and the homothallic production of gymnothecia on suitable media such as pulbarum cereal agar (18).

One further species which was included in these trials was *Myxotrichum deflexum* Berkeley, a common gymnoascac-eous species occasionally occurring as a contaminant in medical mycology specimens (17). This species produces floccose, white mycelium and a red-pigmented reverse and may resemble an aconidal *T. rubrum* within its first 7 to 14 days of growth. Later, it develops the darkened, branching appendages characteristic of the teleomorph (2). Early identification was facilitated on BCP-MS-G medium because the species produced acidic substances, which turned the indicator in the medium beneath the colony a strong yellow. This reaction is not found in any dermatophyte. We have, however, observed it in many contaminating fungi, particularly cellulolytic species such as *Chaetomium globosum* Kunze and *Geomyces pannorum* (Link) Sigler & Carmichael. It is thus useful in screening out such fungi.

**Effect of contamination on BCP-MS-G results.** The contamination of clinical primary isolates of dermatophytes is very common, particularly when *T. rubrum* is isolated (4). Various dermatophyte isolates contaminated with antibiotic-resistant bacteria, yeasts, and nonpathogenic filamentous fungi were observed during routine practice to determine the effect of these organisms on BCP-MS-G test results. In most cases, contaminants had no effect. Isolates contaminated with bacteria or yeasts occasionally gave rise to a yellow (acidic) reaction, as previously demonstrated by Fischer and Kane (4). This seldom hindered the separation of *T. rubrum* and *T. mentagrophytes*, since the former generally produced characteristic restricted growth and, in some cases, red colony reverse pigment, while the latter grew profusely without producing detectable pigment. The yellow color change was thus useful as an early indicator of occult contamination of isolates, which might otherwise have resulted in misdiagnosis owing to false-positive results in the urea broth tests of contaminated *T. rubrum* isolates. Whenever contaminated isolates were examined, colony micro-morphology and macromorphology, as observed on Casamino Acids agar, were particularly useful in confirming presumptive determinations made on the basis of BCP-MS-G results. The auxiliary use of Casamino Acids-erythritol-albumin agar (5) to inhibit bacteria and biotin-requiring yeasts (*e.g.*, *Candida albicans* (Robin) Berkhout) to stimulate red pigment production in *T. rubrum* also proved very useful in work with contaminated isolates.

In rare instances, strongly caseolytic bacteria interfered with the growth of *T. mentagrophytes* on BCP-MS-G. *T. mentagrophytes* isolates contaminated with these bacteria grew slowly, with a thinly powdery colony appearance. Beneath the colonies, the opaque milk solids of the medium were cleared by the contaminants, and the alkaline reaction caused by *T. mentagrophytes* was partially or completely suppressed. Because these isolates had thin spreading colo-nies with unpigmented reverses, and because urea broth tests were positive, the isolates could not be confused with uncontaminated or contaminated *T. rubrum*. The lack of compact colony morphology and red pigment enabled urea-positive *T. raubitschekii* to be ruled out. The isolates were therefore recognizable as bacterially contaminated *T. mentagrophytes* or *Microsporum persicolor* isolates, with the former diagnosis much more likely. In all such instances seen thus far, typical *T. mentagrophytes* conidial structures have allowed immediate determination.

**DISCUSSION**

Our tests on 164 isolates of *T. rubrum* and *T. mentagrophytes* showed that subculturing from peptone-glucose isolation medium to BCP-MS-G medium, in combination with Christensen urea broth and vitamin-free Casamino Acids agar, permitted reliable differentiation of the species within 7 to 10 days. This confirms suggestions made earlier by Fischer and Kane (4) on the basis of studies of a more limited range of isolates. Reliable determination was possible whether or not isolates were contaminated with occult bacteria or yeasts. Results of these tests were in perfect accord with results obtained by testing a subset of isolates with the standard 3-week in vitro hair perforation test. In addition, the BCP-MS-G test facilitated the identification of the *T. rubrum* segregates *T. raubitschekii*, *T. fischeri*, and *T. kanei*, as well as *Microsporum persicolor* and the common contaminant *Myxotrichum deflexum*. The identification of other dermatophyte species was also facilitated with the aid of this test (14).

It appears that the success of the BCP-MS-G test lies in a fundamental difference between *T. rubrum* and its segregates on the one hand and *T. mentagrophytes* on the other. An enzymatic activity of *T. rubrum* and allies, one probably related to the production of ammonium ions from casein, is partially or completely suppressed in the presence of glucose.
in BCP-MS-G. The repression of ammonifying activity ends only after approximately 2 weeks, by which time, presumably, a high proportion of the glucose has been exhausted. In the absence of glucose in the same basal medium, these fungi cause the pH of the medium to rise markedly after no more than 3 days of growth. T. mentagrophytes, in all its biological forms, has a quite different response: with the exception of extremely rare aberrant isolates, it rapidly causes the medium to become alkaline. It is not influenced by the presence or absence of glucose. Although further research is necessary to clarify the nature of this dichotomy, it appears to be highly reliable, rivalling the reliability of the dichotomy elucidated by the in vitro hair perforation test. Thus, the results of the BCP-MS-G test may prove to be of theoretical interest to those who study the differing proteinase activities of the dermatophyte species. For practical purposes in the clinical laboratory, this medium is highly efficacious in facilitating the identification of two of the most common dermatophyte species within 7 to 10 days.

ACKNOWLEDGMENTS

We thank Arvind A. Padhye and Irene Weitzman for contributing cultures, I. Weitzman and David Malloch for assistance in identifying isolates, and Judy Clent for technical assistance.

R.C.S. was supported by a Medical Research Council of Canada postdoctoral fellowship.

LITERATURE CITED