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Improved Procedures for Differentiating *Microsporum persicolor* from *Trichophyton mentagrophytes*

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***Microsporum persicolor*, a zoophilic dermatophyte species, is seldom recorded causing human infections in North America. Its identification has been enhanced as a direct result of the development of improved techniques for its characterization. Identifying characteristics include induction of rough-walled macroconidia on sodium chloride-amended medium, absence of good growth at 37°C, and absence of a pH change during growth on casein glucose medium. In contrast, *Trichophyton mentagrophytes*, a species commonly confused with *M. persicolor*, has smooth-walled macroconidia, grows well at 37°C, and produces an alkaline reaction on casein glucose medium.**

Microsporum persicolor (Sabouraud) Guiart & Grigorakis is a zoophilic dermatophyte of worldwide distribution. It is a causal agent of tinea corporis, tinea capitis, and tinea pedis (17). Infections are acquired primarily through exposure to small rodents, including bank and field voles and mice (1, 2, 4). *M. persicolor* is strikingly similar in both its colonial and microscopic morphology to *Trichophyton mentagrophytes* (Robin) Blanchard, especially the zoophilic variety. These two species were considered synonymous until Stockdale (19) demonstrated that the teleomorph of *M. persicolor* was a distinct species. Recently, the teleomorph of *M. persicolor*, *Nannizzia persicolor*, was transferred to the genus *Arthroderma* (21).

Characteristics which prove helpful in distinguishing *M. persicolor* from *T. mentagrophytes* include the shape and rough cell wall of the macroconidia, development of rosy red-pigmented sectors in colonies grown on media such as pablum cereal agar (16), and development of the teleomorph by mating isolates with tester strains (14). Problems in identification arise when isolates fail to produce the characteristic macroconidia, remain nonpigmented, or fail to produce fertile ascocarps when mated with tester strains. For the diagnostic laboratory where rapid results are expected, the latter procedures are cumbersome and slow, sometimes requiring 6 to 8 weeks for development of fertile ascocarps.

Although *T. mentagrophytes* is among the most common dermatophyte species, *M. persicolor* is isolated only rarely from human infections. There are few reports of *M. persicolor* infection in North America (3, 14); indeed, a recent survey of 59 North American mycological diagnostic laboratories recorded only one isolate of *M. persicolor* among 18,136 dermatophytes isolated between 1979 and 1984 (18). A survey of dermatophyte species isolated or received at the Laboratory Services Branch, Toronto, Canada, during the years 1981 to 1985 showed that *M. persicolor* was isolated from 35 patients with skin lesions clinically diagnosed as dermatophytosis (Table 1). We know of no reason why *M. persicolor* should be more common in Ontario than elsewhere in North America. We find it much more likely that the greater number of isolates of *M. persicolor* obtained in Ontario can be ascribed to the use of physiological tests and careful evaluation of morphological features which allow more accurate identification of the fungus.

To evaluate the potential of our approach in simplifying the problem of distinguishing between *M. persicolor* and *T. mentagrophytes*, we conducted an intensive investigation of the efficacy of tests which had proved useful in the differentiation of other difficult dermatophyte species (13). This paper reports our evaluation of various methods to distinguish 29 known isolates of *M. persicolor* from 35 isolates of *T. mentagrophytes*.

Stock cultures of *M. persicolor* were obtained from the culture collections of the University of Alberta Microfungus Herbarium; the Mycology Section, Laboratory Services Branch, Ontario Ministry of Health; the Institut Pasteur, Paris; and I. Weitzman, New York. Stock cultures of *T. mentagrophytes*, representing granular, velvety, nodular, and cottony variants, were obtained from the first two sources. All stocks were maintained on peptone glucose agar (per liter of distilled water: 40 g of high-glucose corn sugar [Hidex; 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) with added cycloheximide (50 mg liter^{-1}), chloramphenicol (50 mg liter^{-1}), and gentamicin (20 mg liter^{-1}), collectively termed CCG medium.

Several diagnostic tests were evaluated. First, in the bromocresol purple (BCP) casein glucose agar test, as formulated by Fischer and Kane (5) and Kane and Smitka (13), material from the colony surfaces of 7-day-old stocks of *M. persicolor* and *T. mentagrophytes* was inoculated at a single

TABLE 1. Frequency of isolation of *M. persicolor* and *T. mentagrophytes* during the years 1981 to 1986 in Ontario, Canada

Yr	No. of specimens positive for:		Total no. of specimens examined for dermatophytes
	M. persicolor	*T. mentagrophytes*	
1981		542	17,250
1982	2	551	17,755
1983	2	567	18,069
1984	11	628	17,460
1985	11	716	19,575
1986	9	855	18,835

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TABLE 2. *M. persicolor* distinguished from other common dermatophytes by reactions on BCP casein glucose agar (BCPCG) and Christensen urea broth and by growth at 37°C[a]

Dermatophyte species	No. of isolates in present study	Previous BCPCG study	Reactions on BCPCG medium				Urease activity	Growth at 37°C
			Clearing of casein solids	Red pigment formation	Type of growth manifested[b]	pH reaction		
M. audouinii Gruby		Unpublished	+	−	Slow	No change	+	+
M. canis Bodin		10	−	−	Profuse	No change	+	+
M. equinum (Del. & Bod.) Gueguen		10	−	−	Slow	Alkaline	+	+
M. gypseum (Bodin) Guiart. & Grig.		Unpublished	−	−	Profuse	No change	+	+
M. persicolor	29		−	−	Profuse	No change	+	− or +w[c]
T. fischeri Kane		6	−	+	Restricted	No change	−	+
T. kanei Summerbell		20	−	+	Restricted	No change	− or +w[d]	+
T. megninii Blanchard		9	−	−	Profuse	Alkaline	+	+
T. mentagrophytes	35	5, 13	−	−	Profuse	Alakaline	+	+
T. raubitschekii Kane, Salkin, Weitzman & Smitka		11	−	+	Restricted	No change	+	+
T. rubrum (Cast.) Sab.[e]		5, 13	−	+/−	Restricted	No change	−	+
T. verrucosum Bodin		12, Unpublished[f]	+	−	Slow	Alkaline	+	+

[a] Results read at 7 days.
[b] Restricted growth, Growth much slower than that of controls on glucose-free casein agar. Slow or profuse growth, Slow or fast growth rates compared with those seen in controls.
[c] +w, Restricted, subsurface growth.
[d] +w, Weak positive becoming fully positive at 9 to 10 days.
[e] Includes all described variants.
[f] Published studies on *T. verrucosum* (12) used a BCPCG variant with an added 0.5% yeast extract.

point on slants of BCP casein glucose medium (autoclave separately and then mix and dispense: [i] 1,000 ml of distilled water, 80.0 g of Carnation skim milk powder [Carnation Co., Toronto, Ontario, Canada], 2 ml of a 1.6% alcoholic solution of BCP [BDH Chemicals, Toronto, Ontario, Canada]; [ii] 200 ml of distilled water, 40 g of glucose; and [iii] 800 ml of distilled water, 30 g of Difco Bacto-Agar; adjust final pH to 6.8). These slants were held at 25°C for 7 days before examination for growth and indicator color change. Second, a test was conducted for growth and micromorphology on peptone glucose agar, formulated as above and supplemented with 3 and 5% sodium chloride (7, 8). Isolates were examined after 7 to 10 days of growth at 25°C. Third, isolates were tested for growth at 37°C on peptone glucose agar amended with CCG. Growth was evaluated at 7 days. Finally, morphological studies were conducted on cultures grown on peptone glucose and pablum cereal (16) media at 25°C and examined 7 days after inoculation. Mating studies were not done, but have previously been done for many of the isolates.

BCP casein glucose medium. BCP casein glucose medium is frequently used for the demonstration of typical growth of a dermatophyte and detection of contamination (5). On BCP casein glucose medium, both *T. mentagrophytes* and *M. persicolor* produced flat, spreading colonies within 7 days, but the proteolytic activities of the two dermatophytes differed. *T. mentagrophytes* produced an alkaline pH, visible as a purple coloration of the indicator, within 7 days. *M. persicolor*, by contrast, produced no pH change, leaving the medium its original pale blue color. The appearance of profuse growth without concomitant pH change on BCP casein glucose medium distinguishes *M. persicolor* from many other dermatophyte species (Table 2). Note that Table 2 not only includes species tested in the present study, but also summarizes the results for species tested in previous studies. It is interesting, and perhaps taxonomically significant, that the reaction of *M. persicolor* to BCP casein glucose medium is essentially the same as that of other fast-growing *Microsporum* species, including *M. canis* and *M. gypseum*.

Growth at 37°C. *T. mentagrophytes* isolates consistently showed good growth with abundant production of aerial mycelium at 37°C on peptone glucose agar. All *M. persicolor* strains tested grew poorly under these conditions, giving rise to highly restricted colonies lacking aerial mycelium (Fig. 1). This characteristic is highly unusual in a zoophilic dermatophyte (Table 2).

Peptone glucose-sodium chloride medium. Like *T. mentagrophytes*, in which the morphology and number of macroconidia varies with the strain, many isolates of *M. persicolor* do not readily produce macroconidia. A previous study (7) showed that macroconidial development in granular, velvety, and cottony variants of *T. mentagrophytes* was stimulated by the presence of 3 to 5% sodium chloride. On peptone glucose-sodium chloride agar, *M. persicolor* grew rapidly, producing a flat, fluffy to velvety or powdery colony with a rose, brownish-red, or luteous reverse. Microscopic examination of the 29 isolates studied showed that all produced macroconidia in the presence of either 3 or 5% sodium chloride (Fig. 2). When present, the macroconidia provide a useful tool for the identification of an isolate as *M. persicolor*. They are thin walled, three- to seven-septate, clavate to navicular, finely echinulate, or with scattered warty projections. They measure 5 to 9 by 21 to 58 μm. The production of macroconidia and the development of rosy-hued colonies are also enhanced on pablum cereal agar. However, the macroconidia induced on salt-amended peptone-glucose agar are unique in the degree to which they manifest cell wall roughening. Whereas smooth-walled or nearly smooth macroconidia may be common or even predominant on other inductive media (e.g., in our experience, Difco brain heart infusion agar), they are uncommon or absent on salt-amended peptone glucose agar.

Microscopic morphology: additional characteristics. Other characteristics of the microscopic morphology further serve to distinguish *M. persicolor* from *T. mentagrophytes*. In

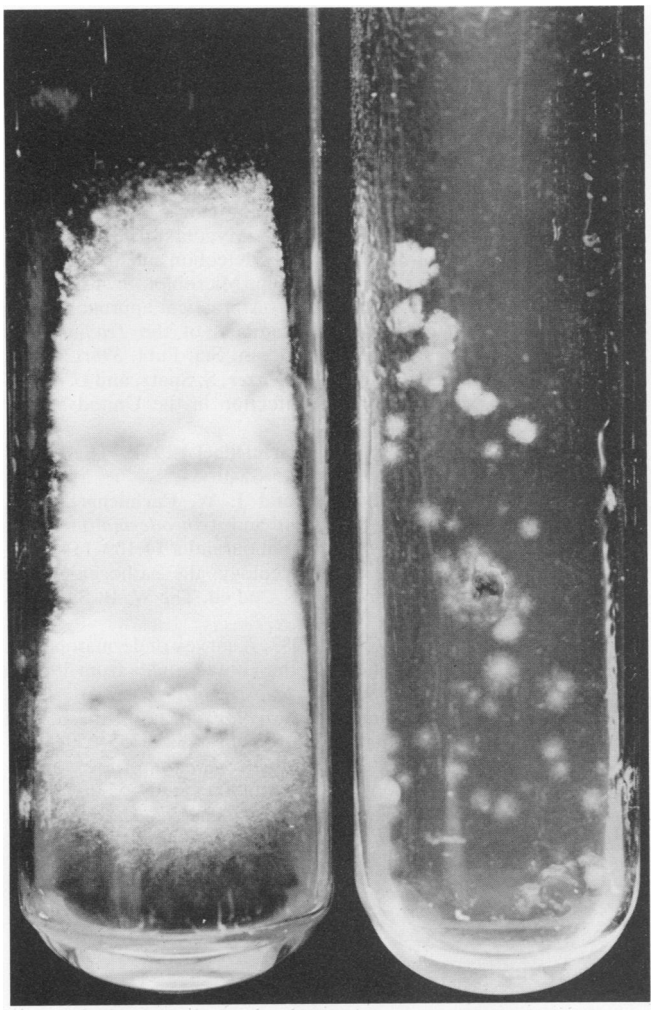

FIG. 1. *T. mentagrophytes* (left) and *M. persicolor* (right) grown on peptone glucose CCG agar at 37°C for 7 days.

zoophilic isolates of the latter, the subglobose or spherical microconidia are sessile or borne on short, swollen pedicels from rather thick (2.5 to 4 μm), fertile hyphae and occur in dense clusters. In *M. persicolor*, the microconidia are pyriform to subglobose with dense cytoplasm and are sessile or borne on short or long, nonswollen pedicels from narrow (1.5 to 2 μm), branched, fertile hyphae (Fig. 3). The conidium-bearing hyphae are surrounded by loose wefts of curved, branched, smooth, broader (3 to 3.5 μm wide) hyphae bearing spiral appendages (Fig. 3). These hyphae resemble the distal branched spiral appendages of fertile ascocarps (19).

Efficacy of determinative techniques. The usefulness of the above techniques and observations for the accurate identification of *M. persicolor* is reflected in Table 1. In 1981 and 1982, before the introduction of these techniques, only two isolates were recognized as *M. persicolor* at the Ontario Ministry of Health. In 1983, with the introduction of the BCP casein glucose test for this purpose, a further two isolates were obtained. In each year since 1983, the number of recognized isolations has been higher (Table 1). The difference in numbers of *M. persicolor* isolates identified in years before the full implementation of the methods described here (1981 to 1983) and subsequent years (1984 to 1986) is highly significant ($P < 0.001$, chi-square test). Since the media used

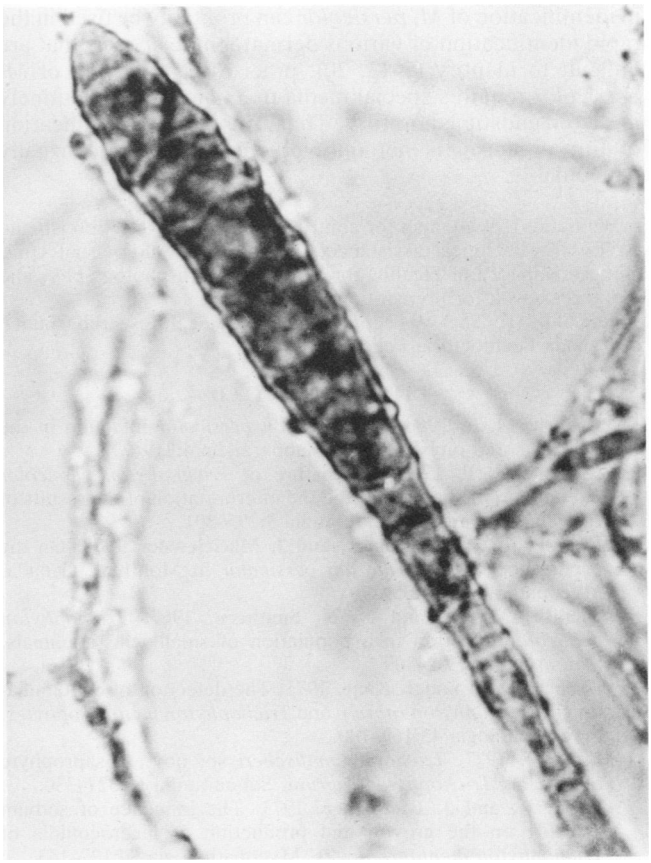

FIG. 2. Rough-walled macroconidium of *M. persicolor* on peptone glucose agar plus 3% sodium chloride. Magnification, ×1,000.

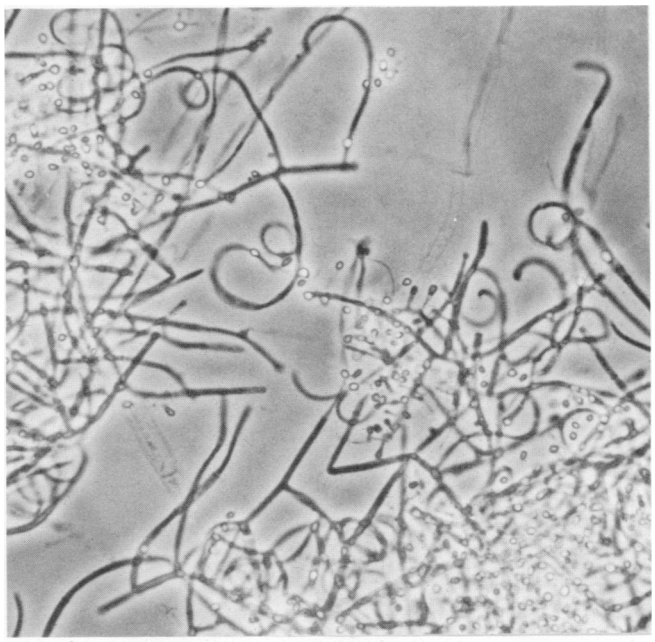

FIG. 3. Pedicellate microconidia and spiral appendages of *M. persicolor*. Magnification, ×460.

in identification of *M. persicolor* can profitably be used in the rapid identification of various dermatophyte species that are difficult to identify (5–13, 20), precise identification of *M. persicolor* requires special media that can be used routinely in the diagnostic laboratory. Thus, the correct identification of this organism is not only practical, but also virtually unavoidable.

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LITERATURE CITED

1. **English, M. P.** 1966. *Trichophyton persicolor* infection in the field vole and pipistrelle bat. Sabouraudia **4**:219–222.
2. **English, M. P.** 1967. The nature of *Trichophyton persicolor* infection in the bank vole and the interpretation of the results of sampling technique. Sabouraudia **5**:295–301.
3. **English, M. P., L. Kapica, and J. Maciejewska.** 1978. On the occurrence of *Microsporum persicolor* in Montreal, Canada. Mycopathologia **64**:35–37.
4. **English, M. P., and N. N. Southern.** 1967. *Trichophyton persicolor* infection in a population of small wild mammals. Sabouraudia **5**:302–309.
5. **Fischer, J. B., and J. Kane.** 1971. The detection of contamination in *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Mycopathologia **43**:169–180.
6. **Kane, J.** 1977. *Trichophyton fischeri* sp. nov., a saprophyte resembling *Trichophyton rubrum*. Sabouraudia **15**:327–330.
7. **Kane, J., and J. B. Fischer.** 1973. The influence of sodium chloride on the growth and production of macroconidia of *Trichophyton mentagrophytes*. Mycopathologia **50**:127–143.
8. **Kane, J., and J. B. Fischer.** 1975. The effect of sodium chloride on the growth and morphology of dermatophytes and some other keratinolytic fungi. Can. J. Microbiol. **21**:742–749.
9. **Kane, J., and J. B. Fischer.** 1975. Occurrence of *Trichophyton megninii* in Ontario. Identification with a simple cultural procedure. J. Clin. Microbiol. **2**:111–114.
10. **Kane, J., A. A. Padhye, and L. Ajello.** 1982. *Microsporum equinum* in North America. J. Clin. Microbiol. **16**:943–947.
11. **Kane, J., I. F. Salkin, I. Weitzman, and C. Smitka.** 1981. *Trichophyton raubitschekii*, sp. nov. Mycotaxon **13**:259–266.
12. **Kane, J., and C. Smitka.** 1978. Early detection and identification of *Trichophyton verrucosum*. J. Clin. Microbiol. **8**:740–747.
13. **Kane, J., and C. M. Smitka.** 1980. A practical approach to the isolation and identification of members of the *Trichophyton rubrum* group. Pan Am. Health Organ. Sci. Publ. **396**:121–134.
14. **Padhye, A. A., F. Blank, P. J. Koblenzer, S. Spatz, and L. Ajello.** 1973. *Microsporum persicolor* infection in the United States. Arch. Dermatol. **108**:561–562.
15. **Padhye, A. A., and J. W. Carmichael.** 1971. The genus *Arthroderma*. Can. J. Bot. **49**:1525–1540.
16. **Padhye, A. A., A. S. Sekhon, and J. W. Carmichael.** 1973. Ascocarp production by *Nannizzia* and *Arthroderma* on keratinous and non-keratinous media. Sabouraudia **11**:109–114.
17. **Rippon, J. W.** 1982. Medical mycology: the pathogenic fungi and the pathogenic actinomycetes, 2nd ed. The W. B. Saunders Co., Philadelphia.
18. **Sinski, J. T., and L. M. Kelley.** 1987. A survey of dermatophytes isolated from human patients in the United States from 1982 to 1984. Mycopathologia **98**:35–40.
19. **Stockdale, P.** 1967. *Nannizzia persicolor*, sp. nov., the perfect state of *Trichophyton persicolor*. Sabouraudia **5**:355–359.
20. **Summerbell, R. C.** 1987. *Trichophyton kanei* sp. nov., a new anthropophilic dermatophyte. Mycotaxon **28**:509–523.
21. **Weitzman, I., M. R. McGinnis, A. A. Padhye, and L. Ajello.** 1986. The genus *Arthroderma* and its later synonym *Nannizzia*. Mycotaxon **25**:505–518.