Differentiation between *Trichophyton mentagrophytes* and *T. rubrum* by Sorbitol Assimilation

A. REZUSTA,*† M. C. RUBIO, AND M. C. ALEJANDRE

Department of Microbiology, Medical Faculty, University of Zaragoza, Zaragoza, Spain

Received 15 February 1990/Accepted 5 October 1990

***Trichophyton rubrum* was easily differentiated from *T. mentagrophytes* by its ability to assimilate sorbitol with an API 20C AUX strip. One hundred percent of 36 *T. rubrum* strains and none of 147 *T. mentagrophytes* strains assimilated sorbitol.**

Pleomorphism and inconsistent conidiation or a lack of conidiation pose major problems for mycologists attempting to identify certain dermatophytes. Until recently, their identification was based almost exclusively on morphologic criteria. In recent years, biochemical tests have proved useful in distinguishing certain dermatophytes.

The recognition of *Trichophyton mentagrophytes* and *T. rubrum* as the predominant species of dermatophytes causing infections in humans (3, 4, 6, 8, 9, 11, 13, 14, 17, 19, 20, 27, 29, 32) has made the differentiation of these two taxa increasingly important. The differentiation of *T. rubrum* and *T. mentagrophytes* is difficult in some situations. Potential solutions include in vitro hair perforation test (1, 2, 23, 32), urea hydrolysis (18, 24), pigment production on cornmeal agar (5), bromocresol purple-milk solids-glucose medium (33), gel immunodiffusion with antisera (10), and others.

In 1923, Hopkins and Iwamoto (15), using the assimilation of various carbon compounds as sole carbon sources, found that differences in the ability to assimilate the compounds depended upon the species as well as the strain. Differences in the techniques used by various authors (16, 22, 25, 28, 34) yielded different results. We have taken advantage of the proven API 20C yeast identification system to differentiate *T. rubrum* from *T. mentagrophytes* by using differences in their ability to assimilate different compounds. In addition to the identification of yeasts, the API 20C system has been used to differentiate some dematiaceous fungi (12) and dermatophytes (7).

One hundred seventy-eight clinical strains isolated by ourselves and five control strains, *T. rubrum* (ATCC 21188), *T. mentagrophytes* (ATCC 21185), *Athroderma benhamiae* (+) (CBS 80872), *A. benhamiae* (–) (CBS 80772), and *A. benhamiae* (+) (11370; Dr. Pereiro's collection), were used in this study. Fungal cultures were identified on the basis of macroscopic and microscopic appearances (21, 26) and in vitro hair perforation (1).

The API 20C AUX system (API, Lyon, France) was used in accordance with the manufacturer's instructions. The strip consists of 1 control cupule and 19 cupules containing different dehydrated substrates: glucose, glycerol, 2-keto-D-gluconate, L-arabinose, xylose, adonitol, xylitol, galactose, inositol, sorbitol, alpha-methyl-D-glucoside, N-acetylglucosamine, cellobiose, lactose, maltose, sucrose, trehalose, melezitose, and raffinose. Mature (7- to 14-day) cultures grown

at 30°C on modified Sabouraud glucose agar were used as a source of inoculum suspensions. Mycelia and conidia were used to prepare a suspension by mixing the fungus with 20 glass balls (3-mm diameter) and 5 ml of sterile distilled water. The suspension was mixed on a Vortex mixer until a density equal to a McFarland no. 3 standard was obtained. From this suspension, 0.5 ml was transferred to 6 ml of API 20C AUX agar medium and inoculated on the strip. We are fairly certain that we did not transfer clumps, but if we did, they were less than 1 mm in size. The cupules, including the control, were filled with the suspension by use of a sterile Pasteur pipette; we ensured that the medium remained convex. The strip was immediately placed in a humid chamber for 7 days and incubated at 30°C. Only the 7-day readings were used for the final analysis.

Of the 19 carbon compounds studied, the following were not assimilated by any of the species tested: glycerol, 2-keto-D-gluconate, L-arabinose, xylose, adonitol, xylitol, inositol, alpha-methyl-D-glucoside, lactose, sucrose, melezitose, and raffinose. The carbon compounds assimilated are shown in Table 1.

Four of the reference strains, *T. mentagrophytes* (ATCC 21185), *A. benhamiae* (+) (CBS 80872), *A. benhamiae* (–) (CBS 80772), and *A. benhamiae* (+) (11370), had the same biotype (glucose, N-acetylglucosamine, cellobiose, and trehalose positive).

For expression of the biotypes, a code was determined in the same manner as that stipulated by the manufacturer for yeasts. We divided the substrates into groups of three and evaluated them in the same manner as the manufacturer, giving a value of 0, 1, 2, and 4 to each of the substrates in each group. For example, 2000340 refers to *T. mentagrophytes* and 2002340 refers to *T. rubrum*. We considered the biotype to be positive when the percentage of positive strains exceeded 84% of all strains studied (30).

We selected an incubation period of 7 days, as it appeared to suit the majority of the species studied. *T. rubrum*, in particular, showed limited growth prior to the end of the incubation period. The API 20C system allows the differentiation of *T. mentagrophytes* and *T. verrucosum* (7). Our results demonstrate that *T. rubrum* and *T. mentagrophytes* can be differentiated on the basis of sorbitol assimilation alone. *T. mentagrophytes* does not grow within 7 days, whereas *T. rubrum* does. A few strains of *T. mentagrophytes* (4.86%) grew to an extremely limited degree because of carry-over in the sorbitol cupule; it was evident, however, that *T. mentagrophytes* did not use this substrate, on the basis of a comparison with the glucose control.

All *T. mentagrophytes* strains were positive and all *T.*

* Corresponding author.

† Present address: Colegio Universitario de Huesca, Pl. Universidad, 3, 22002 Huesca, Spain.

TABLE 1. Assimilation of selected carbon sources

Substrate	No. of isolates that assimilated the carbon source	
	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
Glucose	147	36
Galactose	11	0
Sorbitol	0	36
N-Acetylglucosamine	147	35
Cellobiose	146	36
Maltose	1	0
Trehalose	143	35

rubrum strains were negative in the hair perforation test. The assimilation of sorbitol is as reliable as the hair perforation test, a technique considered excellent (23, 31). The advantage of using the API 20C system as a complement to studies of morphology is that the assimilation results are available in 7 days, in contrast to the 28 days needed by *T. rubrum* for a negative hair perforation test. The application of API 20C for the differentiation of *T. rubrum* and *T. mentagrophytes* reduces time, and the system is easy to use.

REFERENCES

- Ajello, L., and L. K. Georg. 1957. In vitro hair cultures for differentiating between atypical isolates of *Trichophyton mentagrophytes* and *Trichophyton rubrum*. Mycopathol. Mycol. Appl. 8:3-17.
- Ajello, L., and A. A. Padhye. 1984. Dermatophytes and the agents of superficial mycoses, p. 514-525. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
- Alam, S. A., and M. G. Muazzam. 1974. Dermatophytes in Bangladesh. J. Trop. Med. Hyg. 77:267-269.
- Allred, B. J. 1982. Short communication. Dermatophyte prevalence in Wellington, New Zealand. Sabouraudia 20:75-77.
- Bocobo, F. C., and R. W. Benham. 1949. Pigment production in the differentiation of *Trichophyton mentagrophytes* and *Trichophyton rubrum*. Mycologia 41:291-302.
- Bridger, R. C. 1979. Superficial mycoses in a southern New Zealand district. Sabouraudia 17:107-112.
- Calvo, M. A., L. Abarca, and J. Trape. 1981. Estudio comparativo *Trichophyton mentagrophytes* y *Trichophyton verrucosum*. Sabouraudia 19:9-11.
- Del Palacio, A., F. Gonzalez Lastra, and P. Moreno. 1989. Estudio de las dermatofitosis en Madrid durante la década (1978-1987). Rev. Iber. Micol. 6:86-101.
- Doby, J. M., M. Kombila-Favry, J. Chevrant-Breton, and M. T. Boisseau-Lebreuil. 1978. Les dermatophytes isolées au laboratoire de parasitologie du centre hospitalier de Rennes. Arch. Med. L'ouest 10:293-303.
- Dyson, J. E., and M. E. Landay. 1963. Differentiation of *Trichophyton rubrum* from *Trichophyton mentagrophytes*. Mycopathol. Mycol. Appl. 20:81-97.
- English, M. P., and L. Lewis. 1974. Ringworm in the South-West of England 1960-1970, with special reference to onychomycoses. Br. J. Dermatol. 90:67-75.
- Espinell-Ingroff, A., M. R. McGinnis, D. H. Pincus, P. R. Goldson, and T. M. Kerkering. 1989. Evaluation of the API 20 C yeast identification system for the differentiation of some dematiaceous fungi. J. Clin. Microbiol. 27:2565-2569.
- Foged, E. K., and T. Nielsen. 1982. Etiology of dermatophytoses in Denmark based on a material of 1070 cases. Mykosen 25:121-125.
- García de Lomas, J., J. M. Nogueira, C. Segarra, and A. Suay. 1981. *Trichophyton mentagrophytes* var *granulosum*. Actas Dermo-Sif. 72:377-382.
- Hopkins, J. G., and K. Iwamoto. 1923. Fermentation reactions of the ringworm fungi. I. Differentiation of the *Trichophyta* and allied genera from other fungi. Arch. Dermatol. Syphilol. 8:619-624.
- Hopkins, J. G., and K. Iwamoto. 1923. Fermentation reactions of the ringworm fungi. II. Characteristics of three divisions of the *Trichophyton* group. Arch. Dermatol. Syph. 8:839-843.
- Kamalam, A., and A. S. Thambiah. 1976. A study of 3891 cases of mycoses in the tropics. Sabouraudia 14:129-148.
- Kane, J., and J. B. Fischer. 1971. The differentiation of *Trichophyton rubrum* and *T. mentagrophytes* by use of Christensen's urea broth. Can. J. Microbiol. 17:911-913.
- Kilic, M., and S. A. Fazh. 1986. Dermatophytes encountered in skin infections in Kayseri, Central Anatolia. In D. Tümbay (ed.), Abstracts of FEMS Symposium on Dermatophytes and Dermatophytoses in Man and Animals. Bilgeham Publishing House, Bornova, Izmir, Turkey.
- Londero, A. T., and C. D. Ramos. 1980. A twenty-year survey of dermatophytes in the state of Rio Grande Do Sul, Brazil. Proceedings of the Fifth International Conference on the Mycoses. Superficial cutaneous and subcutaneous infections. Scientific publication no. 396. Pan American Health Organization.
- Mackenzie, D. W. R., and C. Philpot. 1981. Isolation and identification of ringworm fungi. Public Health Laboratory Service. Monogr. Ser. 15.
- Mosher, W. A., D. H. Saunders, L. B. Kingery, and J. Williams. 1936. Nutritional requirements of the pathogenic mold *Trichophyton interdigitale*. Plant Physiol. 11:795-806.
- Padhye, A. A., C. N. Young, and L. Ajello. 1980. Hair perforation as a diagnostic criterion in the identification of *Epidermophyton*, *Microsporum* and *Trichophyton* species, p. 115-120. Proceedings of the Fifth International Conference on the Mycoses. Scientific publication no. 396. Pan American Health Organization.
- Philpot, C. 1967. The differentiation of *Trichophyton mentagrophytes* from *T. rubrum* by a simple urease test. Sabouraudia 5:189-193.
- Philpot, C. 1977. The use of nutritional tests for the differentiation of dermatophytes. Sabouraudia 15:141-150.
- Rebell, G., and D. Taplin. 1974. Dermatophytes. Their recognition and identification. University of Miami Press, Miami.
- Rezusta, A. 1987. Tesis. Estudio de las dermatofitosis en Aragón, aportación al comportamiento bioquímico de los Géneros *Microsporum* y *Trichophyton*. Medical Faculty, University of Zaragoza, Zaragoza, Spain.
- Rezusta, A., and M. C. Rubio Calvo. 1988. Differentiation of dermatophytes isolated from clinical specimens with the API 20 C AUX. X Congress of the International Society for Human and Animal Mycology. Rev. Iber. Micol. 5(Suppl. 1):118.
- Rubio, C., A. Rezusta, J. Gil, S. Salvo, and R. Gómez-Lus. 1986. Incidence of dermatophytes in Zaragoza, Spain. In D. Tümbay (ed.), Abstracts of FEMS Symposium on Dermatophytes and Dermatophytoses in Man and Animals. Bilgeham Publishing House, Bornova, Izmir, Turkey.
- Runyon, E. H., L. G. Wayne, and G. P. Kubika. 1974. Family II. *Mycobacteriaceae* Chester 1897, 63, p. 681-701. In R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
- Sinski, J. T., D. V. Avermaete, and L. M. Kelley. 1981. Analysis of tests used to differentiate *Trichophyton rubrum* from *Trichophyton mentagrophytes*. J. Clin. Microbiol. 13:62-65.
- Sinski, J. T., and K. Flouras. 1984. A survey of dermatophytes isolated from human patients in the United States from 1979 to 1981 with chronological listings of worldwide incidence of five dermatophytes often isolated in the United States. Mycopathologia 85:97-120.
- Summerbell, R. C., S. A. Stanley, and J. Kane. 1988. Rapid method for differentiation of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and related dermatophyte species. J. Clin. Microbiol. 26:2279-2282.
- Swartz, H. E., and L. K. Georg. 1955. The nutrition of *Trichophyton tonsurans*. Mycologia 47:475-493.