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Early Detection and Identification of *Trichophyton verrucosum*

JULIUS KANE* AND CATHERINE SMITKA

Laboratory Services Branch, Ontario Ministry of Health, Toronto, Ontario M5W 1R5

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A new medium for the early detection and identification of *Trichophyton verrucosum* has been formulated. The key ingredients of the medium are 4% casein and 0.5% yeast extract. *T. verrucosum* is recognized by its early hydrolysis of casein and very slow growth. Microconidia were produced by 19 out of 35 isolates (54%), and macroconidia were produced by 8 out of 35 isolates (23%). All isolates formed chains of chlamydospores at 37°C, and 24 out of 35 isolates formed chains at 28°C. Nutritional requirements of all 35 strains of *T. verrucosum* were confirmed. The medium was evaluated by isolating 570 suspected *T. verrucosum* from skin scrapings. The early detection of hydrolysis, formation of characteristic chains of chlamydospores, and restricted slow growth of this dermatophyte differentiate it from *T. schoenleinii*.

Trichophyton verrucosum is the common cause of ringworm in cattle in Ontario. Infections occur most frequently during late winter and early spring and present a hazard to those persons caring for cattle.

In this laboratory, human isolates of *T. verrucosum* have been grown most frequently from the trunk, hands, scalp, and face. However, other areas of the body, such as the feet, legs, groin, anus, fingernails, and ears, have also been infected.

T. verrucosum grows poorly on peptone dextrose agar (Sabouraud), and sometimes the growth is hardly noticeable even after 4 weeks of incubation. The slow growth of this zoophilic fungus has presented a serious problem in its isolation and identification. In the past, identification has rested largely upon gross morphology (glabrous), lack of sporulation, capacity to grow well at 37°C (4), and a nutritional requirement for thiamine and inositol. This nondescript appearance is shared by *T. schoenleinii*, but it does not have the nutritional requirement. With either organism, the lack of a characteristic appearance makes detection in primary cultures problematic. Following the introduction of bromocresol purple (BCP) milk dextrose agar in the identification of *T. rubrum*, *T. mentagrophytes* (1), and *T. megninii* (3), studies were made to determine the value of this medium in the identification of other dermatophytes. *T. verrucosum* produced a rapidly clearing zone of hydrolysis around the primary growth. This was emphasized by the BCP indicator and was found to be very useful in indicating the presence of this fungus. Frequently the hydrolysis became apparent before any growth was visible. Rosenthal and Sokolsky (5) noted the hydrolysis of casein by *T. verrucosum* while studying the enzymatic activities of pathogenic fungi.

The purpose of this study is to report the efficacy of BCP casein yeast extract agar medium in the isolation and identification of *T. verrucosum*.

MATERIALS AND METHODS

Cultures. Thirty-five recent human primary isolates from skin scrapings and hair submitted by local physicians to our diagnostic laboratory were used in the experimental study. As our controls, we used 19 cultures of other dermatophytes to compare the degree of casein hydrolysis and rate of growth on the new medium with *T. verrucosum*. Nutritional requirements for inositol and thiamine were determined for all 35 isolates of *T. verrucosum*. All cultures were checked for purity (1). Skin scrapings and hair from patients living in rural areas, or when *T. verrucosum* was otherwise suspected, have been cultured on the new medium since 1972.

Media. Media used in this study were composed of the following substances: (i) BCP 0.25% yeast extract base with 0.5, 1.0, 2.0, and 4.0% casein. (ii) BCP 4% casein base with 0.1, 0.3, 0.5, and 0.7% yeast extract. (iii) BCP with 4% casein and 0.5% yeast extract agar (BCPCYA). (iv) BCPCYA with 0.5 mg of cycloheximide per ml, 50 μg of chloramphenicol per ml, and 20 μg of gentamicin per ml (BCPCYA-CCG). (v) BCPCYA with 2% dextrose. (vi) BCPCYA with 0.25, 0.5, and 0.7% Lab-Lemco beef extract powder (Oxoid code L29). (vii) Peptone dextrose CCG agar (Sabouraud) (1).

Procedures. Thirty-five isolates of *T. verrucosum*

were selected for testing. The cultures were inoculated on BCP yeast extract base slants with various concentrations of casein, and the tubes were incubated at 28°C in order to determine the optimal concentration of casein for the growth of *T. verrucosum*. In the same manner, the isolates were tested on BCP casein base with various concentrations of yeast extract. Once the optimal concentrations of both substances necessary for the earliest detection of the fungus were determined, the effects of dextrose and beef extract on the degree of growth, hydrolysis, and sporulation of *T. verrucosum* were observed. The completely formulated medium was then tested with all isolates at 28°C as well as at 37°C.

Fifteen cultures of other dermatophytes, as well as four isolates of *T. schoenleinii*, were inoculated onto BCPCYA for comparison with *T. verrucosum* and incubated at 28°C. All cultures were checked on a daily basis for 3 weeks.

RESULTS

The results of comparing the growth of 35 isolates of *T. verrucosum* on various concentrations of casein is shown in Table 1. The best growth was obtained with 2 to 4% casein. The addition of 0.3 to 0.5% yeast extract to casein base improved the growth of 7 of the 20 cultures tested. Neither the addition of 2% dextrose nor beef extract to the medium stimulated the growth of *T. verrucosum* as compared to sugar- and beef extract-free media.

Hydrolysis by all 35 isolates of *T. verrucosum* was distinct at 28°C as well as at 37°C (Table 2). Most isolates showed hydrolysis as early as 48 h at both incubation temperatures. Figure 1 shows the hydrolysis of a primary isolate after 6 days of incubation at 28°C. The colony shown is very limited, and its diameter did not outgrow the original inoculum. Findings of the microscopic examination of the tiny colonies produced in 10 days are shown in Fig. 2 and 3. Microconidia were produced by 19 of 35 isolates (54%), and macroconidia were produced by 8 of 35 organisms (23%) at 28°C. Twenty-four isolates formed chains of flattened chlamydospores at 28°C, and all 35 isolates produced these chains at 37°C.

All of the 19 other dermatophytes used as controls for *T. verrucosum* growth on the new medium hydrolyzed casein in 3 to 5 days at 28°C. The aerial mycelium became pronounced during this short time, and its diameter exceeded the original inoculum several times (Fig. 4–7).

The final formula for the BCPCYA medium used for the early detection of *T. verrucosum* is: casein (powdered skim milk), 40 g; yeast extract powder, 5 g; agar, 15 g; BCP (1.6% in alcohol), 1 ml; distilled water, 1,000 ml; cycloheximide, 0.5 mg/ml; chloramphenicol, 50 μg/ml; gentamicin, 20 μg/ml.

TABLE 1. *Growth of 35 isolates of T. verrucosum on yeast extract base with various concentrations of casein in 2 weeks at 28°C*

Degree of growth[a]	No. of isolates in indicated concn of casein (%):			
	0.5	1	2	4
–	11	8	0	0
Tr	6	7	8	7
+ and ++	18	20	25	26
+++	0	0	2	2

[a] –, No growth; Tr, trace; +, 2 to 3 mm; ++, 4 to 5 mm; +++, 6 to 7 mm.

TABLE 2. *Response of 35 isolates of T. verrucosum on BCPCYA medium after 2 weeks of incubation*

Response	No. of isolates at:	
	28°C	37°C
Hydrolysis	35	
Microconidia	19	
Macroconidia	8	
Chlamydospores	24	35

The yeast extract powder is dissolved in 50 ml of the distilled water and filter sterilized. The rest of the ingredients are combined and heated to dissolve them. The pH is adjusted to 7.0, and the medium is autoclaved for 10 min at 15 lb/in^2 pressure. The antibiotics and yeast extract are then added to the medium, which is immediately dispensed into tubes (153 by 30 mm) and slanted.

DISCUSSION

The hydrolysis of casein by *T. verrucosum* is a dependable characteristic which is produced at the onset of metabolism and is clearly shown on BCPCYA. The development of the aerial mycelium of *T. verrucosum* is restricted and slow, and therefore clearly offset by the zone of hydrolysis. The early detection of hydrolysis-compensates for this phenomenon and enables one to suspect the presence of the dermatophyte in only a few days. The microscopic examination of the tiny colonies discloses typical chains of flattened chlamydospores that are characteristic of *T. verrucosum*. The production of chains of chlamydospores on BCPCYA is more rapid at 37°C and frequently observed in 2 to 3 days. The production of micro- and macroconidia from the described medium is also helpful in the identification of *T. verrucosum*. On rare occasions pleomorphic isolates are seen (4 out of 570 primary isolates), and the requirements for thiamine and inositol must be determined. A recent pleomorphic isolate, F6243/78, showed hydrolysis and restricted whitish growth, but did not show any chlamydospores. Three consecutive

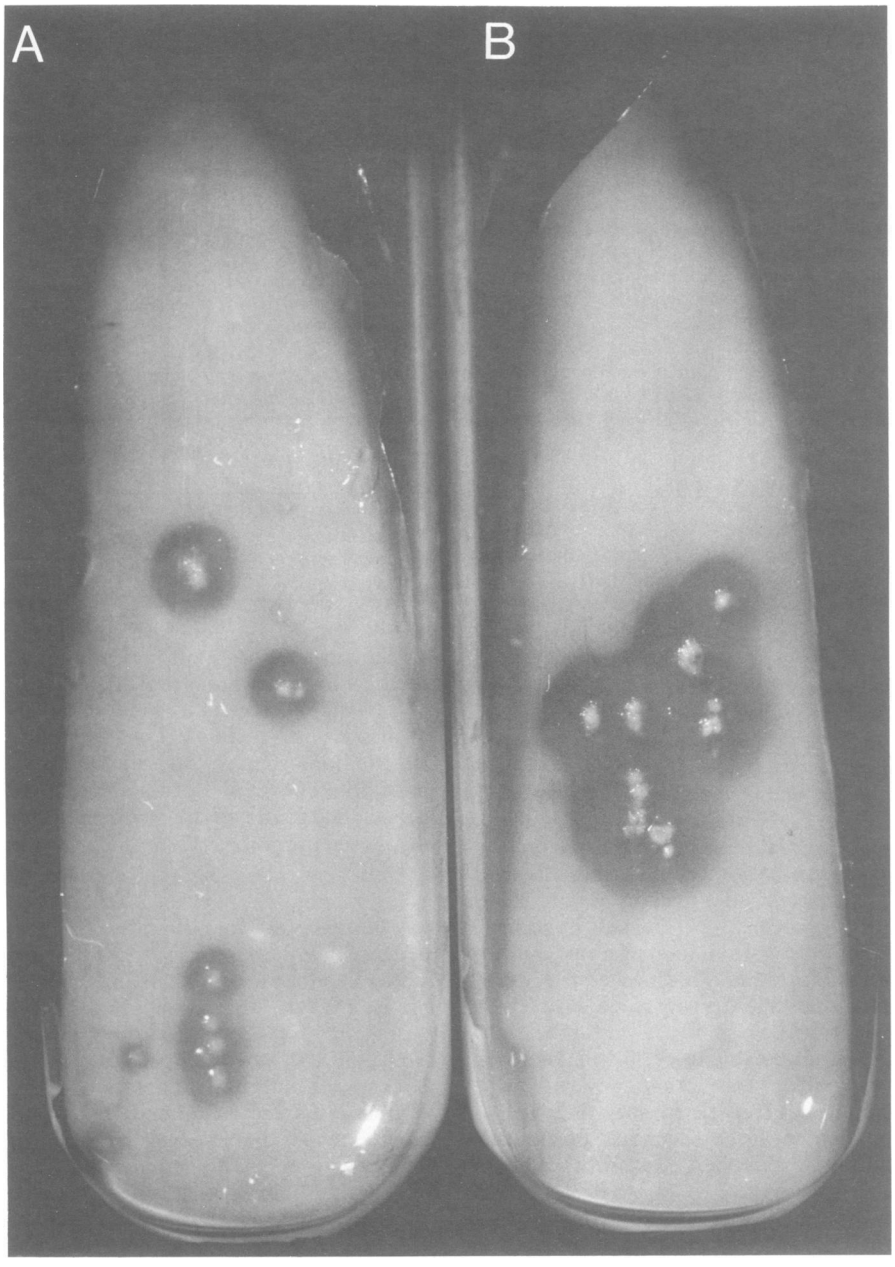

FIG. 1. *Hydrolysis of casein by primary growth of T. verrucosum on BCPCYA in 6 days at 28°C. (A) Single colonies. (B) Group of colonies.*

subcultures on BCPCYA resulted in the production of chains of chlamydospores. A nutritional test confirmed *T. verrucosum*.

Previous studies (1) have shown that dextrose retards the growth of *T. rubrum* in a casein base. By following the findings of Georg (2) with yeast extract, dextrose was replaced by 0.5% yeast extract for the primary isolation of *T. verrucosum*.

Other dermatophytes are able to hydrolyze casein in BCPCYA; however, compared to *T. verrucosum*, their aerial mycelium does not remain restricted to the center but rather covers the hydrolyzed portion of the medium as well.

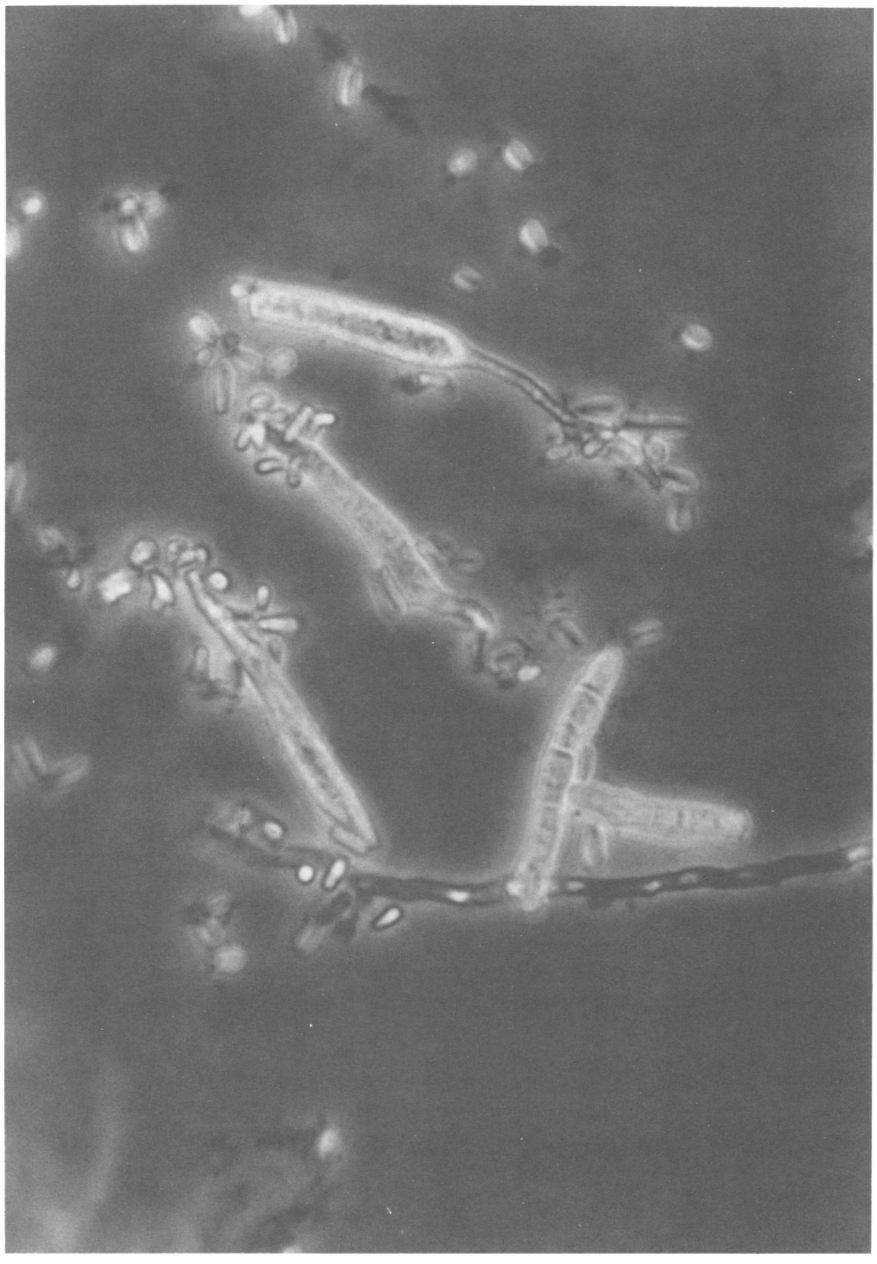

FIG. 2. *Microconidia and macroconidia produced by T. verrucosum on casein yeast extract agar in 10 days at 28°C (phase contrast, ×400).*

The growth of *T. verrucosum* on the new medium is unique and characteristic when compared with that of other dermatophytes.

The growth of a frequent contaminant, *Scopulariopsis brevicaulis*, is often restricted due to CCG present in BCPCYA. This fungus hydrolyzes casein in the medium, but microscopic examination differentiates it easily from *T. verrucosum*. Some bacteria may also hydrolyze casein, but these colonies are soft compared to *T. verrucosum*.

The comparison of growth of *T. verrucosum* and *T. schoenleinii* is shown in Fig. 7. *T. schoenleinii* produces spreading growth on the

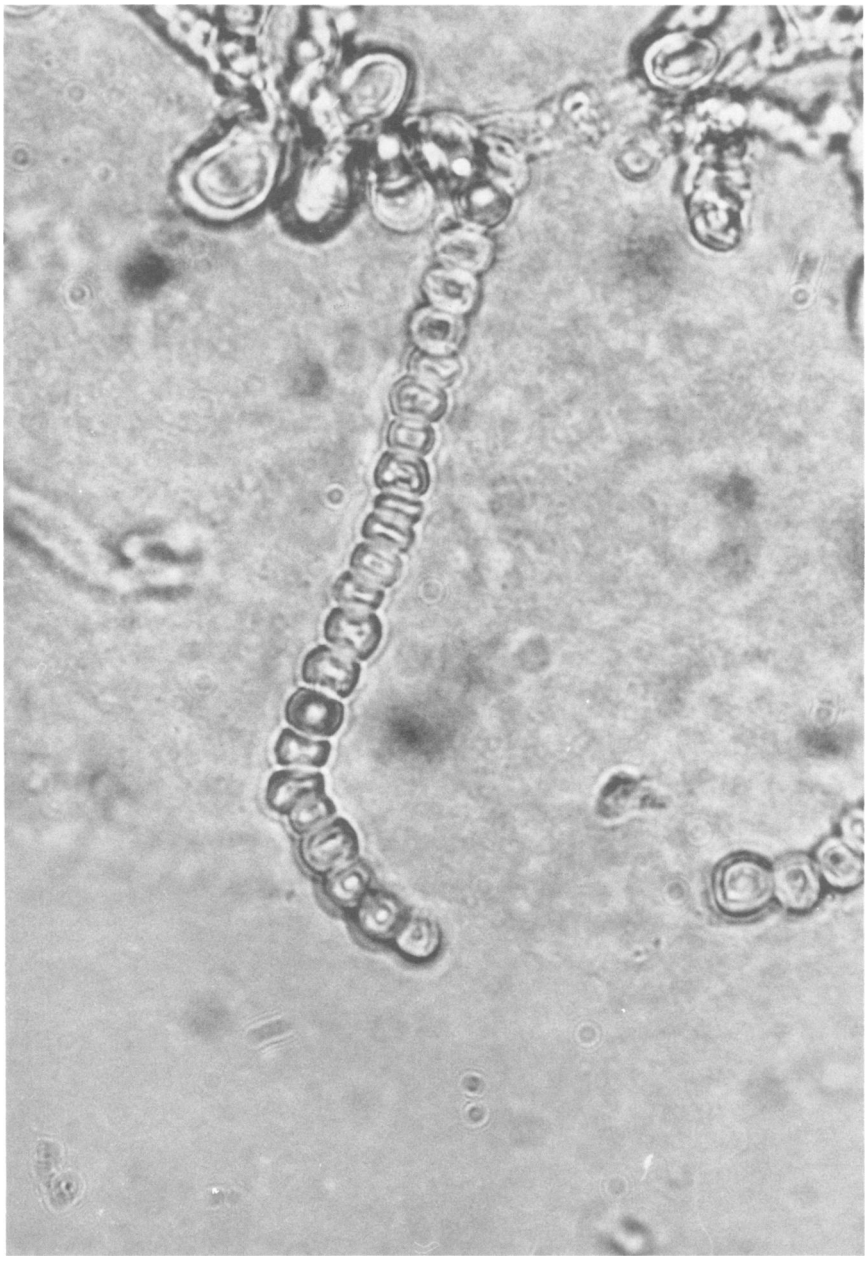

FIG. 3. *Characteristic chlamydospores produced by T. verrucosum on casein yeast extract agar (×400).*

BCPCYA in contrast to the restricted colony growth of *T. verrucosum*. Also microscopic examination of *T. schoenleinii* reveals neither the presence of chains of chlamydospores nor typical macroconidia.

The experimental results with 35 confirmed strains of *T. verrucosum* encouraged adoption of BCPCYA medium for use in our diagnostic work. All skin scrapings and hair of patients from rural areas and where *T. verrucosum* is otherwise suspected are set up on this medium in addition to peptone dextrose antibiotic agar (1). From 1972, when the use of BCPCYA medium began, until 1977, 570 isolates of *T. verrucosum* were detected and identified. Only 4 of these isolates required the nutritional tests de-

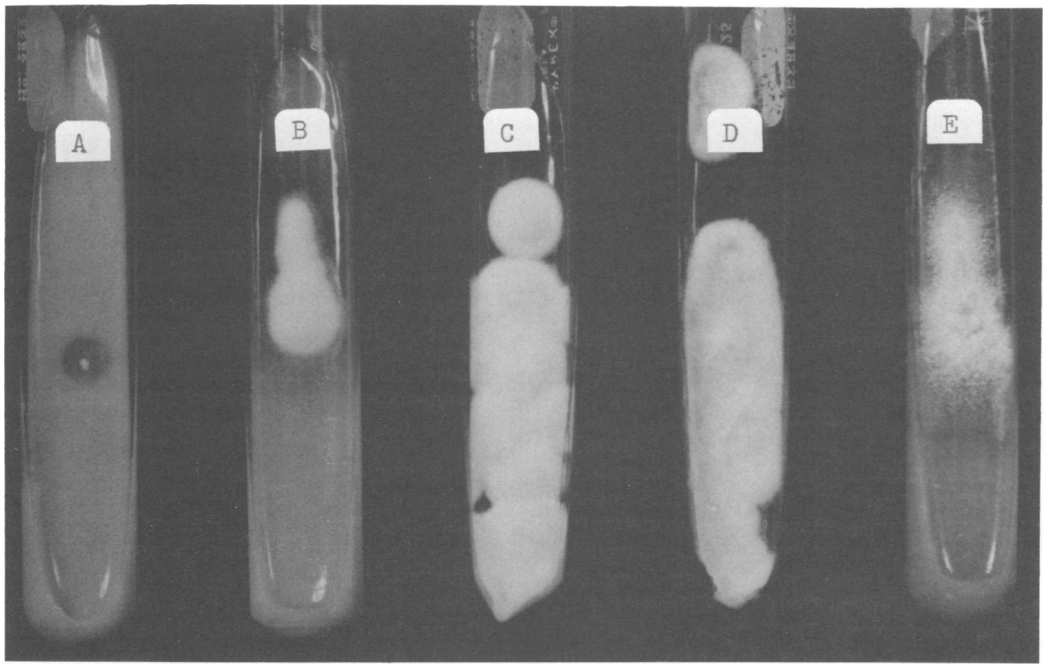

FIG. 4. *Appearance of dermatophytes on BCPCYA in 10 days at 28°C. (A) T. verrucosum; (B) T. rubrum; (C) T. mentagrophytes (cottony type); (D) T. mentagrophytes (velvety type); (E) T. mentagrophytes (granular type). Note unique restricted growth and large zone of hydrolysis in T. verrucosum.*

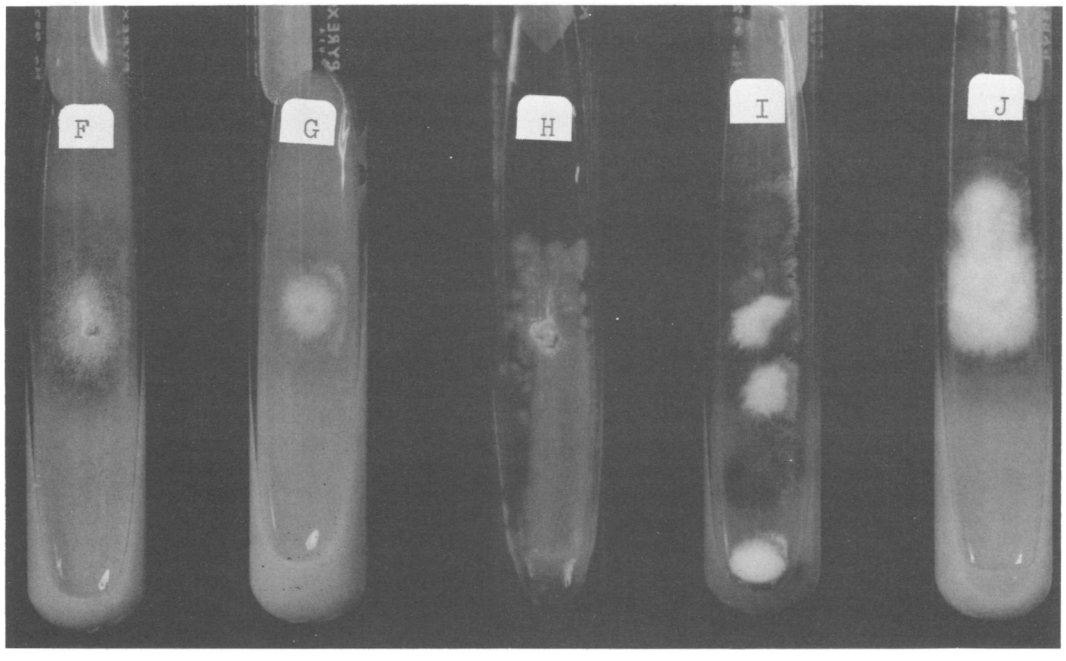

FIG. 5. *Appearance of dermatophytes on BCPCYA in 10 days at 28°C. (F) T. tonsurans; (G) T. megninii; (H) T. schoenleinii; (I) T. soudanense; (J) T. equinum. Note extensive aerial growth covering zone of hydrolysis.*

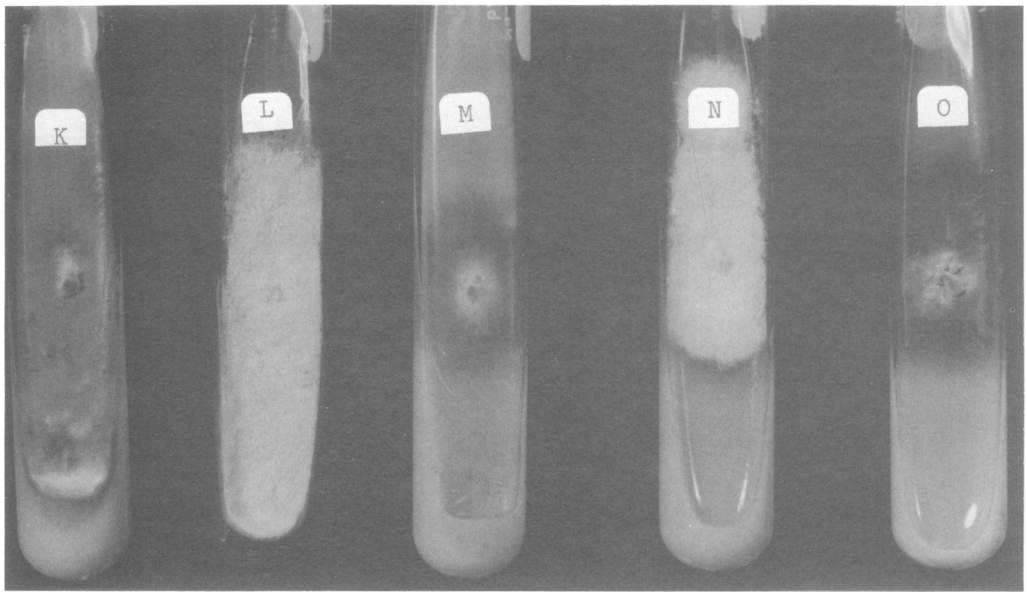

FIG. 6. *Appearance of dermatophytes on BCPCYA in 10 days at 28°C. (K) Microsporum canis; (L) M. gypseum; (M) M. audouinii; (N) M. nanum; (O) Epidermophyton floccosum. Note spreading aerial mycelium in all five dermatophytes.*

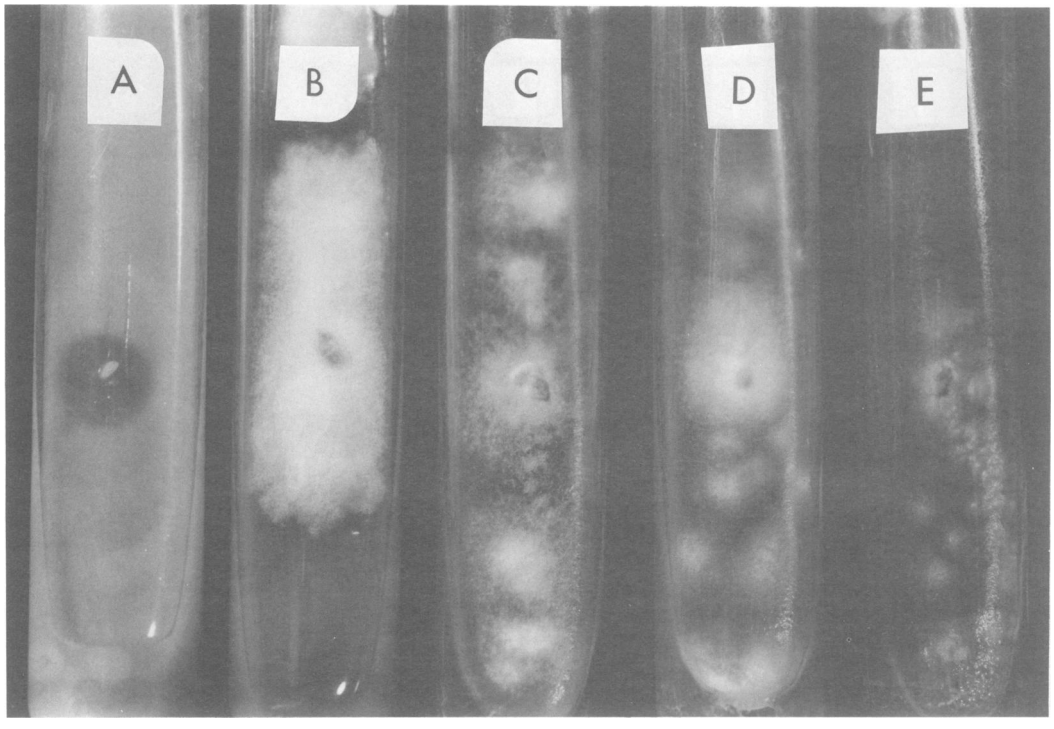

FIG. 7. *Comparison of growth of T. verrucosum (A) with T. schoenleinii (B, C, D, and E). Note the unique restricted growth and large zone of hydrolysis of T. verrucosum.*

scribed by Georg (2). The remainder were identified as *T. verrucosum* by the production of early hydrolysis, very restricted growth, and formation of chains of chlamydospores from primary isolates at 28°C or subsequent incubation at 37°C for 2 to 3 days. The majority of scrapings from which *T. verrucosum* was isolated had mycelium present in the hydroxide mount. In most cases the presumptive diagnosis of the physician was stated as cattle ringworm. As mentioned previously, almost all isolates of *T. verrucosum* come from rural areas where most of the population comes into contact with cattle. A small number of *T. verrucosum* isolates were identified from employees of meat-packing companies. In the identification of *T. verrucosum*, the following criteria are considered: source of specimen, clinical diagnosis, geographic area, hydrolysis of casein, slow, confined growth, chlamydospore production in typical chains, and enhanced formation of typical macroconidia on this new medium. We wish to emphasize that the adoption of BCPCYA in diagnostic work alleviates the fear of overlooking this important dermatophyte.

ACKNOWLEDGMENTS

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