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THE GENUS *DERMATOPHILUS*

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ABSTRACT

GORDON, M. A. (New York State Department of Health, Albany). The genus *Dermatophilus*. J. Bacteriol. **88**:509-522. 1964.—Seventeen strains of *Dermatophilus* originating in skin lesions of cattle, sheep, horses, deer, and man were compared as to conditions for growth, colonial characteristics under varying conditions, microscopic morphology, and biochemical reactions. All grew well aerobically at 37 C and were facultatively anaerobic. They were morphologically similar in both gross and microscopic appearance, and most produced motile spores. Stable gray variants often appeared among the orange-yellow "wild-type" colonies. Acid without gas was produced consistently from glucose and fructose, and transitorily from galactose, but was produced from none of eight other carbohydrates except belatedly by some strains from maltose. Almost all strains hydrolyzed casein, most of them digested BCP milk with varying rapidity, and the majority liquefied gelatin, but there was considerable variation in this last property. Differences crossed both host and geographic lines. It is concluded that all isolates can be accommodated in the species *D. congolensis* Van Saceghem 1915, emend. 1916, 1934, with *D. dermatonomus* and *D. pedis* falling into synonymy.

Microorganisms of the genus *Dermatophilus* were not known to exist in the United States until 1961, when three independent reports of their occurrence appeared. Bridges and Romane (1961) found this bacterium in Texas in skin lesions of two calves, one of them represented by a biopsy taken in 1952; Bentinck-Smith, Fox, and Baker (1961) recovered it in culture from 1 of 15 cases of equine infection in New York and Vermont; and Dean et al. (1961) obtained cultures from lesions in a deer and in two of four cases among men who handled the animal. These are thus far the only reported cases of streptotrichosis in humans. (The commonly employed spelling is streptothricosis. P. K. C. Austwick pointed out to me that streptotrichosis is orthographically the correct form, with which I agree since the combining form of the suffix *-thrix*, taken from the invalid generic designation *Streptothrix*, is *-trichos*.) Despite continuing surveillance at check stations of deer killed by hunters during the past two seasons, conducted by personnel of the New York State Conservation Department and of this Division, no additional infections in animals have been discovered in this state. However, the disease has been found widespread among cattle in Iowa (Pier, Neal, and Cysewski, 1963) and in Kansas (Kelley et al., 1964).

Elsewhere in the Western hemisphere, in addition to an isolate from sheep attributed by Austwick (1958) to Ault in Argentina, cultures have been obtained by Perez Catán and Di Rocco (1963) from "lano de palo" of Argentinian sheep and by Barnum (Ontario Veterinary College, Guelph; *personal communication*, 1961 and 1962) from diseased Canadian cattle. Magallanes et al. (1959) reported many cases among sheep in Uruguay, substantiated by the occurrence of structures characteristic of the microorganism in smears from skin lesions, but got no cultures.

Original descriptions of the three accepted species, *D. congolensis* Van Saceghem 1915, emend. 1916, 1934, *D. dermatonomus* Austwick 1958 (*Actinomyces dermatonomus* Bull 1929), and *D. pedis* Austwick 1958 (*Polysepta pedis* Thompson and Bisset 1957), were made without reference to either of the others. Subsequent isolates have been assigned to species largely on the basis of host animal and type of lesion, for no direct comparison has been made of morphological and physiological characteristics of strains from various sources in widely separated localities, although Bugyaki (1959) stated, without offering supporting evidence, that one species, *D. congolensis*, is responsible for contagious dermatitis of cattle, horse, and sheep in the Congo and Ruanda-Urundi. The present studies were undertaken to supply this lack and thereby to determine whether there are indeed three valid species of *Dermatophilus*.

MATERIALS AND METHODS

The cultures studied are listed in Table 1. In several instances, two descendants of the same original isolate were available, as indicated. These were handled as separate strains for determination of variability. Cultures were maintained by serial transfer on Brain Heart Infusion (BHI) Agar slants incubated aerobically at 36 C (±1) for 3 to 7 days and then stored at room temperature. Stocks were successfully lyophilized in skim milk suspension after growth for 4 days in beef infusion-peptone broth (F5A broth) containing 0.1% glucose and 0.5% sodium chloride. Anaerobic culture was in Brewer jars with illuminating gas, and an atmosphere of varying carbon dioxide content was provided in a cabinet at 37 C by blocks of Dry Ice. Topographic details of colonies on blood-agar plates were examined with the aid of an inverted 10× microscope ocular.

Motility was determined macroscopically by "stab" culture in a semisolid "motility test medium" consisting of 0.3% beef extract, 1% peptone, 0.5% NaCl, and 0.4% agar at pH 7.4. It was read on an arbitrary scale of 1+ to 4+, according to degree of radial extension of growth from the stab. All cultures in this medium were also examined microscopically for motile spores, as were cultures of several strains in F5A broth and on blood-agar (beef heart infusion-agar with 1% peptone, 0.5% NaCl, and 5% defibrinated horse blood). The catalase test was performed by touching one-half loopful of growth on BHI Agar to a drop of Superoxol (30% hydrogen peroxide) on a glass slide and observing for evolution of bubbles.

The sugar fermentation media contained 1% peptone, 0.5% NaCl, 1% of the test carbohydrate, and Andrade's indicator at pH 7.4 to 7.5. Proteolysis test media included: bromocresol purple milk (1 ml of 1.6% alcoholic solution of indicator per liter of skim milk), beef extract gelatin (0.03% beef extract, 0.05% peptone, and 12% gelatin, at pH 7.8), and Loeffler's coagulated serum slants. Urea agar slants consisted of 0.5% NaCl, 0.2% KH_2PO_4, 0.1% peptone, 0.1% glucose, and 2% agar; the mixture was adjusted to pH 6.9, and 6 ml of 0.2% phenol red solution were added per liter. Agar plates containing casein, tyrosine, xanthine, and starch were prepared and read as described by Gordon and Smith (1955) and Gordon and Mihm (1957), except that Lugol's iodine was used to test for hydrolysis of starch.

Inoculum for all of the above media was pipetted from a 4- to 5-day broth culture, except that material for the gelatin stabs, Loeffler's slants, and catalase test was taken from BHI Agar slants. All media were incubated at 36 C (±1), and readings were made at 48 hr, 5 days, 1 week, and 2 weeks. Proteolysis tests often were held longer.

In the preparation of specimens for electron micrography of flagella, strain A-21 was grown on a blood-agar plate at 36 C for 48 hr. A loopful of growth was suspended in one drop of Tryptone broth on a hollow-ground slide and thoroughly mixed with one drop of 0.1% solution of egg albumin. After an interval of 5 to 10 min, one drop of the supernatant was mixed in a second hollow-ground slide with two drops of 3% phosphotungstic acid adjusted to pH 7.0 with 0.1 N KOH, and one drop of this mixture, containing numerous spores, was placed immediately upon a carbon-coated copper grid. After standing a few seconds, excess liquid was withdrawn with a capillary pipette and the grid was placed upon filter paper in a petri dish. Finished specimens were examined with a Siemens Elmiskop I electron microscope at original magnifications of 5,000 to 40,000 ×.

For serological studies, *Dermatophilus* was grown in F5A broth in shake culture at 36 C for 1 week. After centrifugation, the supernatant fluid was Seitz-filtered and added to twice its volume of cold acetone; the resulting precipitate was removed by centrifugation (1,000 × *g*), freed from acetone by vacuum distillation, and dissolved in normal saline to one-half the volume of the original supernatant fluid. This solution was used as antigen in gel-precipitin tests. For preparation of antiserum, mycelium from the above cultures was killed by suspension in 0.5% formalinized normal saline at 4 C for 24 hr; it was then centrifuged, washed, resuspended in saline, heated at 56 C for 30 min, and stored in 0.1% formalin in the cold. Rabbits received a 1:30 (v/v) suspension (washed free from formalin) intravenously, 1, 2, and 5 ml, respectively, being given on the first 3 days of each of 3 consecutive weeks. Blood was drawn 1 week after the last injection.

TABLE 1. *Strains of Dermatophilus studied**

Culture no.	Name and strain no. as received	Source
A-1	*D. dermatonomus* V. 973 α	Isolated from facial lesions of Blackface sheep in Scotland in 1954 by D. I. Nisbet.
A-2, A-8	*D. dermatonomus* V. 973 β	
A-3	"Congolensis group" V. 1240 (A)	Isolated in 1937 by A. W. Stableforth from a horse in London; NCTC 5175.
A-4, A-9	*D. congolensis* V. 1328/1	Isolated in 1956 by P. K. C. Austwick from scabs of cattle (bovine streptotrichosis) sent by S. A. Hutchings from Northern Rhodesia; "designated as neotype"; ATCC 14637.
A-5, A-11	*D. dermatonomus* V. 2623	Isolated in 1957 by D. A. Stewart, New So. Wales, Australia, from skin lesions of sheep (mycotic dermatitis); "designated as neotype"; ATCC 14640.
A-6, A-12	*Dermatophilus* sp. V. 3076	Isolated from a case of mycotic dermatitis in a sheep at Veterinary Investigation Centre, Cardiff, Wales.
A-7	*Polysepta pedis*	Received from R. E. M. Thompson, Bland-Sutton Institute of Pathology, London; ATCC 14641.
A-10	*Dermatophilus* sp. V. 2145	Isolated in 1958 by P. K. C. Austwick from skin lesions on a horse, Wandsworth, London.
A-13	*Dermatophilus* sp. V. 3860	Isolated in 1961 from bovine streptotrichosis by D. A. Barnum, Ontario Veterinary College, Guelph, Canada.
A-14	*D. congolensis* V. 3881/2	Isolated by T. J. Wiktor, Elizabethville, Belgian Congo, from bovine streptotrichosis; received by Austwick in 1961.
A-15	*D. pedis*? V. 3758/A	Isolated in 1961 by G. A. Pepin from strawberry foot rot in a lamb in Staffordshire, England.
A-16	*D. dermatonomus* 294-61	Isolated in 1961 at Guelph, Canada, from lesions on a Holstein cow; received from D. A. Barnum (same strain as A-13).
A-17	*D. congolensis* 1PA 2118	Isolated in 1962 from "lana costrosa" of a sheep from the province of Santa Cruz, Argentina; received from E. Perez Catán, Instituto Nacional de Tecnologia Agropecuaria, Castelar (B.A.).
A-18	*D. congolensis* 1PA 2441	Isolated in 1962 from "lana costrosa" of a sheep from the Province of Chubut, Argentina; received from E. Perez Catán.
A-19	*D. dermatonomus* 450-62	Isolated in 1962 at Guelph, Canada, from skin infection in a bovine; received from D. A. Barnum.
A-20	*Dermatophilus* "5"	Received in 1963 from Donald C. Kelley, D.V.M., Kansas State University, U.S.A. Isolated from cattle.
A-21	*D. congolensis*	Isolated in December 1963, at the Division of Laboratories and Research, N.Y. State Dept. of Health, Albany, from a sample of "lano de palo" (lumpy wool) received from E. Perez Catán July 22, 1963.
Deer	*Dermatophilus* sp. M6444	Isolated in 1960 at the Division of Laboratories and Research from lesions of a dead deer; ATCC 14638.
o	*Dermatophilus* sp. M6444 (orange)	Color variants of the deer isolate.
g	*Dermatophilus* sp. M6444 (gray)	
Sev	*Dermatophilus* sp. M6446	Isolated in 1960 at the Division of Laboratories and Research from furuncles on arm of a man who had handled the dead deer (M6444); ATCC 14639.

* Strains A-1 through A-6 and A-8 through A-15 were received from P. K. C. Austwick, Central Veterinary Laboratory, Weybridge, Surrey, England. Where two culture numbers are listed in the left-hand column for the same strain, duplicate transfers were received at an interval of 8 months, and all were examined independently.

RESULTS

Conditions for growth. All strains of *Dermatophilus* grew well aerobically at 36 C on blood-agar, BHI Agar, Tryptone broth (Difco Tryptone, 1%; NaCl, 0.5%; pH 7.2), and F5A broth. On blood-agar, growth at 27 C was initiated more slowly, but its volume approximated that at 36 C after several days; anaerobic growth at 36 C was substantial but much less than that produced aerobically. No growth was obtained at either temperature on Sabouraud Dextrose Agar (Difco) or on Potato Dextrose, Czapek's solution, or tomato paste-oatmeal agars (Pridham et al., 1957). The organisms were readily recovered in F5A broth from lyophilization in skim milk. Survival during storage on agar slants was irregular, varying from a few weeks to 2 years, but was more prolonged at room temperature than at 4 or 36 C.

Colonial morphology and growth characteristics. There was great variation in colonial topography, a single strain often giving rise to diverse forms on different media, under varying conditions, from one time to another, and even on the same agar plate. Most of the forms appeared under each of the conditions of growth except that of anaerobiosis. Variants included colonies describable as pulvinate to umbonate (A-3 only, Fig. 2), annelliform (Fig. 3), butterfly (Fig. 4), molar tooth (Fig. 5), cake crumb (Fig. 6), umbilicate (Fig. 7), convolute (Fig. 8), montane-ridged (Fig. 9), conical-crateriform (Fig. 10), acuminate-crateriform (Fig. 11), rosebud (Fig. 12a, b), medallion (Fig. 13, 14), conical-montane (Fig. 15), compound conical (Fig. 16), pustular-crateriform (Fig. 17), wheel-crateriform (Fig. 18), wheel-conical (Fig. 19), and cerebriform (Fig. 20). Figure 1 is an example of a mixture of colonial types shown by a single strain.

At 24 hr, aerobic cultures on blood-agar plates incubated at 36 C typically showed tiny (0.5 to 1.0 mm), round to square or irregular, grayish-white, raised, very rough granular colonies (Fig. 21), tough and adherent and usually pitting the medium. Upon further incubation, these generally became yellowish, and then orange, developing pigment first in the more crowded areas, where β-hemolysis also appeared early. Some strains developed pustular-crateriform colonies about 1 mm in diameter within 48 hr. Freshly isolated deer and human strain colonies attained a diameter of 4 to 6 mm in 7 days, with many becoming mucoid apically (Fig. 14). Both deer and human isolates, as well as some of the strains received from other sources (A-7, A-12, A-19), soon developed segregants that remained gray (g) through numerous passages. At first all gray colonies of the deer strain were crateriform and all orange (o) colonies were acuminate, but these later intergraded. Strains A-1 through A-6 were gray or white when first received (on screw-capped agar slants of unknown composition), but all became orange upon blood-agar on first transfer. Subsequently, A-2 and A-5 tended to remain gray on BHI Agar. All strains grown aerobically at 36 C were β-hemolytic; none showed aerial hyphae. No attempt is made here to define the color of the colonies of *Dermatophilus* in very strict terms, since there is considerable variation within the ranges of orange-yellow on the one hand and grayish-white on the other. However, pigment of colonies on blood-agar often appeared to correspond to either Munsell Color Co., Inc. (1929) notation 7.5 YR 6/6 ("cinnamon"; Ridgway, 1912), or Munsell notation 5 YR 6/12 ("orange"; Ridgway, 1912). On Tryptone Agar, our o strain and the A-6 isolate were read as Munsell 10.0 YR 8/6 and matched Maerz and Paul's (1930) "antimony yellow" (but not Ridgway's antimony yellow, which corresponds to Munsell 1 YR 7.4/7.5). Bull (1929) recorded that at 22 C the color in his colonies was close to antimony yellow of Ridgway (1912).

Colonies on blood-agar plates incubated aerobically at 27 C were pinpoint in size at 48 hr, and either gray or orangish. After 7 days, isolated colonies of most strains were 1 to 2 mm in diameter; however, those of two sheep isolates (A-6, 18) were less than 1 mm, and one of the cattle isolates had reached 3 to 4 mm and the g strain, 3 mm. All were hemolytic. Incubation in CO_2 at 37 C resulted in growth comparable to that attained aerobically but with greater variation in rate of growth among strains. The variation crossed host and "species" lines. Colonies displayed a wide range of topographical sculpturing, often including radiating ridges and valleys, and all produced hemolysis.

All strains grew anaerobically, forming colonies of diameter comparable to those grown aerobically but much less voluminous. At 48 hr, they were 0.2 to 1 mm in diameter, typically adherent, with a white umbo sloping to a translucent periphery (Fig. 24). Culture A-5, however, had a

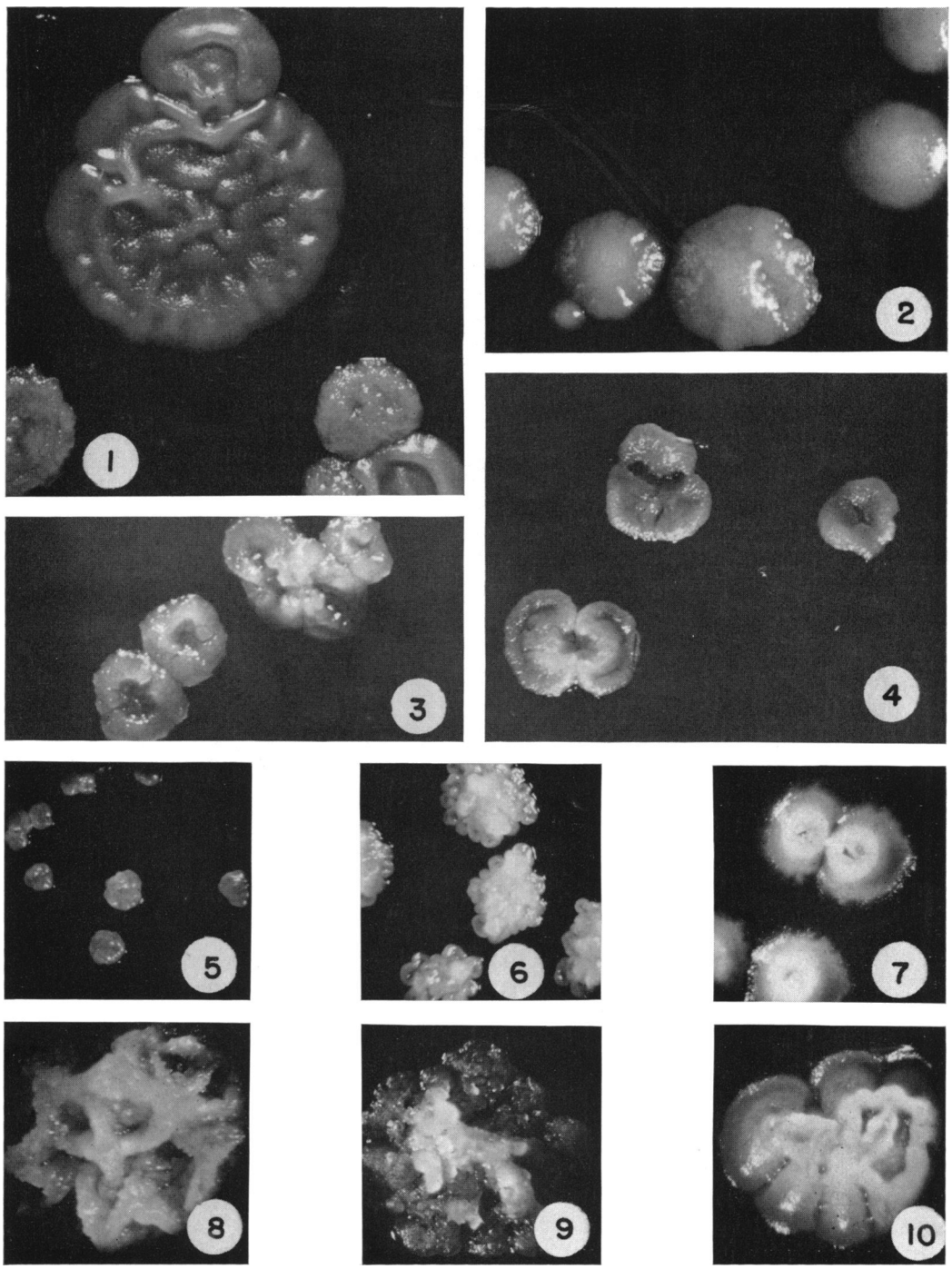


FIG. 1–10. *Colonial variation in Dermatophilus congolensis on blood-agar plates; see text for nomenclature. Figures 1, 3, and 4 (A-4, -11, -6) incubated at 27 C for 7 days. Figures 2 and 5 to 10 (A-3, -11, -11, -20, -19, -12, -7) incubated at 37 C with CO_2: Fig. 5, 2 days; Fig. 6, 7, and 10, 3 days; Fig. 8 and 9, 7 days; Fig. 2, 11 days.* × *9.6.*

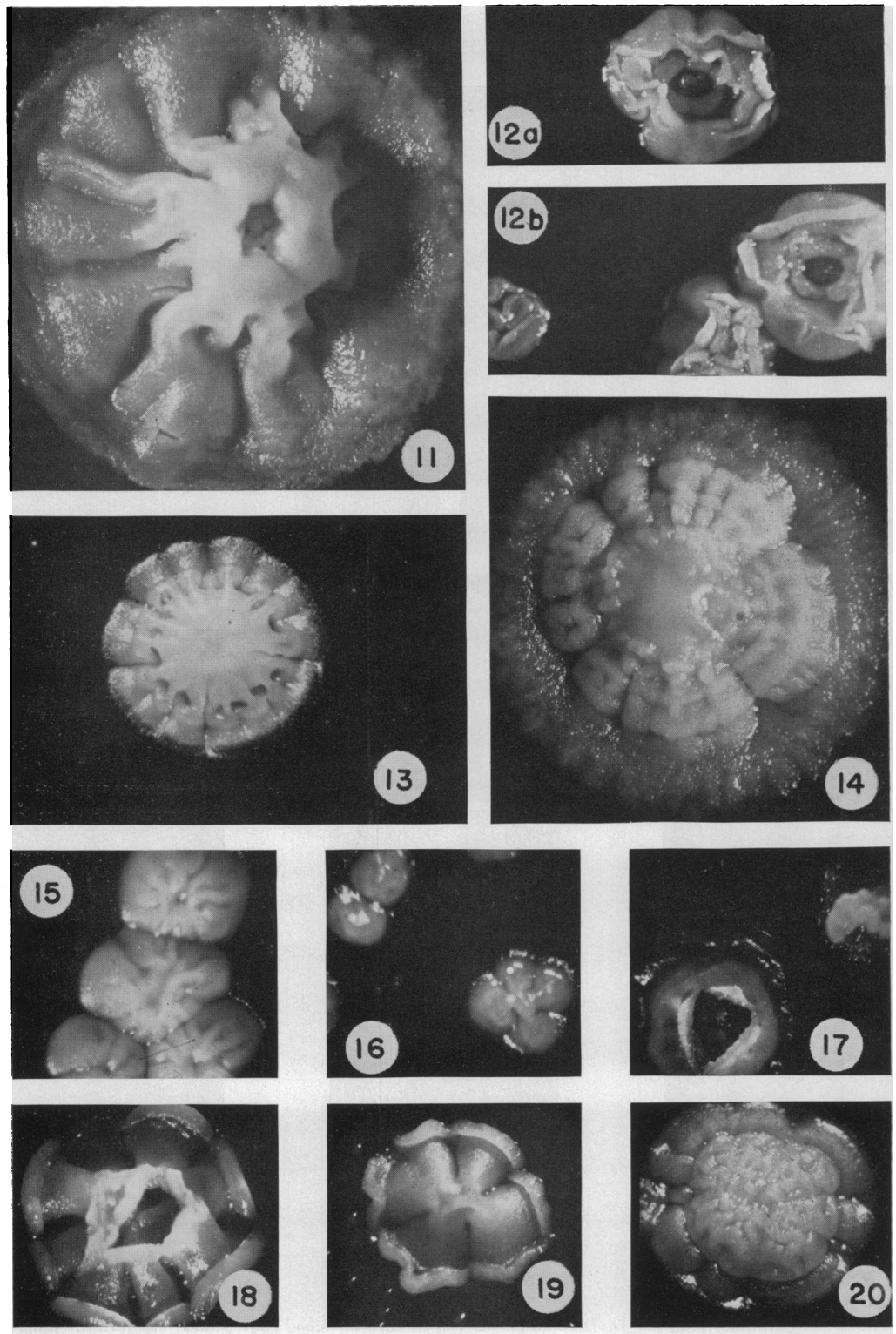


FIG. 11–20. *Colonial variation in Dermatophilus congolensis on blood-agar plates incubated at 37 C with CO_2; see text for nomenclature. Fig. 15 (deer), 3 days; Fig. 12a, b (Sev), 4 days; Fig. 13 and 16 (A-7-g and A-4), 5 days; Fig. 18 and 19 (A-7 and A-6), 6 days; Fig. 17 and 20 (A-12 and deer), 7 days; Fig. 11 and 14 (A-20 and deer), 11 days.* × *9.6.*

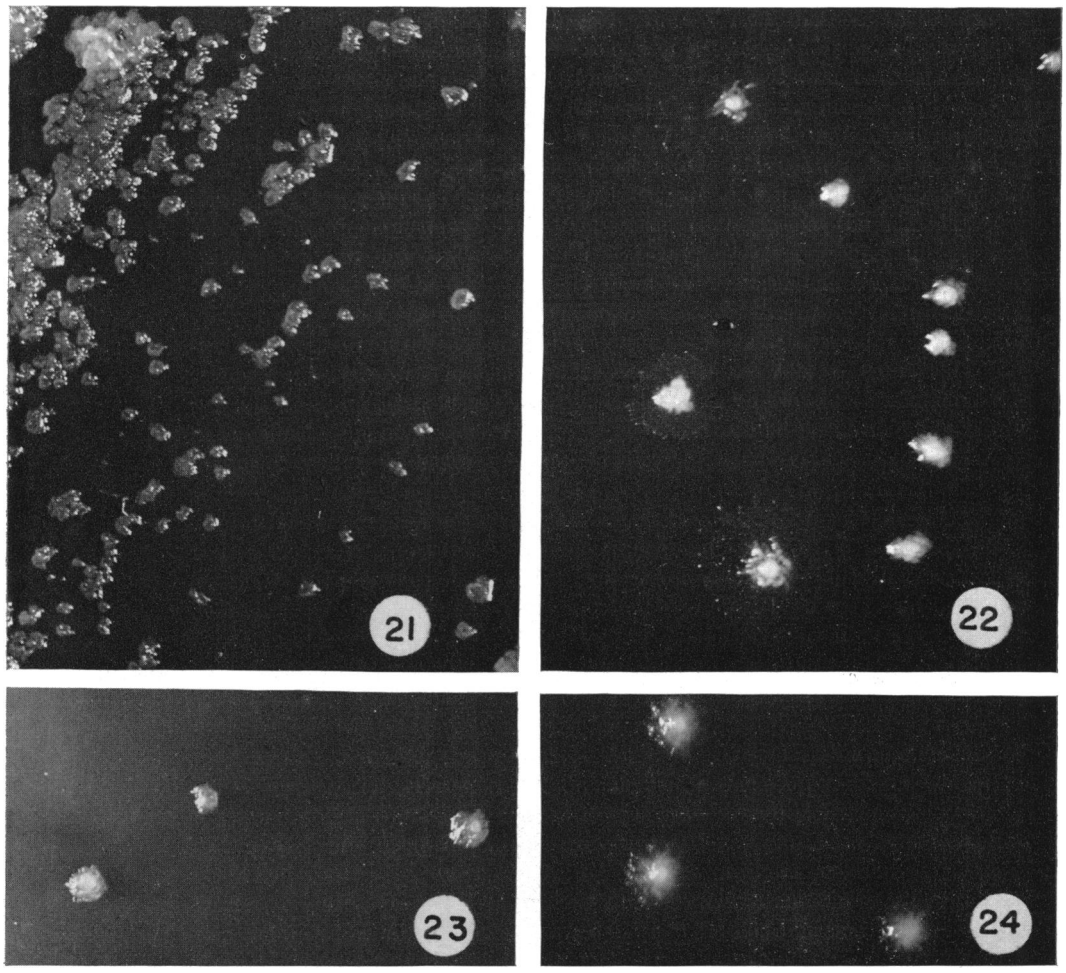


FIG. 21–24. *Young colonies of Dermatophilus congolensis on blood-agar plates. Figure 21 (A-5), aerobic, 24 hr. Figure 22 (A-5), anaerobic, 7 days. Figures 23 and 24 (A-5 and A-19), anaerobic, 48 hr.* × 9.6.

heaped, irregular center (Fig. 23), and A-11, 13S (smooth), 17, 18, and g were molar-tooth type in miniature. There was no hemolysis at this time. After 7 days, colonies varied from 1 to 4 mm in diameter; most were white, umbonate to pulvinate, and radially filamentous, becoming translucent and arborescent peripherally (Fig. 22). Often the apices were yellow or yellowish. Exceptional strains displayed glabrous, molar-tooth to irregularly lobate, nonarborescent colonies, pigmented either yellow or white; those of A-9 and A-17 were annelliform. The centers of larger colonies tended to become convolute or to be surrounded by a circular groove. Hemolysis was present in some strains.

The color and texture of the several isolates took on a somewhat different aspect on BHI Agar slants, where, as a result of heavy inoculation, confluent growth was the rule. After 3 days, this growth was white, gray, yellow, or orange, and of gross appearance variously soft-granular, dry-granular, dry-membranous, or waxy-membranous. When tested with a needle, it was found to vary from completely butyrous (rarely) to soft and occasionally membranous, to very coherent and membranous, being in general markedly adherent to the substrate. Several strains had either a butyrous or a caseous and friable overlay on an adherent base.

In F5A broth, all isolates within 1 week produced a thick sediment, with clear supernatant fluid. Strain o sometimes formed a hard, adherent

ring and A-14 a soft, flocculent ring at the surface, whereas A-5 and g customarily grew in numerous small, hard, discrete colonies, adherent to the submerged wall of the tube. The sediment was generally flocculent, either granular or flaky, but in some strains (A-3) it was ropy or taffy-like. Its appearance varied in the earlier stages of growth, however, depending upon state of maturation (i.e., completion of life cycle), which in turn was a function of strain differences and amount of inoculum. There was no consistent relationship between source of an isolate and time span of its life cycle.

Microscopic morphology. For descriptions and illustrations of sequential stages in the life cycle of *D. congolensis*, recent publications (Roberts, 1961; Dean et al., 1961; and Gordon and Edwards, 1963) should be consulted. The present studies resulted in observations similar to these and revealed no important differences in microscopic morphology among the putative three species. In addition, we succeeded in obtaining electron micrographic evidence of possession of flagella by the motile spores of some strains, the number observed varying from one to several per cell (Fig. 25). They resemble those illustrated by Thompson (1954), and are similar in structure to true bacterial flagella (Edwards, 1963).

Owing to the filamentous nature and coherence of the colonies, and the variations in respective stages of the life cycle, it was difficult to prepare uniform suspensions for comparative microscopy. Stained smears of a given culture might present any of the following appearances microscopically, depending upon age and strain: (i) myriad free coccoid "spores" (Fig. 26), motile or not (these are not conceived to represent the usual bacterial spore); (ii) germinating spores, singly or clustered (Fig. 27); (iii) branching filaments in any or all stages of segmentation (Fig. 28, 29); (iv) mucoid packets or clusters of cocci, either in clumps (Fig. 30) or scattered; (v) nonmucoid clusters, either cubical or irregular (Fig. 31); or (vi) combinations of the above. Cocci or spores predominated in the smoother, softer colonies, and sporulation appeared to be favored by lower temperatures (27 C as against 36 C). After repeated transfers on agar, sporulation tended to decrease or disappear, and germ tubes arose from cells within coccal clumps or fragments of filaments. In F5A broth, hyphae of strain A-5 at times developed round to oval, terminal or sub-

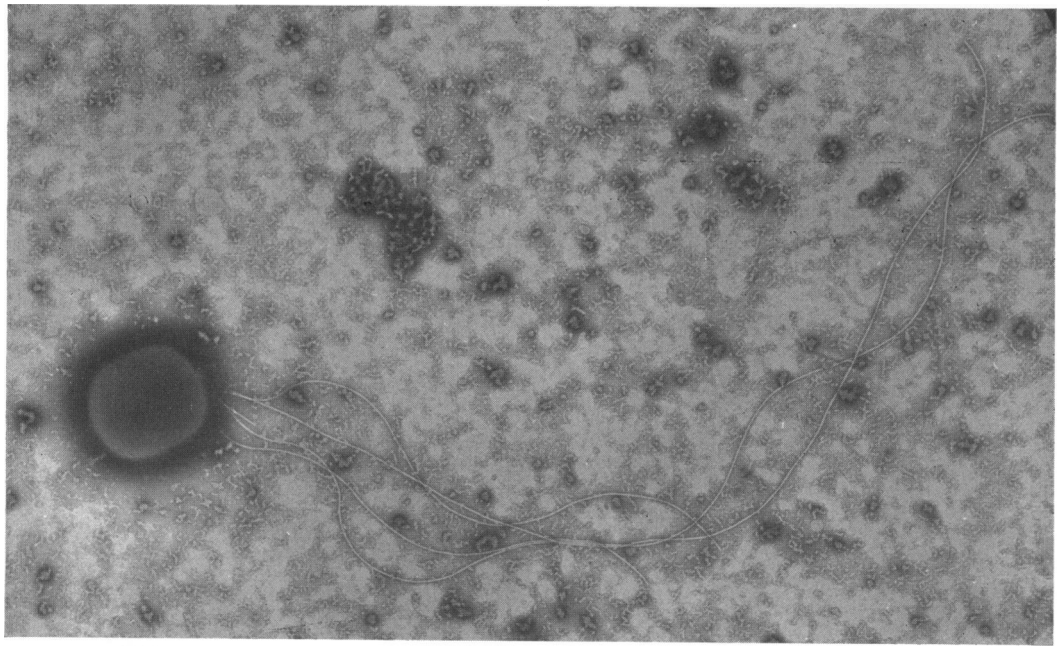


FIG. 25. *Encapsulated motile spore of Dermatophilus congolensis strain A-21, showing multiple bacterial flagella; negative stain.* × *30,000.*

terminal swellings (Fig. 32); A-16 and A-20 displayed an especially broad mature-segmented stage (Fig. 33). Degree of encapsulation (Fig. 34), as determined in India ink mounts, was variable, even between segregants of the same isolate.

Dermatophilus cells tended to retain the crystal violet of the Gram stain rather too heavily for observation of details. They were shown well by methylene blue and Giemsa stain and in infected tissues by the Grocott-Gomori silver and periodic acid-Schiff stains. The organism was not acid-fast when stained by the Ziehl-Neelsen method with decolorization for 10 sec, or by the BH technique (Mariat, 1962). The latter proved excellent, however, for revealing microscopic detail.

Motility. In semisolid "motility test medium," the following cultures displayed motility macroscopically after incubation for 48 hr at 36 C: A-6, 7, 7-g (gray), 9, 14, 16, 17, 18, 19, 21, and o. After an additional 5 days of incubation, A-1, 7-o (orange), 13, and Sev had acquired motility. Serial transfers on this medium failed to induce motility in the remaining strains except for numbers A-4 (second and third transfers after 2 weeks of incubation, and fourth transfer after 1 week), A-12 (as with strain 4; in addition, the first transfer became positive in 16 days), and A-15 (second transfer, after 1 week; third after 48 hr). Another gray variant, 19-g, remained nonmotile, and 13 R (rough) was much more belatedly motile than was 13S (smooth). Numbers A-2, 3, 5, 8, 11, 20, and g also consistently failed to show motility. The stock culture of A-10 was lost before determinations could be made. Hanging-drop examination for motility confirmed its presence or absence in the respective cultures.

Biochemical tests. None of the strains listed in Table 1 formed gas in carbohydrate media. All produced acid in glucose and fructose within 48 hr (5 days for A-2, 8), the acid reaction enduring for at least 2 weeks. None produced acid at any time from sucrose, lactose, xylose, dulcitol, mannitol, sorbitol, or salicin. All but A-2 (slightly acid at 1 week) showed acid in galactose at 48 hr, but this progressively diminished until there was none or only a trace after 2 weeks. On the other hand, all were read as negative in maltose at 48 hr, but most became slightly or distinctly acid in 1 or 2 weeks.

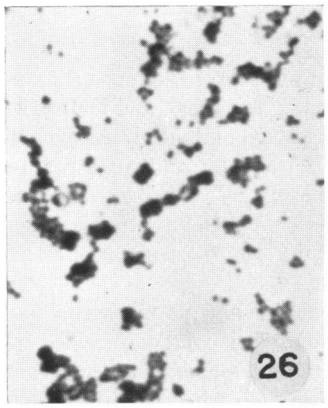
All isolates hydrolyzed urea within 24 hr, produced catalase within 5 days (7-o stronger than 7-g; o stronger than g and deer), and hydrolyzed starch within 1 week. None hydrolyzed tyrosine or xanthine, reduced nitrate to nitrite, produced indole, or gave methyl red or Voges-Proskauer reactions (all of these were read at 1 and 2 weeks). Proteolytic properties varied among strains and are summarized in Table 2.

Serology. On Ouchterlony plates, serum from rabbits hyperimmunized to o antigen yielded two precipitin lines with both o and g antigens. These two antigens also reacted with sera from sheep, goats, and rabbits experimentally infected with the deer strain, producing one to three lines. They failed to react with normal or antihistoplasma rabbit serum. Serum from the goat formed two lines with both o and g antigen (which showed mutual identity) but none with antigens of *Nocardia asteroides* or *Actinomyces bovis*.

Host specificity. Although these experiments have been reported previously (Dean et al., 1961), results are mentioned here because of their pertinence to the question of classification of species by host preference. It was demonstrated by pure culture inoculation of animals that deer and human isolates attacked cattle, sheep, goats, deer, rabbits, and mice impartially. Similar observations by others will be discussed below.

DISCUSSION

Properties of the three species of *Dermatophilus* as described by their respective authors fall within the range of variation ascribed herein to *D. congolensis*. Van Saceghem's (1934) isolate failed to digest milk, but most cattle strains in our hands were found to be strongly proteolytic. He also reported acid from sucrose and none from levulose. Thompson (1954) characterized *Polysepta pedis* as an obligate aerobe, but the culture that Dr. Thompson sent us was found to grow anaerobically under the conditions of our laboratory. Thompson and Bisset (1957) stated that *P. pedis* failed to liquefy gelatin, whereas we found that different segregants of *P. pedis* varied in this respect, some being strongly proteolytic. Bugyaki's (1959) *D. congolensis* was reported to reduce nitrates to nitrites, but none of our strains did so. Mémery (1961) tabulated morphological and biochemical characteristics determined in his own and preceding studies of microorganisms cultured from streptotrichosis of cattle, sheep, goats, and horses, all apparently referable to the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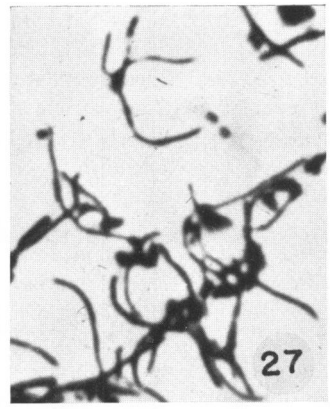
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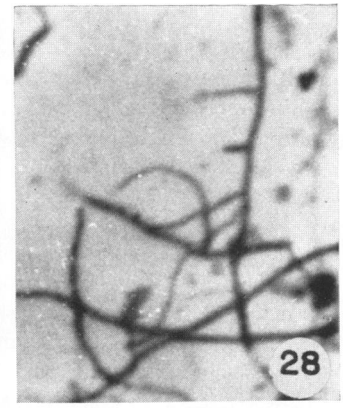
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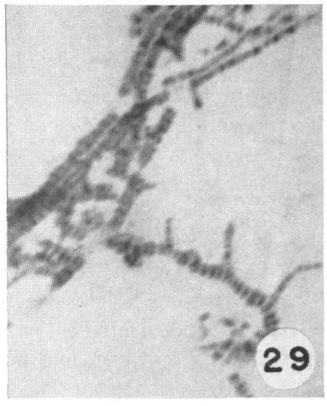
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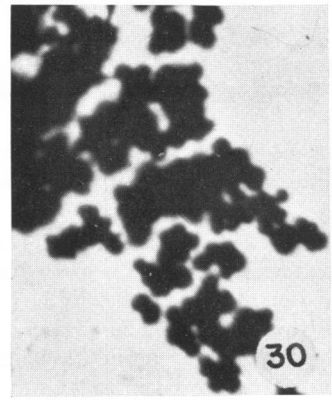
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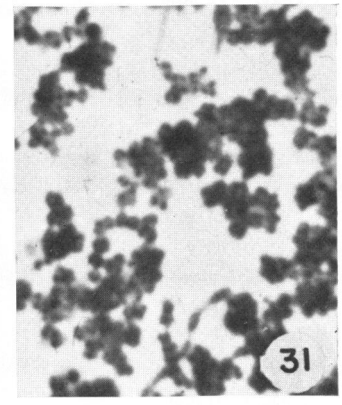
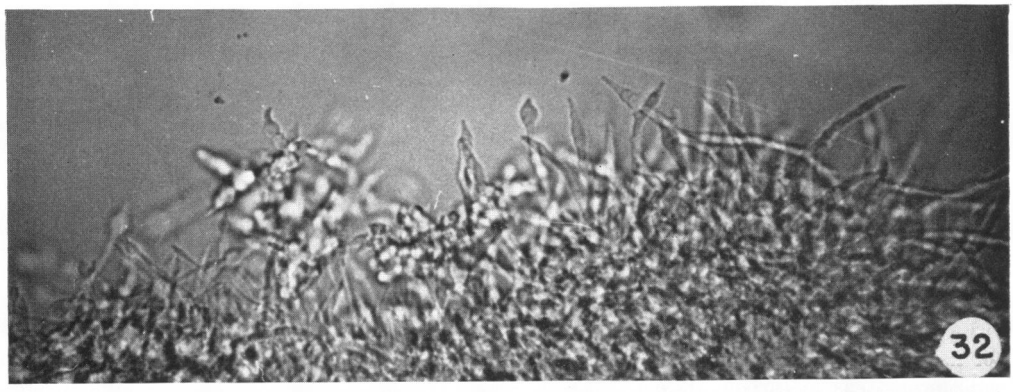
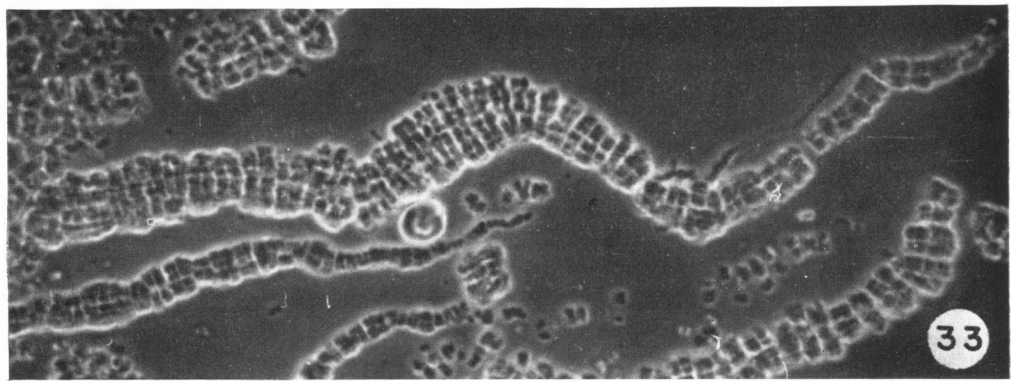

31


32


33

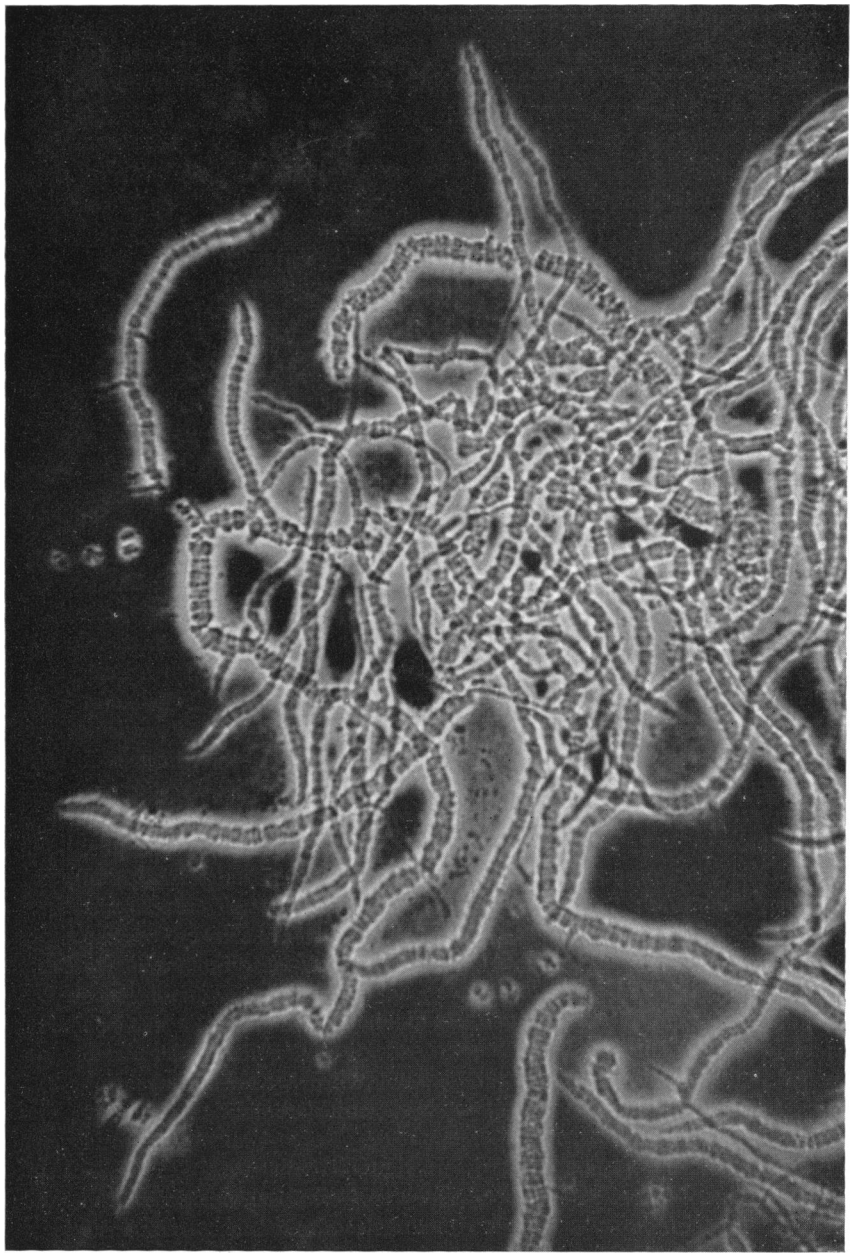


FIG. 34. *A-20, 8-day culture in F5A broth, displaying encapsulation; India ink mount. × 500.*

FIG. 26–33. *Cyclical and strain variation in microscopic appearance of Dermatophilus congolensis. (26) A-13, free coccoid spores from BHI Agar, 7 days; Gram stain. × 1,200. (27) A-16, germinating clustered spores, BHI Agar, 3 days; Gram stain. × 1,200. (28) Deer strain, early branching filamentous stage, blood-agar, 48 hr; methylene blue stain. × 1,200. (29) A-6, early segmentation stage, BHI Agar, 3 days; BH (acid-fast) stain. × 1,200. (30) Sev, mucoid coccal packets, BHI Agar, 3 days, Gram stain. × 1,200. (31) A-3, nonmucoid coccal clusters, BHI Agar, 3 days, BH stain. × 1,200. (32) A-5, edge of colony in F5A broth at 24 hr, displaying subterminal hyphal swellings; wet mount. × 500. (33) A-20, broad, mature-segmented hyphae in F5A broth at 24 hr; wet mount, dark phase-contrast. × 500.*

TABLE 2. *Proteolytic activity of Dermatophilus cultures**

Strain no.	Gelatin		Loeffler's serum medium		BCP milk		Casein agar	
	1 week	3 weeks	1 week	3 weeks	1 week	3 weeks	1 week	2 weeks
A-1		0		0	0	1+		0
A-2		0		0	0	1+	1+	1+
A-3	0	1+	0	0	0	2+	3+	3+
A-4	0	4+	1+	1+	2+	4+	4+	
A-5		0		0	0	2+		0
A-6	0	3+	0	3+	4+		4+	
A-7	2+	4+		0	2+	4+	4+	
A-7-o		0	0	1+		0	4+	
A-7-g	1+	4+	0	2+	2+	4+	4+	
A-8		0		0	0	2+	2+	4+
A-9	4+		2+	3+	0	1+	4+	
A-10	4+		2+	4+	4+		4+	
A-11		0		0	0	4+	1+	3+
A-12		0		0	2+	4+	3+	4+
A-13	4+		2+	3+	4+		4+	
A-14	4+		2+	3+	4+		4+	
A-15	4+			0	2+	4+	4+	
A-16	4+		1+	1+	4+		4+	
A-17	0	4+	1+	3+	2+	4+	4+	
A-18	2+	4+	1+	1+	0	4+	4+	
A-19	4+		1+	1+	4+		4+	
A-19-g		0		0	4+		4+	
A-20	4+		0	1+	4+		4+	
Deer	4+		0	4+	4+		4+	
o	4+		1+	2+	4+		4+	
g	0	3+	1+	3+	2+	4+	4+	
Sev	1+	4+	2+	3+	4+		4+	

* Scale of 0 to 4+ refers, respectively, to degrees of: (1) liquefaction of gelatin stab; (2) clearing and softening of Loeffler's coagulated serum slant; (3) peptonization of bromocresol purple milk; and (4) clearing of casein agar. Suffixes o and g represent orange and gray variants, respectively, of the correspondingly numbered strains.

genus *Dermatophilus*. All are compatible with our strains with minor differences, mostly in biochemical determinations, readily ascribable to variations in media, techniques, and methods of reading results in the respective laboratories. Mémery, for example, added 10% serum to his carbohydrate media and observed acid formation in some of the substrates reported elsewhere as nonreactive. He concluded, nevertheless, that the differences among strains of all origins were less real than apparent, and that exceptions to the general pattern did not permit separation into distinct biochemical types.

The most obvious variations among our strains occurred in their colonial pigmentation and in proteolytic ability, but these differences could not be correlated with host species and, in fact, were in some cases observed among variants of a given isolate. There was a tendency, however, for sheep isolates to be slower than others in liquefying gelatin and peptonizing milk. Snijders and Jansen (1955), comparing *Streptothrix bovis* of cattle with *Actinomyces dermatonomus* of sheep, concluded that slight morphological and biochemical differences between the two were mainly the result of more active growth of the cattle isolates. In our hands, o and g variants of a given isolate often differed in microscopic morphology and in biochemical characteristics (e.g., intensity of catalase reaction and rate of proteolysis), as did other independent descendants of a single isolate, e.g., A-1 versus A-2, A-4 versus A-9, A-5 versus A-11, and A-6 versus A-12. The reactions reported by Bentinck-Smith et al. (1961) for their culture, whose major characteristics were considered identical with those of Stableforth (1937), differ from those of our A-3, a descendent of Stableforth's isolate, in motility, rapid liquefaction of gelatin and peptonization of milk, and in growth on Sabouraud medium. The reactions of Pier's cattle isolate were practically identical with those of our deer strain when compared directly (Pier et al., 1963). Cultures of *A. dermatonomus* and *Streptothrix congolense* sent to me by Peter W. Thorold from Kenya in 1952 displayed soft, granular, respectively orange and yellowish bacterial-type colonies, and appeared microscopically to be composed exclusively of coccal packets until early examination of subcultures revealed the presence of branching mycelial forms.

Susceptibility to infection with the various isolates, as well as differences in colonial morphology and pigmentation, microscopic morphology, and biochemical activities, cuts across host lines. Van Saceghem (1916) observed his organism in the skin of sheep and goats as well as cattle; later (Van Saceghem, 1934), he stated that horses, goats, and sheep can be infected with *D. congolensis* but not so severely as are cattle. Stableforth (1937) found his equine isolates to be closely related to those obtained from cattle and sheep in culture characteristics, microscopic morphology, and fermentation reactions, but to produce different lesions in experimentally inoculated rabbits. Bovine and ovine strains

from Kenya, studied by Hudson (1937), afforded cross-protection against each other in rabbits. Edgar and Keast (1940) experimentally infected a sheep and a calf with a horse isolate that they called *Actinomyces dermatonomus*. Dean et al. (1961) succeeded in infecting a deer, a calf, and two sheep with both human and deer isolates. Pier's cattle strain was able to infect a calf, goat, lamb, and pony (Pier et al., 1963).

Van Saceghem (1916) considered his organism to be a filamentous bacterium composed of a zooglea with fine (0.3 μ) granulations in the interior. There is now considerable support (Austwick, 1958; Roberts, 1961; Gordon and Edwards, 1963) for the inclusion of *Dermatophilus* in the order Actinomycetales (Buchanan 1917, 1918). Within this order, Austwick created a new family, Dermatophilaceae, to be grouped together with the family Actinomycetaceae, or fragmenting actinomycetes, of Waksman and Henrici (1943). Mémery (1961) held that *Dermatophilus* may be included within the Actinomycetaceae according to the classification of Prévot (1958), obviating the need to create a new family, but the unique manner of spore formation in *Dermatophilus*, as well as the motility of the spores, seems to me to constitute sufficient reason to erect a separate family.

Taken together with the results of our comparative studies, the differences among *D. congolensis*, *D. dermatonomus*, and *D. pedis*, as summarized by Austwick (1958), appear to fall well within intraspecific bounds. It is, therefore, suggested that the synonymy of *Dermatophilus congolensis* be expanded to include *D. dermatonomus* (Bull, 1929) Austwick 1958; *Polysepta dermatonomus* Thompson and Bisset 1957; *Nocardia dermatonomus* Henry 1952; *D. pedis* Austwick 1958; *Polysepta pedis* Thompson and Bisset 1957; and *Rhizobium* sp. Thompson 1954. Austwick's description of *D. congolensis* would then be expanded as follows. Mycelial filaments nonseptate at apices, 0.5 to 1.5 μ in diameter, up to 5.0 μ wide after several transverse and longitudinal divisions. Colonies on solid media rough or smooth, grayish-white becoming yellowish or orange with age and sometimes viscous; adherent to medium. Aerobic and facultatively anaerobic, growing more rapidly at 37 C than at room temperature. Mycelium and spores gram-positive, not acid-fast. Acid produced within 48 hr in glucose, fructose, and transitorily in galactose; often belatedly from maltose. No fermentation or acid production in sucrose, lactose, xylose, dulcitol, mannitol, sorbitol, or salicin. Catalase, urease, and amylase are produced. Casein is hydrolyzed; Loeffler's coagulated serum medium is liquefied at varying rates or not at all; bromocresol purple milk is peptonized by most strains within 1 week; gelatin is liquefied very rapidly by some strains and slowly or not at all by others; causes streptotrichosis in cattle, sheep, horse, goat, deer, man, and other mammals.

Sarcinomyces would have been a fitting name for *Dermatophilus* but has been pre-empted by Lindner (1898) for organisms that are reminiscent of *Dermatophilus* but cannot be identified with it on the basis of Lindner's description. A culture of *Sarcinomyces crustaceus* obtained by us from the Institute for Fermentation, Osaka, Japan, in 1958 proved to be *Pullularia pullulans*. Cooke (1962) placed both these latter names in synonymy with *Aureobasidium pullulans*. *Mycosarcina* is suggested as a replacement if *Dermatophilus* should be rejected for any reason.

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