Geodermatophilus, a New Genus of the
Dermatophilaceae (Actinomycetales)

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Organisms having a complex life cycle similar to the animal pathogen Dermatophilus congolensis have been isolated from soils of the United States. The soil isolates grow readily on agar media producing a black, friable, or sooty colony; in broth, the isolates produce a thick sediment with a clear supernatant fluid and occasionally a black surface pellicle. The generic taxon Geodermatophilus is proposed for these soil isolates. Geodermatophilus differs morphologically from Dermatophilus in thallus shape, ensheathment, and branching, and physiologically in respect to blood hemolysis, casein hydrolysis, carbohydrate utilization, acid production, and media requirements. The type species proposed is G. obscurus. Three subspecies are recognized.

During the isolation of microorganisms from soil samples, cultures of a black, friable organism appeared repeatedly from a soil sample obtained in the Amargosa Desert of Nevada. Macroscopically, these cultures resembled some of the black, sooty molds, and microscopically they were composed of greenish-black, cushion-shaped aggregates of small, cuboidal cells. The cultures were found to be insensitive to antifungal agents but sensitive to antibacterial antibiotics.

The morphological development of the organism could not be ascertained from a study of the colonial development on agar plates or growth in shaken broth culture. Still cultures composed of a few sterile alfalfa seeds in 10 ml of sterile tap water were inoculated with some of the black cellular material. After 1 month, a water mount was made of some of the black granular material at the bottom of the tube; with a phase-contrast microscope, in addition to the cushion-shaped aggregates of greenish-black cells, a few small filaments were found. These filaments were transversely separtate and were composed of cells which appeared as "longitudinally compressed spheres," as described by Roberts (8) in characterizing young filaments of Dermatophilus dermatonomus (Dermatophilaceae, Actinomycetales). With this clue as to the possible identity of these organisms, additional broth still cultures were studied and the remaining morphological forms in the life cycle of these puzzling isolates were discovered.

Literature reviews of the genus Dermatophilus have been published by Austwick (1) and Gordon (3). Roberts (8) has studied the life cycle of D. dermatonomus in detail by light microscopy, and Gordon and Edwards (2) have studied D. congolensis by electron microscopy.

MATERIALS AND METHODS

The following cultures and isolates were used in this study: D. congolensis ATCC 14638; D. dermatonomus ATCC 14640; D. pedis ATCC 14641; soil isolates G-7, G-12, G-13, and G-20, obtained from a soil sample from the Amargosa Desert, Nev.; soil isolate G-5, obtained from a soil sample from Westgard Pass, Calif.; and soil isolate G-17, obtained from a soil sample from Zion National Park, Utah.

Cultures of Dermatophilus were maintained on Difco Brain Heart Infusion (BHI) agar (3) and on a medium composed of 0.5% Yeast Extract (Difco), 5.0% glycerol, 0.1% CaCO₃ and 1.5% agar (5-g medium). Soil isolates (referred to by the proposed taxon Geodermatophilus) were maintained on a medium consisting of 0.5% Yeast Extract (Difco), 0.5% NZ Amine type A (Sheffield Chemical Co., Norwich, N.Y.), 1% glucose, 2% Soluble Starch (Difco), 0.1% CaCO₃ and 1.5% agar (6b medium). For morphological studies in still culture, a broth medium composed of 0.1% yeast extract, 0.1% glucose, 0.1% Soluble Starch (Difco), and 0.1% CaCO₃ (1111 broth) was used, in addition to an alfalfa seed broth and 6b broth. A malt extract medium which would support the growth of Geodermatophilus but not Dermatophilus contained 0.5% Yeast Extract (Difco), 1.5% Malt Extract Broth (Difco), 1% Soluble Starch (Difco), 1% reagent grade sucrose (Merck Sharp and Dohme Research Laboratories, Rahway, N.J.), 0.2% reagent grade CaCO₃ (Merck), and 2% Difco agar (YMS55 medium).

An inoculum was prepared by suspending material scraped from abundantly growing agar cultures,
20 to 40 days old, into 10 ml of sterile tap water and pipetting visible quantities of this coarse suspension into experimental media. Microscopic observations were made with a standard GFL Zeiss microscope equipped with a phase-contrast condenser and Neofluar phase contrast objectives. Motility was observed by placing a drop of broth on a slide and covering it with a cover slip. Fixed wet mounts used for photographically recording detail were made by spreading a thin layer of the culture material over the surface of a clean cover slip and allowing it to air dry. A drop of water or dilute stain was placed on a microscope slide and the coverslip with the dried material face down was brought carefully into contact with this drop on the slide. For internal structure, a few drops of 0.5% safranin solution (Fisher Scientific Co., Pittsburgh, Pa.) was added to 10 ml of sterile tap water and was used directly on the slide. For external detail, a small drop of gentian violet solution (Fisher Scientific Co.) was allowed to diffuse under the cover slip of a water mount. For the photomicrographs, Kodachrome X 35-mm film and a Leica Micro IIso photographic attachment were used.

Carbohydrate utilization studies. Carbohydrates were autoclaved separately to minimize decomposition; they were added to the basal medium in 1% concentration, except for glycerol, which was added in 2% concentration. A basal medium composed of 0.25% Yeast Extract (Difco), 0.1% reagent grade CaCO₃ (Merck), and 1.5% agar (Difco) was dispensed in 100-ml portions in screw-cap bottles and was sterilized by autoclaving for 15 min at 15 lb of pressure. Test carbohydrates were dispensed dry in 1-g quantities in metal-cap (Morton closures) tubes (25 by 150 mm) and were autoclaved for 12 min at fast exhaust, which was generally satisfactory for sterilizing high-purity carbohydrates. A 10-ml amount of sterile tap water was added aseptically to the sterilized carbohydrate, and the tubes were agitated for 20 min on a shaker. The yeast extract-agar base was cooled to 60°C, and the carbohydrate solutions or suspensions were aseptically added to the agar, mixed, dispensed in 5-ml quantities in metal-cap tubes (16 by 150 mm), and slanted. Utilization was judged by comparing the amount of colony growth on the test carbohydrate surface to the negative (basal medium only) and positive (glucose tube) control standards at 7, 14, and 30 days.

Acid production from carbohydrates was detected by use of the following media: (i) Phenol Red Agar Base (Difco); (ii) 1% peptone (Difco), 1.5% NaCl, 1.5% agar, and 10 ml of Andrade's indicator per liter (3); (iii) the carbohydrate utilization medium-yeast extract base with Andrade's indicator. Andrade's indicator was composed of 0.15 g of acid fuchsin (Coleman and Bell) and 16 ml of 1 N NaOH (the remaining volume being brought up to 100 ml with distilled water). This mixture was added to medium adjusted to pH 7.4 to 7.5 at 10 ml per liter (M. Gordon, personal communication). Carbohydrates were added in 1% concentration, according to the method described above.

The hydrolysis of casein and the decomposition of tyrosine were performed according to the methods of Gordon and Smith (4); the hydrolysis of gelatin and starch and the reduction of nitrate to nitrite were performed according to the methods of Gordon and Mihn (5).

Growth under anaerobic conditions was determined with a Gaspask Anaerobic Jar (BBL). Sensitivity to antibiotics was determined by an agar diffusion method, with antibiotic sensitivity discs on a medium consisting of 0.5% Yeast Extract (Difco), 1.0% dextrose, 0.1% CaCO₃, and 0.85% Ionagar no. 2 (Oxoid). Nystatin, griseofulvin, and tolunaate discs (6.35 mm), prepared from ethyl alcohol solutions of these antifungal agents, contained approximately 20 μg of antifungal agent per disc.

An attempt was made to establish skin infections in guinea pigs and rabbits. An area on the animal's flank was shaved, scarified, and swabbed with heavy cell suspensions of a centrifuged broth culture of the soil isolate being tested. Animals were observed at 3, 6, and 10 days for signs of lesions.

 RESULTS

Microscopic. The results of the examination of broth cultures of D. congolensis ATCC 14638 and D. pedis ATCC 14641 are consistent with Gordon's observations (3) that these two organisms are similar.

D. dermatonomus ATCC 14640 differs in its present form from the two cultures mentioned above. This organism was not observed to form zoospores, germ tubes, or the long mycelial-like, gelatinous sheathed filaments. Instead, small masses of cuboidal cells appeared to produce short, rigid filaments with pronounced transverse septations and a few longitudinal septations (Fig. 13). Physiologically, this organism behaves as the two other strains of Dermatophilus studied.

Since a reconstruction of the life cycle of D. congolensis, from information in the literature and from cultural observation, is necessary for an interpretation of the soil organisms to be described, an illustrated account follows. The thallus in Dermatophilus is usually a long tapered filament composed of cuboid or cocoid cells (Fig. 1 to 4). The outer wall of the thallus disintegrates, liberating the cocoid and cuboid elements in a gelatinous matrix (Fig. 4–6). The cocci become zoospores (Fig. 54). After a period of motility, the zoospores germinate (Fig. 7 to 10). In Fig. 8, one may observe the acropetal formation of septa as noted by Roberts (8). According to Roberts (8), branching occurs almost simultaneously with transverse septation in an area towards the spore end of the filament (Fig. 11).

Morphological cycle of soil isolates. A developmental pattern observed primarily in isolate G-12 is shown in Fig. 14 to 25. The thallus is a tuber or cushion-shaped aggregate of cells (Fig. 14, 15); the cells are angular or cuboidal (Fig. 15). When
a thallus is disrupted, packets of these cuboidal cells, with their characteristic net septation, appear (Fig. 16). This particular isolate is characterized by direct germination of cuboidal cells into new thalli and occasionally by the formation of rarely branched tubular filaments (Fig. 17 to 20). A zoospore stage was observed infrequently. Within the tubular filaments, transverse septa appear. The outer tubular wall appears like a tight sausage casing (Fig. 20). Laterally compressed spheres, as noted by Roberts (8) in D. dermatonomus filaments, may be seen in the single filaments in Fig. 21 and 22. The first stages in longitudinal septation appear in Fig. 23. The larger divisions of the multisepate filament (Fig. 24) are reminiscent of Roberts’ (8) observation that the cells created by each transverse septum can undergo binary fission in both planes so as to give rise to a mulberry-shaped cluster of coccii, or cuboidal cells, as in the case of these isolates.

Soil isolates with a zoospore stage. The zoospore stage of soil isolates which produce large greenish-black thalli is preceded by a phase in which certain thalli attain a degree of transparency and appear to be made up of almost indistinguishable dots (Fig. 26). The thallus then loses its distinct outline and looks very much like an indistinct mass of cellular debris (Fig. 27). Inspection of this mass with the aid of a dilute crystal violet stain reveals intensely staining coccii (Fig. 28, 29). Zoospores accompany such stages. Figure 30 shows a typical lanceolate zoospore, a large coccus possibly in the process of transforming into a zoospore, and another large nonrefractile coccus. In Fig. 31, one can see what may be the origin of the zoospores from the coccii. At 4 o’clock, a small, almost empty thallus (sporangial) casing which contains two darker, spheroidal cells can be seen. Toward the center are three or four less dense spheroidal cells of similar diameter to the ones at 4 o’clock. At 1 o’clock, one can see a cluster of three spheroidal cells and two lanceolate zoospores. I believe that the lanceolate zoospores are derived from the spheroidal cells possibly by two processes, one in which the spheroidal cell loses its rigidity and assumes the lanceolate shape of the thallus, and the other in which the rigid spheroidal cell develops a small pore through which the protoplasm emerges and undergoes a metamorphosis into a zoospore. This pore method of zoospore emergence was suggested by Austwick (1). That such a pore mechanism exists seems to be indicated by the lanceolate-pedicellate structures attached to single cells of small thalli (Fig. 34, 38, and 39). In wet mounts, these pedicellate cells were often observed in motion in what looked like an attempt to break away from the larger nonmotile thallus cell. In Fig. 40, this attempt apparently was futile and both cells developed into larger thick-walled thalli.

In Fig. 32, a mixture of large almond-shaped postmotile spores and some slender elliptical to lanceolate zoospores may be seen. The budding zoospore in the center is a commonly observed phenomenon. Zoospores appear to bud off motile replicas of themselves, and all stages of these developing buds can be found. In Fig. 33, a zoospore pair may be seen.

Postmotile stages of development differ among the soil isolates. Isolate G-5 was commonly found to produce large postmotile lanceolate cells that simply increased in size and developed a transverse and eventually longitudinal septations (Fig. 34). Germ tubes and filaments were not common. The short, blunt dictyosporotype thallii developed by this isolate can be seen in Fig. 36 and 37. Figure 35 shows zoospore germ tube formation in isolate G-20. The “rabbit ear” germination type in the upper right of Fig. 35 generally involves the emergence of a germ tube and a pedicellate elliptical cell. A more conventional germ tube has been produced by the lanceolate cell in the lower left, and a double-ended germination occurs in the upper left.

Figures 41 and 42 show young tubular filaments in which the outer cell wall is independent of the cellular contents developing within. A twocelled spore may be seen emerging from the partially empty tube in Fig. 42.

Branched filaments. Isolate G-17 occasionally develops a branched tubular thallus. This isolate produces abundant lanceolate zoospores that germinate to produce irregularly constricted, often branched, filamentous tubes (Fig. 43–51). Germ tube emergence occurs at any point on the surface of the spore. In Fig. 48, one can see what appear to be twin postmotile spores that failed to separate but which produced germ tubes independently. Figure 51 shows a filament within which the protoplasm has condensed in a sausage-like manner. Figure 52 shows an example of longitudinally compressed spheres, and Fig. 53 shows the onset of longitudinal septation. Figures 55 and 56 are electron micrographs of a flagellated zoospore and a flagellated germinating zoospore of isolate G-17.

Physiological reactions. Cultures of Dermatophilus and Geo dermatophilus did not grow on Czapek Sucrose Agar, Sabouraud Dextrose Agar, or Potato Dextrose Agar. They did not produce a color reaction on tyrosine agar or H2S agar. Cultures of Dermatophilus grown for 14 days in a broth composed of 1% yeast extract, 1% glucose,
FIG. 1 and 2. ATCC 14638, 5-day 6b broth still culture. In Fig. 1 to 53, the scale maker represents 60 μm.
Fig. 3. ATCC 14638, 5-day 6b broth still culture.
Fig. 4. ATCC 14641, 4-day 6b agar culture.
Fig. 5. ATCC 14638, 5-day 6b broth still culture.
Fig. 6. ATCC 14641, 3-day 6b broth still culture.
Fig. 7. ATCC 14638, 9-day BHI broth shake tube culture.
Fig. 8. ATCC 14638, 7-day 6b broth still culture.
Fig. 9. ATCC 14641, 15-day 6b broth still culture.
Fig. 10. ATCC 14638, 9-day BHI broth shake tube culture.
Fig. 11. ATCC 14638, 20-day mannitol broth still culture.
Fig. 12. ATCC 14638, 7-day 6b broth still culture.
Fig. 13. ATCC 14640, 3-day 6b broth shake tube culture.

FIG. 15-17. Soil isolate G-9, 11-day alfalfa seed broth still culture.

FIG. 18. Soil isolate G-12, 21-day water still culture.

FIG. 19 and 20. Soil isolate G-9, 11-day alfalfa seed broth still culture.

FIG. 21-23. Soil isolate G-12, 40-day alfalfa seed broth still culture.

FIG. 24 and 25. Soil isolate G-12, 20-day yeast extract-mannitol broth still culture.
Fig. 26-28. Soil isolate G-13, 21-day alfalfa seed broth still culture.
Fig. 29. Soil isolate G-24, 9-day alfalfa seed broth still culture.
Fig. 30. Soil isolate G-5, 6-day 6b agar culture, smear.
Fig. 31. Soil isolate G-17, 14-day 6b broth still culture.
Fig. 32. Soil isolate G-7, 10-day starch broth still culture.
Fig. 33. Soil isolate G-17, 14-day 6b broth still culture.
Fig. 34. Soil isolate G-5, 2-day 6b broth shake tube culture.
Fig. 35. Soil isolate G-20, 5-day alfalfa seed broth still culture.
Fig. 36 and 37. Soil isolate G-3, 9-day alfalfa seed broth still culture.
Fig. 38 and 39. Soil isolate G-5, 2-day 6b broth shake tube culture.
Fig. 40. Soil isolate G-7, 24-day Czapek broth still culture.
Fig. 41 and 42. Soil isolate G-6, 8-day alfalfa seed broth still culture.

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and 0.1% CaCO₃ emitted a characteristic acrid odor, whereas *Geodermatophilus* cultures were either neutral or dank in odor. Cultures of *Dermatophilus* responded more rapidly to an incubation temperature of 37 C than to 26 C, whereas *Geodermatophilus* isolates responded better at 26 C. Both groups of organisms were found to grow at 26 and 37 C but not at 50 C. Thompson (9) reported that cultures of *D. pedis* were destroyed by exposure to 60 C for 15 min. Heat lability was also observed in this study with *Dermatophilus* cultures. For the *Geodermatophilus* isolates, however, several colonies would occasionally develop from cell suspensions subjected to 30-, 60- or 90-min exposures at 60 C, probably representing not more than one cell in 100,000.

Gordon (3) reported *Dermatophilus* cultures...
to be facultatively anaerobic, growth being substantial but much less than that produced aerobically. In the present study under anaerobic conditions, *Dermatophilus* cultures produced negligible growth on 6b or 5g agar, and scant to negligible growth on blood agar but definite zones of \( \beta \)-hemolysis. *Geodermatophilus* cultures produced no hemolysis and negligible growth under these conditions.

Cultures of *Dermatophilus* grew well on Brain Heart Infusion Agar (Difco), whereas *Geodermatophilus* did not grow at all. In contrast, *Dermatophilus* cultures did not grow on a medium composed of yeast extract, starch, sucrose, and malt extract, whereas *Geodermatophilus* cultures grew abundantly on this medium. Casein, whether incorporated into an agar medium or as bromocresol purple milk, was rapidly hydrolyzed by *Dermatophilus*, whereas no hydrolysis or growth occurred with *Geodermatophilus* isolates. Gelatin was hydrolyzed by *Dermatophilus* cultures and by some of the *Geodermatophilus* isolates.

\( \beta \)-Hemolysis of blood agar (10% human blood) and growth occurred with *Dermatophilus*, whereas no \( \beta \)-hemolysis or growth occurred with *Geodermatophilus*. Nitrate was not reduced to nitrite either in inorganic or organic nitrate broth by *Dermatophilus*. With *Geodermatophilus*, nitrate reduction did occur sporadically in isolate G-20 and was vigorous in isolate G-17 with both inorganic and organic nitrate broth.

For the three strains of *Dermatophilus* studied, acid production from carbohydrates was consistent with the phenol red indicator system or with Andrade's indicator system for D-glucose, D-levulose, and D-ribose, usually within 48 to 72 hr. An acid reaction on glycerol, maltose, and sucrose was sporadic and was not observed on galactose with the phenol red indicator system. On the two media with Andrade's indicator, an acid reaction was transitory on galactose, similar to the report of Gordon (3).

The production of acids from carbohydrates by isolates of *Geodermatophilus* did not generally occur before 5 days and frequently required 20 to 30 days for an indicator change; final readings were made at 60 days. Acid production accompanied by negligible growth was characteristic of isolates of *Geodermatophilus* on \( \beta \)-rhamnose, D-ribose, and D-arabinose. In Table 1, acid detection data are given along with the scoring for carbohydrate utilization.

Carbohydrate utilization patterns were reliable for *Geodermatophilus* and a differential diagnosis could be made among isolates. Although a carbohydrate utilization pattern was obtained for *Dermatophilus* cultures, it was considered to be of a quality not suitable for differential diagnosis.

Antibiotic sensitivity data were used to help establish the bacteria-like nature of the soil isolates in contrast to a fungal relationship such as *Sarcinomyces* (3). Isolates of the proposed genus *Geodermatophilus* and strains of *Dermatophilus*

### Table 1. Carbohydrate utilization patterns and acid detection

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<th>Carbohydrates</th>
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<th>G-7</th>
<th>G-12</th>
<th>G-13</th>
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<sup>a</sup> Key: 0, no growth; ±, poor growth; +, fair growth; ++, good growth. A, detection of acid production relatively consistent; a, sporadic or transitory detection of acid production.

<sup>b</sup> *D. congoensis* ATCC 14638.
were found to be insensitive to the antifungal agents nystatin, griseofulvin, and tolnaftate, but sensitive to the antibacterial antibiotics streptomycin, neomycin, and gentamicin, and very sensitive to chlortetracycline, novobiocin, and erythromycin.

Although three attempts were made to infect guinea pigs with the soil isolates and one attempt was made to infect rabbits, neither the lesions nor the pustular-type dermatitis described by other investigators (7, 9) was observed.

DISCUSSION

This report extends the family Dermatophilaceae, which at present consists of a single species, *D. congoensis* (3), known only from a dermatitis caused in wild and domestic animals and man. Attempts to isolate *D. congoensis* from the soil have thus far failed (8). At first, it was thought that the organisms reported in this study might represent a saprophytic soil form of *Dermatophilus*. Morphologically, there is a degree of similarity between the life cycle of these two groups of organisms. Nevertheless, although these similarities unite these organisms and separate them from other actinomycetes, the differences between them appear sufficient to merit the recognition of a distinct taxon to include these soilborne relatives of *Dermatophilus*; the generic name *Geodermatophilus* is proposed for these soilborne organisms.

I believe that members of the same genus should be readily identifiable with each other in terms of morphology and, to a degree, in terms of physiology. The soil isolates used in this study are related to *Dermatophilus* only through a cryptic filamentous phase; it is difficult to relate them physiologically since they do not seem to grow on the same medium. *Geodermatophilus* forms a natural group in which the development of the filamentous phase has not evolved a true mycelium. Filament formation is similar to the formation of a primitive pseudomycelium in certain yeasts in that it is not often encountered but, when found, appears as loosely united filaments representing elongations of buds characteristically pinched and constricted at irregular intervals (Fig. 17, 18, 35, 41–46, 49, 50). These filaments or tubes act like holocarpic sporangia (Fig. 19–22, 31, 37, 42, 51–53) producing within sporangiopores, which, when liberated, may germinate directly or become zoospores. The thallus, which usually remains intact until conditions are favorable for germination, is composed of angular or cuboidal cells that are generally darkly pigmented. Capsular material is not demonstrable in India ink preparations.

In *Dermatophilus*, spores germinate to produce filaments similar to a true mycelium in that the filaments are frequently encountered, are long, are relatively uniform in diameter, are septate in an acropetal manner, and appear to be branched (Fig. 7, 8, 10, 11). The thallus usually breaks up shortly after formation into spherical spores which are often retained in a gelatinous matrix. Thallus cells are hyaline. Capsular material is demonstrable in India ink preparations.

The morphological characters separating *Dermatophilus* from *Geodermatophilus* are as follows.

(i) In *Dermatophilus*, filaments are commonly produced from spores and appear to be similar to a true mycelium. These filaments produce secondary branching perpendicular to the axis of the main filament and are reminiscent of in situ germination. Zoospores are spherical; division appears to be by fission (6). Germinating spores produce a long, relatively rigid, uniform filament within which transverse septation appears to occur in an acropetal manner.

(ii) In *Geodermatophilus*, filaments are not commonly produced from spores; when present, the filaments appear to be rudimentary, similar to a primitive pseudomycelium. The filaments produce secondary branching which is not restricted to a plane perpendicular to the main axis of the filament; branching appears to arise in a manner similar to budding. Zoospores are elliptical to lanceolate, and division appears to occur by budding. Germinating spores become directly septate or produce a short tube or occasionally a longer, flexible filament within which transverse septation appears to occur in a simultaneous manner.

Preliminary infection studies in rabbits and guinea pigs have not established the pathogenicity of the *Geodermatophilus* isolates. Whether other species may be susceptible and whether better methods of inducing an infection may be necessary to establish pathogenicity require further study.

The concept of species in *Geodermatophilus* is based upon the morphological and physiological characteristics of 20 isolates, of which 6 were selected for the present study. With organisms having a complex life cycle, certain isolates are found that omit or emphasize certain phases of the cycle. Some isolates produce few or no zoospores, the thallus cells germinating directly to produce filaments, whereas other isolates produce zoospores, but these cells, after losing motility, divide directly to produce a thallus and rarely produce a germ tube or filament.

The type species proposed for the genus
Geodermatophilus is *G. obscurus*; this species has been chosen to represent the group of greenish-black thallus-producing organisms. The three subspecies described below, that were isolated from dry desert areas of the western United States, are distinct but are considered best placed within the *G. obscurus* species concept.

**Geodermatophilus** gen. n. Gr. noun *Ge.* earth and dermatophilus, earth dermatophilus.

Organisms producing a muciform, tuber-shaped, noncapsulated, holocarpic thallus consisting of masses of cuboid cells averaging 0.5 to 2.0 μm in diameter. The thallus breaks up, liberating cuboid or coccioid nonmotile cells and elliptical to lanceolate zoospores. Germinating cells may divide directly to produce a thallus or may produce a germ tube and an irregularly constricted filament which may branch at various angles. The contents of these tubes first divide transversely by septa that do not appear to involve the outer layer of the cell wall, giving rise to a tube of longitudinally compressed cells. Septa formed later in horizontal and vertical longitudinal planes give rise to rows of cuboidal cells. Mycelium rudimentary, aerial mycelium not produced. Organisms aerobic, gram-positive, and of cell wall type III (H. Lechevalier, personal communication). Colonies usually turn dark brown, dark gray, or black in old cultures.

**Geodermatophilus obscurus** sp. n. L. adj. dark, obscure, indistinct, unintelligible (ATCC 25078).

Colony growth on yeast extract-casein hydrolysate-agar or yeast extract-glycerol-agar good; colony plicate, granular, dark brown periphery, black center; odor dank. Thallus appearing greenish-black by transmitted light. Thallus varying in size from a few cells to many cells arranged in cushion or tuber-shaped aggregates. Individual thallus cells cuboidal. Thallus disintegrates giving rise to cuboidal and coccioidal cells and elliptical to lanceolate zoospores. Zoospores often budding while motile, giving rise to two- or three-celled motile units. Postmotile spores may produce germ tubes and filaments or may undergo enlargement, septation, and thallus formation without an intervening germ-tube stage.

Does not hydrolyze casein or gelatin, hemolyze blood, or grow on Brain Heart Infusion Agar. Nitrate reduction weak or not at all. Starch is hydrolyzed, growth is good on yeast extract-starch-sucrose-malt extract agar. Growth optimal at 24 to 28 C, reduced at 37 C; none at 50 C. In aqueous suspension, few cells survive 30 min at 60 C.

Carbohydrates utilized for growth are: L-arabinose, D-galactose, D-glucose, glycerol, inositol, D-levulose, D-mannitol, sucrose, and xylose. Carbohydrates utilized poorly or not at all are: D-arabinose, dulcitol, β-lactose, melezitose, α-melibiose, raffinose, L-rhamnose, and D-ribose. Acid reaction on D-arabinose, L-arabinose, D-glucose, glycerol, D-levulose, D-ribose, L-rhamnose, and D-xylose.

Type designated: Isolate G-20, obtained from soil sample from the Amargosa Desert of Nevada. On the basis of morphology, isolates G-7 and G-13 are included here.

**Geodermatophilus obscurus** sub. sp. n. amargosae (ATCC 25081).

Colony growth on yeast extract-casein hydrolysate-agar good; colony plicate, granular, dark brown periphery, black center; odor dank. Colony growth on yeast extract-glycerol-agar good; colony granular, black; odor dank. Thallus appearing greenish-black by transmitted light. Thallus usually large, tuber-shaped, composed of cuboidal cells. Thallus breaks up into cuboidal clusters of cells. Individual cuboidal cells may give rise to long slender filaments which enlarge and undergo septation to produce relatively broad, blunt-ended thalli or cuboidal cells may enlarge, septate, and form thalli directly. Rarely observed to produce zoospores. Similar in physiological reactions to *G. obscurus*, except that gelatin weakly hydrolyzed, nitrate not reduced, growth on inositol and L-rhamnose fair but not good. Acid reaction on L-arabinose, D-galactose, D-glucose, glycerol, D-levulose, L-rhamnose, D-ribose, and D-xylose.

Type designated: Isolate G-12, obtained from soil sample from the Amargosa Desert of Nevada.

**Geodermatophilus obscurus** sub. sp. n. utahensis (ATCC 25079).

Colony growth on yeast extract-casein hydrolysate-agar fair; colony flat, granular, black; odor dank. Growth on yeast extract-glycerol-agar good; colony raised granular, black; odor dank. Thalli appearing greenish-black by transmitted light. Thallus varying from a few cuboidal cells to large cushion or tuber-shaped cellular masses. Thallus disintegration gives rise to cuboidal and coccioidal cells and many elliptical to lanceolate zoospores. Zoospores terminally bud new zoospores to produce motile trains of two to three zoospores. Postmotile spores typically produce a germ tube and a tubular filament which may branch at various angles and is characteristically pinched or constricted. Transverse and longitudinal septations occur within this tubular filament to produce a multicellular thallus. Similar in physiological reactions to *G. obscurus* except for vigorous reduction of nitrate to nitrite; poor growth on L-arabinose and glycerol; fair growth on D-arabinose, inositol, and D-levulose. Acid reactions sporadic but have been detected on

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D-arabinose, D-glucose, D-mannitol, and D-ribose.

Type designated: Isolate G-17, obtained from soil sample from Zion National Park, Utah.

From additional studies, the apparent discrepancy between good growth on yeast extract-glycerol-agar and poor growth in carbohydrate utilization studies with glycerol appears to reside in the 5% concentration of glycerol in the yeast extract-glycerol-agar medium versus the 2% glycerol concentration in the carbohydrate study. Glycerol concentrations of 5 and 10% support good growth, whereas concentrations of 1 to 3% do not.

Geodermatophilus obscurus sub. sp. n. dictyosporus (ATCC 25080).

Colony growth on yeast extract-casein hydrolyzate-agar good; colony plicate, dark grayish-brown; odor dank. Growth on yeast extract-glycerol-agar good; colony granular, plicate, strong brown; odor dank. Colonies on some media in early stages of development have a salmon pink peripheral border which later turns brown dark or black. Thalli appearing olive green to greenish-black by transmitted light. Thallus varying in size, often small, superficially resembling dictyospores of higher fungi. Thallus composed of cuboidal cells which, when released, may form zoospores or develop directly into a new thallus. Germ tubes may be produced from postmotile spores, or postmotile spores may undergo septation directly to form new thalli. The thalli formed directly from postmotile spores are small and tend to characterize this particular isolate. Often pedicellate lanceolate cells arise from cuboidal cells of small dictyothalli. Similar in physiological reactions to G. obscurus, except that gelatin is hydrolyzed, inositol is not utilized, and β-lactose and melitose are utilized. Acid reaction on D-arabinose, L-arabinose, D-glucose, glycerol, D-levulose, L-rhamnose, D-ribose, and D-xylose.

Type designated: Isolate G-5, obtained from soil sample from Westgard Pass, Calif.

In retrospect, on first view, these organisms appeared to be composed of an amorphous mass of debris often difficult to recognize as cellular. However nondescript these cultures first appeared, the one positive fact was that the biomass did grow, and the problem was resolved into discovering how this development took place. The use of still culture methods, based upon consideration of the aquatic actinomycetes in conjunction with the previously reported data on the complex cycle of development in Dermatophilus, has made possible an understanding of the soil isolates described in this study.

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Literature Cited