APPLIED MICROBIOLOGY, July 1969, p. 114-121
Copyright © 1969 American Society for Microbiology

Vol. 18, No. 1
Printed in U.S.A.

# Predominant Catalase-negative Soil Bacteria[1]

## II. Occurrence and Characterization of *Actinomyces humiferus*, sp. N.

WILLIAM E. GLEDHILL[2] AND L. E. CASIDA, JR.

*Department of Microbiology, The Pennsylvania State University, University Park, Pennsylvania 16802*

Received for publication 27 March 1969

A microorganism resembling an *Actinomyces* species was found to be a numerically predominant inhabitant of various organically rich soils. This organism forms a hyphal-like structure with true branching that fragments into gram-positive diphtheroid and coccoid elements. Its cells ferment carbohydrates and contain both lysine and ornithine as the major basic amino acids of the cell wall. It is catalase-negative, microaerophilic to aerobic, and sensitive to lysozyme, and it is dependent on an organic nitrogen source and incubation at 30 C for optimum growth. Based on these characteristics, a new species, *Actinomyces humiferus*, is proposed. The ecological and medical implications of a large soil population of this microorganism are discussed.

The genus *Actinomyces* includes those microorganisms which are facultative to anaerobic, require $CO_2$ for growth, ferment carbohydrates, grow best at 37 C, have cell walls containing lysine as a major basic amino acid, and form a mycelium with true branching that fragments into diphtheroid and coccoid elements (24). These microorganisms occur among the normal bacterial flora of the oral cavity, and they have been associated with various pathological conditions in warm-blooded animals (2, 12, 13, 17, 18, 23, 26). In contrast, their presence in soil and similar natural sources has only rarely been noted (3, 19, 20, 29).

By employing a modified dilution-frequency isolation procedure (6), we have now demonstrated that an *Actinomyces*-like organism constitutes a numerically dominant population of certain soils. Although this organism possesses certain major characteristics in common with those presently ascribed to *Actinomyces* species, it differs significantly in other important aspects. The present communication, therefore, describes various microbiological properties of our isolates, and recommends that they be designated as a new species, *Actinomyces humiferus*.

### MATERIALS AND METHODS

Except where noted, the methods employed in this investigation were those used previously (15). The 26 soils (15) were collected from many locations throughout the United States, and they ranged from organically rich to barren desert soils with pH values from 3.8 to 9.1, moisture contents from 0.7 to 51.9%, and organic matter contents from 0.6 to 25.2%. The modified dilution-frequency isolation technique of Casida (6) was employed for isolation of the *Actinomyces*-like organism described in this study.

Acid products resulting from glucose fermentation were analyzed by gas chromatography. For this determination, the volatile and nonvolatile acids were extracted from the medium (21); then the volatile acids were regenerated from their sodium salts by adding 4% aqueous phosphoric acid containing 5% formic acid (7). Separation and identification of the volatile fermentation acids were accomplished by direct injection of aqueous solutions into a model 5300 Barber-Coleman gas chromatograph with a hydrogen flame ionization detecter and containing a stainless-steel column [6 feet by 1/8 inch outside diameter (183 by 0.32 cm)] packed with Chromosorb 102 (Applied Science Laboratories, State College, Pa.). Methyl esters of nonvolatile acids (1) were chromatographed on a stainless-steel column [6 feet by 0.25 inch outside diameter (183 by 0.32 cm)] containing 10% diethylene glycol adipate and 2% phosphoric acid on Chromosorb W (Applied Science Laboratory). All samples were chromatographed isothermally at 160 C with nitrogen as the carrier gas (50 ml per min).

Lactic acid was also determined colorimetrically (22) after extraction from the medium.

### RESULTS

**Occurrence.** An *Actinomyces*-like microorganism was isolated regularly from soils with organic matter contents of 6.5% or greater and *p*H values ranging from 6.4 to 7.9. Forty-one strains were isolated for the present study. These strains

[1] This research was authorized for publication as paper no. 3564 in the journal series of the Pennsylvania Agricultural Experiment Station on March 17, 1969.

[2] Present address: Microbiological Research, Tenneco Chemicals, Inc., Piscataway, N. J. 08854

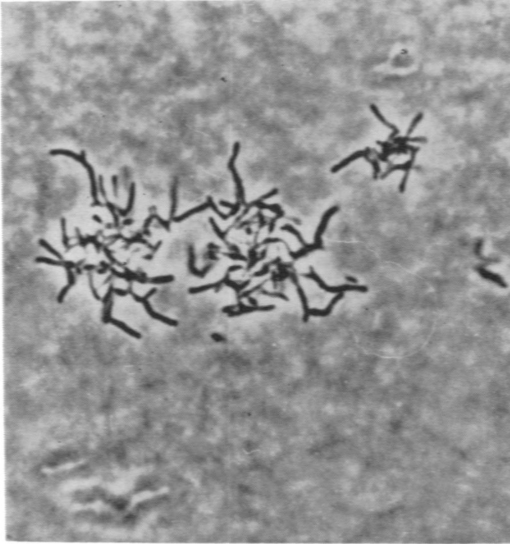

FIG. 1. *Branched mycelium observed at 12 hr of incubation. Phase contrast.* × *1,630.*

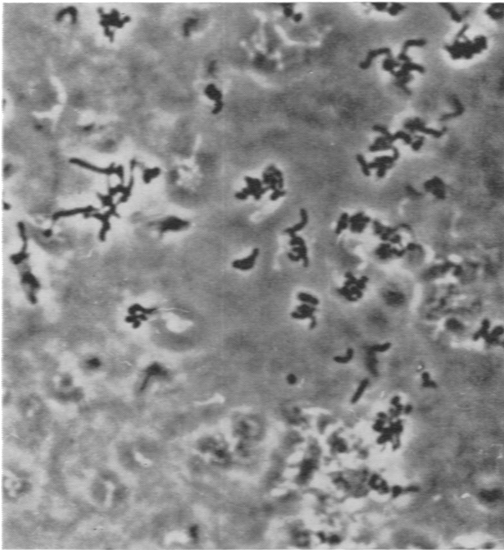

FIG. 2. *Fragmentation of 36-hr-old mycelium to yield coccoid and diphtheroid cells. Phase contrast.* × *1,630.*

represented a numerically predominant segment of the bacterial flora, since they were recovered from those soil dilutions 1 and 2 logarithmic units beyond the dilution range for platable microorganisms (6). Attempts to isolate this organism from a wide variety of soil types by the use of standard plating techniques were unsuccessful.

**Cellular morphology.** During the first 24 hr of growth on agar and liquid media, most of the cells became filamentous with true branching (Fig. 1), and they often displayed swollen terminal regions. For most strains, fragmentation to diphtheroid and coccoid cells of varied size and shape (Fig. 2) occurred between 24 and 48 hr. This fragmentation prevented the formation of an extensive hyphal structure, although occasional rough colony variants formed a more extensive mycelium; in this instance, fragmentation was not initiated until later in their growth cycle. Cultures of physiologically older cells (48 hr), similar to those in Fig. 2, divided in the diphtheroid and coccoid states without mycelial development. However, when these cells were placed in fresh medium, hyphal formation was initiated by the development of "buds" at 1 or more points on the parent coccoid or diphtheroid cell (Fig. 3). Cells remained gram-positive throughout their development cycle.

**Colony morphology.** Growth on Heart Infusion Agar (24 hr) appeared as branched, filamentous, "spider-like" microcolonies (Fig. 4), which are typical of members of the genus *Actinomyces* (5, 18). At 48 hr, as fragmentation of older segments of the hyphal structure ensued, the colonies formed a dense central region (Fig. 5). Mature colonies (7 to 10 days) of most strains when examined by transmitted light were small (less than 1 mm), opaque, entire, and convex, and

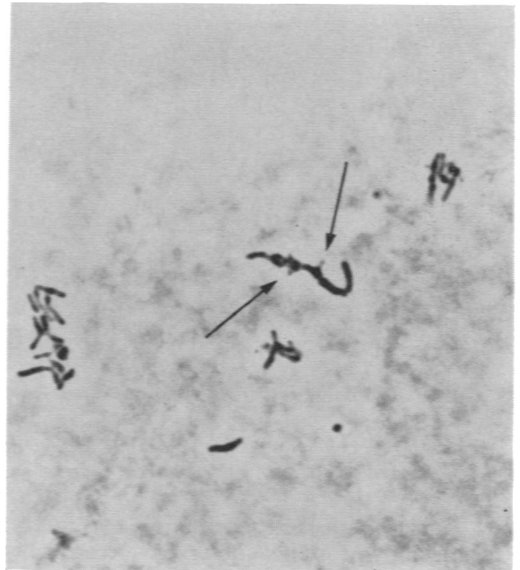

FIG. 3. *Growth initiation by the formation of "buds" at 8 hr of incubation. Phase contrast.* × *1,630.*

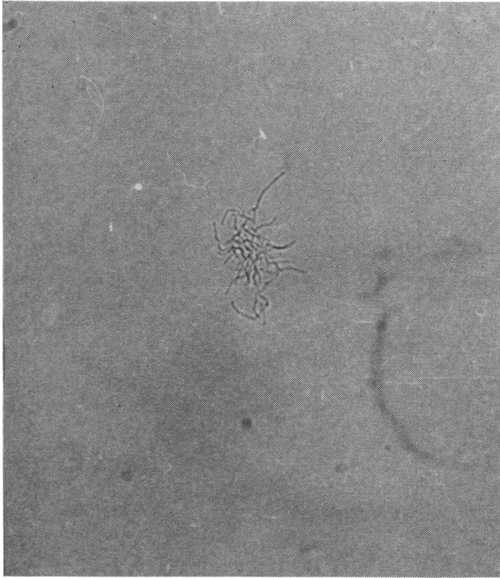

FIG. 4. *"Spider-like" microcolony at 24 hr of incubation.* × *650.*

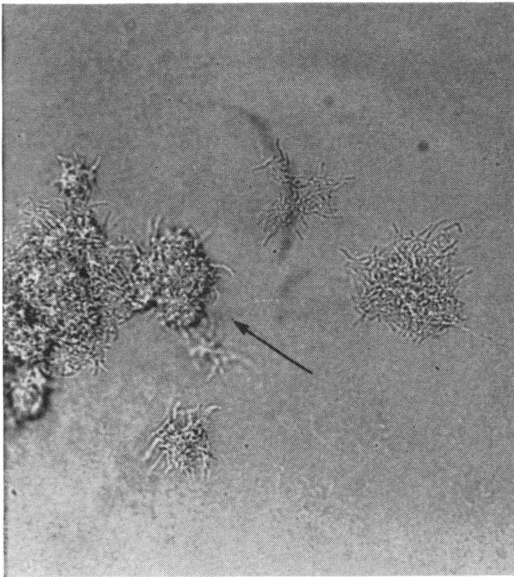

FIG. 5. *Microcolony with dense central region at 48 hr of incubation.* × *650.*

they displayed a dark central area (Fig. 6). Rough-colony variants observed occasionally were irregular and heaped, and they were smaller and more convex than the smooth colonies. Further cultivation of these variants was possible, but they tended to revert to the smooth form. Pigment production has not been observed for either smooth or rough strains.

**Oxygen requirements and growth characterization.** The soil isolates were microaerophilic to aerobic. Growth in thioglycollate broth was restricted primarily to a distinct band of finely granular or flocculent consistency at or just below the interface between the oxidized and reduced zones, with comparatively less growth in the oxidized region. Growth in Heart Infusion Broth containing 0.08% agar also occurred mainly as a diffuse band 0.5 cm below the surface of the medium, with decreasing turbidity at higher and lower regions of the tube. In Heart Infusion Agar shake tubes, maximum growth occurred in the upper 1 cm of agar, with isolated light patches of growth in lower portions of the tube. A white granular-to-flaky sediment of growth without turbidity was produced in Heart Infusion Broth. Growth was not observed under anaerobic conditions, and the development of all strains either was not affected or was inhibited by the presence of an increased $CO_2$ tension.

The *p*H range for optimum growth in media no. 1 and 2 was 7.2 to 7.5. The optimum growth temperature for all isolates was approximately 30 C, and this value did not change during continued cultivation on laboratory media. At initial isolation from soil, all strains were unable to grow at 37 C. However, after prolonged laboratory cultivation a few isolates did display feeble growth at this temperature, although successive transfers at this temperature resulted in death of the cultures.

**Biochemical characteristics.** As may be seen in Tables 1 and 2, these soil organisms can be characterized as catalase-negative, nonacid-fast, susceptible to lysis by lysozyme, not reducing

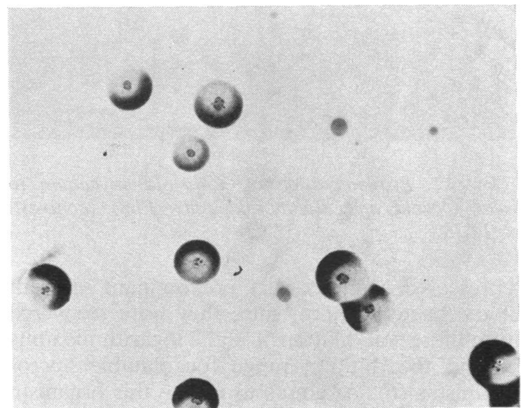

FIG. 6. *Mature colony at 8 days.* × *6.*

TABLE 1. *Biochemical properties of soil isolates*[a]

| Property | Positive strains (%) | Property | Positive strains (%) |
|---|---|---|---|
| Catalase activity | 0 | Glucose fermentation | 100 |
| Benzidine test | 0 | Acid from | |
| Oxidase test | 0 | Glycerol | 74 |
| Nitrate reduction | 0 | Arabinose | 96 |
| Hydrolysis of | | Ribose | 11 |
| Starch | 100 | Xylose | 89 |
| Gelatin | 44[b] | Fructose | 100 |
| Tributyrin | 0 | Galactose | 93 |
| Casein | 85 | Glucose | 100 |
| Esculin | 89 | Mannose | 96 |
| Decomposition of | | Rhamnose | 96 |
| Tyrosine | 0 | Salicin | 78 |
| Xanthine | 0 | Adonitol | 0 |
| Urea | 0 | Dulcitol | 0 |
| Methyl red test | 100 | Inositol | 7 |
| Voges-Proskauer test | 15 | Mannitol | 93 |
| Lysozyme sensitivity | 93 | Sorbitol | 4 |
| Utilization of | | Cellobiose | 67 |
| Acetate | 0 | Gentiobiose | 85 |
| Propionate | 0 | Lactose | 59 |
| Gluconate | 26 | Maltose | 96 |
| Oxalate | 0 | Melibiose | 93 |
| Citrate | 0 | Sucrose | 100 |
| Lactate | 0 | Trehalose | 70 |
| Succinate | 0 | Turanose | 82 |
| Fumarate | 100 | Melezitose | 96 |
| Pyruvate | 100 | Raffinose | 89 |
| α-Ketoglutarate | 97 | Stachyose | 96 |
| Litmus milk | | Dextrin | 100 |
| Acid and reduction | 82 | Inulin | 4 |
| No reaction | 18 | Starch | 100 |
| Acid fast | 0 | Growth in presence of | |
| Production of | | 1% bile | 33 |
| $NH_3$ from peptone | 0 | 3% bile | 0 |
| $NH_3$ from arginine | 0 | 4% NaCl | 52 |
| Indole | 0 | | |
| $H_2S$ | 67 | | |

[a] Results based on 27 strains.
[b] These strains showed only a very narrow zone of hydrolysis.

TABLE 2. *Various properties of soil isolates*

| Glucose fermentation products[a] | | Major cell wall components[b] | | | DNA base composition[c] | |
|---|---|---|---|---|---|---|
| Acid | Total acids | Amino acids | Amino sugars | Sugars | CsCl density | Per cent GC[d] |
| | % | | | | | |
| Lactic | 91–97 | Alanine | Glucosamine | Rhamnose | 1.7315 | 73.0 |
| Succinic | <5 | Glutamic acid | Muramic acid | | | |
| Acetic | <5 | Lysine | | | | |
| Propionic | 0 | Ornithine | | | | |
| | | Aspartic acid | | | | |

[a] Average of 12 strains.
[b] Determinations based on 14 strains.
[c] Data from one representative strain.
[d] Moles per cent guanine plus cytosine of the total bases.

nitrate, not producing indole, able to utilize certain organic acids for growth, and fermenting a broad spectrum of carbohydrates without gas formation. Lactic acid, their major end product of glucose fermentation, usually was produced in quantities just great enough to provide a positive methyl red test.

Based on the benzidine test (Table 1), iron porphorin compounds, such as the cytochromes, were absent or present only to a minor extent. Gelatin either was not attacked or was only weakly hydrolyzed. In contrast, casein was readily decomposed, and it markedly stimulated the growth of most of the isolates. Many of the strains produced $H_2S$, but this product could only be detected by suspending lead acetate paper strips above growth on Brain Heart Infusion Agar slopes supplemented with 0.1% cysteine.

Cell wall composition (Table 2) was similar to other *Actinomyces* species (4, 8, 10) in that lysine and ornithine were the only basic amino acids detected. Rhamnose was the predominant cell wall sugar in all strains, although a few of the isolates also displayed trace amounts of glucose and fucose.

The deoxyribonucleic acid base composition (Table 2), characterized by a high per cent guanine plus cytosine, lies within the 64 to 80% range of values reported for other members of the order *Actinomycetales* (27, 28).

**Nutritional requirements.** The soil isolates and cultures of established *Actinomyces* species did not grow on media lacking organic nitrogen. Also, little if any growth occurred in certain chemically defined media or media containing simple peptones; these media were Conn's glycerol asparagine, Sabouraud dextrose, Czapek-Dox, and nutrient broths. The addition of soil extract to medium BAVT (15) slightly stimulated the growth of 21 of the 27 strains studied.

**Pathogenicity.** The soil isolates did not induce experimental infections in mice as have been described for various *Actinomyces* species (5, 24). Examination of 14 mice sacrificed 3 weeks after intraperitoneal injection of washed saline cell suspensions revealed no significant pathology.

**Serology.** The five strains examined by the fluorescent antibody approach displayed no serological cross-reactivity with specific antisera to accepted *Actinomyces* and *Rothia* species. However, two of the isolates did show a slight positive, but apparently nonspecific, reaction with *Bacterionema* antiserum.

**Taxonomy.** From the results presented above, it is evident that our soil isolates display major characteristics in common with the genus *Actinomyces*. These characteristics are morphology, nutrition, cell wall composition, catalase reaction, and carbohydrate fermentation. However, our isolates differ from the described species of this genus as regards oxygen requirements, optimum growth temperature, and lysozyme sensitivity. Therefore, pending final consideration by the Subcommittee on Microaerophilic Actinomycetes, a new species, *Actinomyces humiferus*, is proposed for these organisms.

**Actinomyces humiferus,** sp. n. Gledhill and Casida, 1969. L. noun *humus* soil; L. verb *fero* to bear; M.L. adj. *humiferus* soil-borne.

Microcolonies on agar and initial growth in liquid media composed of branched, often filamentous cells eventually fragmenting into coccoid and diphtheroid elements. Mature colonies small, opaque, smooth, entire, convex, with a dark central region. Rough-colony variation occasionally observed. Pigmentation not evident. Gram-positive, nonacid-fast, no aerial mycelium or spores, nonmotile. Growth in broth containing 0.08% agar primarily as a diffuse band just beneath the surface of the medium. Organic nitrogen required for growth. Catalase-negative; microaerophilic to aerobic, poor or no growth anaerobically. Oxidase-negative, benzidine-negative. Growth not stimulated by increased $CO_2$ tension. Temperature optimum at approximately 30 C, poor or no growth at 37 C. Sugars fermented with lactic acid as a major product; no gas formation. Cell walls contain lysine, ornithine, alanine, glutamic acid, aspartic acid, glucosamine, muramic acid, and rhamnose as major components. Cells sensitive to lysis by lysozyme.

Casein, esculin, and starch hydrolyzed; gelatin weakly hydrolyzed but not liquified. Litmus milk acidified and reduced. Nitrates not reduced to nitrities. Indole not produced from tryptophan; ammonia not produced from peptone or arginine. Methyl red test positive; Voges-Proskauer test negative. Xanthine, tyrosine, and urea not decomposed. Hydrogen sulfide produced. No growth in the presence of 4% NaCl. Utilization of pyruvate, fumarate, $\alpha$-ketoglutarate, and gluconate. Acid from arabinose, xylose, fructose, galactose, glucose, mannose, rhamnose, salicin, mannitol, cellobiose, gentiobiose, maltose, melibiose, sucrose, turanose, melezitose, raffinose, stachyose, dextrin, and starch. No acid from ribose, adonitol, dulcitol, inositol, sorbitol, and inulin. Variable acid production from glycerol, lactose, and trehalose.

**Habitat.** Soil. The type culture was deposited at the American Type Culture Collection as ATCC 25174 (our isolate number PSU-16S).

## DISCUSSION

This study has demonstrated the occurrence of an *Actinomyces*-like microorganism as a numer-

TABLE 3. *Selected characteristics of Actinomyces and Rothia species*[a]

| Property | *Actinomyces humiferus* 25174[b] | *A. bovis* 13683 | *A. israelii* 10048 | *A. naeslundii* 12104 | *A. odontolyticus* 17929 | *A. viscosus* 15987 | *A. eriksonii* 15423 | *R. dentocariosa* 17931 |
|---|---|---|---|---|---|---|---|---|
| Habitat | Soil | Animals | Animals | Animals | Animals | Animals | Animals | Animals |
| Catalase activity | − | − | − | − | − | + | − | + |
| Requirement for | | | | | | | | |
| Oxygen[c] | i | ii | ii | iii | ii | iv | v | vi |
| Organic nitrogen | + | + | + | + | + | + | + | + |
| Carbon dioxide | − | + | + | + | + | + | + | − |
| Lysozyme sensitivity[b] | + | − | − | − | ND[d] | ND | − | − |
| Nitrate reduction | − | − | + | + | + | + | − | + |
| Hydrolysis of | | | | | | | | |
| Casein | + | −[b] | −[b] | −[b] | − | − | ND | − |
| Starch | + | + | + or − | + or − | − | + | + | − |
| Gelatin liquefaction | − | − | − | − | − | − | − | + |
| Methyl red test | + | + | + | + | − | ND | + | + |
| Voges-Proskauer test | − | − | − | − | − | − | − | + |
| Production of | | | | | | | | |
| Indole | − | − | − | − | − | − | − | − |
| Hydrogen sulfide | + | − | − | + | − | − | + | − |
| Growth temperature optimum | 30 C | 37 C | 37 C | 37 C | 37 C | 37 C | 37 C | 37 C |
| Cell wall rhamnose[e] | + | + | − | + | − | + | − | − |
| Glucose fermentation | + | + | + | + | + | + | + | + |
| Acid from | | | | | | | | |
| Fructose | + | + | + | + | + | + | + | + |
| Glucose | + | + | + | + | + | + | + | + |
| Lactose | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + |
| Glycerol | + | − | − | − | − | − | − | + |
| Mannitol | + | − | + | − | − | − | + | − |
| Xylose | + | − | + | − | − | − | + | − |
| Cellobiose | + | − | + | − | − | ND | + | − |
| Melezitose | + | − | − | − | − | − | + | − |
| Rhamnose | + | − | − | − | − | − | − | − |
| Inositol | − | + | + | + | − | + | − | − |
| Starch | + | + | − | − | − | + | + | − |

[a] Data, unless otherwise stated, are compiled from ref. 2, 9, 11, 13, 14, 16, 17. Numbers identifying species are American Type Culture Collection numbers.

[b] Data obtained from the present investigation.

[c] (i) Microaerophilic to aerobic, (ii) microaerophilic to anaerobic, (iii) facultative, (iv) facultative to aerobic, (v) anaerobic, (vi) aerobic.

[d] Not determined.

[e] Present as a major component.

ically predominant inhabitant of certain soils. The major properties of this organism, such as morphology, catalase reaction, organic nitrogen requirement, cell wall composition, and carbohydrate fermentation, are in agreement with those commonly associated with the genus *Actinomyces*. However, at the species level our isolates are microaerophilic to aerobic, inhibited or at least not stimulated by increased $CO_2$ tension, and sensitive to lysis by lysozyme. Also, their optimum growth temperature is approximately 30 C, and they are able to hydrolyze casein. Since these characteristics differ significantly from those of previously described *Actinomyces* species (Table 3), it is proposed that a new species, *Actinomyces humiferus*, be created to encompass the properties of these soil isolates.

Of the various unique properties displayed by

*A. humiferus*, lysozyme sensitivity is of primary significance. Of the strains examined, 93% were susceptible to lysozyme lysis during growth, a phenomenon that we have not been able to demonstrate for other *Actinomyces* or *Rothia* species. Also, protoplasts could be prepared by adding lysozyme to actively growing or early stationary phase cells in an osmotically protective medium. Thus, although the general chemical composition of *A. humiferus*, as determined after acid hydrolysis, is similar to that for other *Actinomyces* species, its lysozyme sensitivity indicates a fundamental difference in cell wall structure.

*A. humiferus* displays more characteristics in common with *A. eriksonii* (Table 3) than with other *Actinomyces* species. However, major differences are that *A. eriksonii* is an obligate anaerobe, its cell walls contain galactose as the major sugar (13), and it does not ferment rhamnose.

The generally aerobic nature of *A. humiferus* is shared by the genus *Rothia*. This similarity could prove important from a phylogenetic standpoint if it is considered that *A. humiferus* might represent a transitional group between the anaerobic, catalase-negative *Actinomyces* species and the aerobic, catalase-positive species of *Rothia*. In addition to its catalase reaction, *Rothia* differs from *A. humiferus* by its ability to reduce nitrate, inability to grow on organic acids (11), lysozyme insensitivity, proteolytic nature, cell wall composition, and fermentative ability.

The occurrence of an *Actinomyces* species as a major population of certain soils is of both ecological and medical importance. Since this species is a numerically dominant soil inhabitant, it must possess certain unique capabilities which allow population maintenance at a high level. One might postulate either dormancy of the cells in soil, or the presence in soil of a food source of unique composition: one that is not readily decomposed by other soil microflora and is constantly present in sufficient quantities to sustain this large population. Since *A. humiferus* is proteolytic (at least for casein) and has only been found in soils with relatively high organic contents, humiclike soil material might offer an attractive possibility for a natural nutrient source. Detailed ecological studies must be initiated to provide answers pertaining to the nutrient source and also to establish if these organisms are as functionally important in soil as their numbers might suggest.

Further study of the relation of *A. humiferus* to medically important *Actinomyces* species could eventually lead to a clarification of the epidemiology of the diseases caused by the latter organisms. It is hoped that with the development and use of additional suitable isolation techniques, such as that employed in this study, other soil *Actinomyces* species can be isolated which will be of a more anaerobic nature than *A. humiferus* and better able to adapt to growth at 37 C. The concept of soil as a natural reservoir for infectious *Actinomyces* species does not seem so remote when one considers the greater prevalence of *Actinomyces* infections in grazing as opposed to carnivorous animals and rural as opposed to urban populations (30).

ACKNOWLEDGMENTS

The immunological relationships of our isolates to various genera of the *Actinomycetaceae* were determined by J. M. Slack and M. A. Gerencser (Medical Center, University of West Virginia). Deoxyribonucleic acid base compositions were determined by M. Mandel (M. D. Anderson Tumor Clinic, University of Texas).

LITERATURE CITED

1. Alcock, N. W. 1965. A simple procedure for the extraction and esterification of some organic acids. Anal. Biochem. 11:335–341.
2. Batty, I. 1958. *Actinomyces odontolyticus*, a new species of actinomycete regularly isolated from deep carious dentine. J. Pathol. Bacteriol. 75:455–459.
3. Beerens, H., and N. C. Hvid-Hansen. 1952. Presence dans les eaux sulfureuses francaises de bacteries reductuies de sulfate et d'une variete proteolytique d'*Actinobacterium israeli*: *A. israeli* var. *liquefaciens* (nov. sp.). Compt. Rend. 234:480–482.
4. Boone, C. J., and L. Pine. 1968. Rapid method for characterization of actinomycetes by cell wall composition. Appl. Microbiol. 16:279–284.
5. Buchanan, B. B., and L. Pine. 1962. Characterization of a propionic acid producing actinomycete, *Actinomyces propionicus*, sp. nov. J. Gen. Microbiol. 28:305–323.
6. Casida, L. E. Jr. 1965. Abundant microorganism in soil. Appl. Microbiol. 13:327–334.
7. Cottyn, B. G., and C. V. Boucque. 1968. Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. J. Agr. Food Chem. 16:105–107.
8. Cummins, C. S. 1962. Chemical composition and antigenic structure of cell walls of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Actinomyces*, and *Arthrobacter*. J. Gen. Microbiol. 28:35–50.
9. Davis, G. H. G., and J. H. Freer. 1960. Studies upon an oral aerobic actinomycete. J. Gen. Microbiol. 23:163–178.
10. DeWeese, M. S., M. A. Gerencser, and J. M. Slack. 1968. Quantitative analysis of *Actinomyces* cell walls. Appl. Microbiol. 16:1713–1718.
11. Georg, L. K., and J. M. Brown. 1967. *Rothia*, gen. nov., an aerobic genus of the family *Actinomycetaceae*. Intern. J. Syst. Bacteriol. 17:79–88.
12. Georg, L. K., G. W. Robertstad, and S. A. Brinkman. 1964. Identification of species of *Actinomyces*. J. Bacteriol. 88:477–490.
13. Georg, L. K., G. W. Robertstad, S. A. Brinkman, and M. D. Hicklen. 1965. A new pathogenic anaerobic *Actinomyces* species. J. Infec. Dis. 115:88–99.
14. Gerencser, M. A., and J. M. Slack. 1967. Isolation and characterization of *Actinomyces propionicus*. J. Bacteriol. 94:109–115.
15. Gledhill, W. E., and L. E. Casida, Jr. 1969. Predominant catalase-negative soil bacteria. III. *Agromyces*, gen. nov.

Microorganisms intermediary to *Actinomyces* and *Nocardia*. Appl. Microbiol., *in press*.

16. Howell, A., Jr., and H. V. Jordon. 1963. A filamentous microorganism isolated from peridontal plaque in hamsters. III. Physiological and biochemical characteristics. Sabouraudia 3:93–105.

17. Howell, A., Jr., H. V. Jordon, L. K. Georg, and L. Pine. 1965. *Odontomyces viscosus*, gen. nov. spec. nov., a filamentous microorganism isolated from peridontal plaque in hamsters. Sabouraudia 4:65–68.

18. Howell, A., Jr., W. C. Murphy III, F. Paul, and R. M. Stephans. 1959. Oral strains of *Actinomyces*. J. Bacteriol. 78:82–95.

19. Hvid-Hansen, N. 1951. Anaerobic actinomycetes (*Act. israelii*) in ground-water. Acta Pathol. Microbiol. Scand. 29:335–338.

20. Kalakutskii, L. V. 1960. Study of anaerobic proactinomycetes. I. Isolation of pure cultures from nature. Microbiology (Transl.) 29:59–63.

21. Li, Y. F., and L. K. Georg. 1968. Differentiation of *Actinomyces propionicus* from *Actinomyces israelii* and *Actinomyces naeslundii* by gas chromatography. Can. J. Microbiol. 14:749–753.

22. Markus, R. L. 1950. Colorimetric determination of lactic acid in body fluids utilizing cation exchange for deproteinization. Arch. Biochem. 29:159–165.

23. Melville, T. H. 1965. A study of the overall similarity of certain actinomycetes mainly of oral origin. J. Gen. Microbiol. 40:309–315.

24. Pine, L., and L. Georg. 1965. The classification and phylogenetic relationships of the *Actinomycetales*. Int. Bull. Bacteriol. Nomencl. Taxon. 15:143–163.

25. Pine, L., A. Howell, Jr., and S. H. Watson. 1960. Studies on the microbiological, physiological, and biochemical characteristics of *Actinomyces bovis*. J. Gen. Microbiol. 23:403–424.

26. Rosebury, T. 1944. The parasitic actinomycetes and other filamentous microorganisms of the mouth. A review of their characteristics and relationships, of the bacteriology of actinomycosis, and of salivary calculus in man. Bacteriol. Rev. 8:189–223.

27. Tewfik, E. M., and S. G. Bradley. 1967. Characterization of deoxyribonucleic acids from streptomycetes and nocardiae. J. Bacteriol. 94:1994–2000.

28. Tewfik, E., S. G. Bradley, S. Kurodo, and R. Y. Wu. 1968. Studies on the deoxyribonucleic acids from streptomycetes and nocardia. Develop. Ind. Microbiol. 20:242–249.

29. Waksman, S. A., and E. R. Purvis. 1932. The microbiological properties of peat. Soil Sci. 34:95–113.

30. Wilson, G. S., and A. A. Miles. 1957. Topley and Wilson's principles of bacteriology and immunity, vol. 2, p. 1461–1468. Williams & Wilkins Co., Baltimore.