RAPIDLY GROWING, ACID FAST BACTERIA[1]

I. Species' Descriptions of Mycobacterium phlei Lehmann and Neumann and Mycobacterium smegmatis (Trevisan) Lehmann and Neumann

RUTH E. GORDON and MILDRED M. SMITH

Department of Microbiology, Rutgers University, New Brunswick, New Jersey

Received for publication January 12, 1953

The studies of Thomson (1932), Gordon (1937), and Gordon and Hagan (1938) on the acid fast bacteria that grow with comparative rapidity at 28 C or 37 C on ordinary media divided these organisms into three major groups, mainly on the bases of temperature relationships and carbohydrate utilization. No attempt was made to define the species encountered in the three groups although the homogeneity of groups Ia and IIIa clearly indicated the presence of two recognizable species.

The purpose of the work reported in part here was to reassemble a collection of the rapidly growing, acid fast bacteria[2] and to prepare reliable, useful descriptions of the species represented.

MATERIALS AND METHODS

For the purposes of convenience and completeness, all media used are listed below:

Bennett's agar. Yeast extract, 1 g; beef extract, 1 g; N-Z Amine A, 2 g; glucose, 10 g; distilled water, 1,000 ml; pH 7.3 (Jones, 1949).

Glucose asparagine agar. Glucose, 10 g; asparagine, 0.5 g; K_2HPO_4, 0.5 g; agar, 15 g; distilled water, 1,000 ml; pH 6.8 (a modification of Conn's glycerol asparaginate agar—Conn, 1921).

Yeast extract agar. Yeast extract, 10 g; glucose, 10 g; agar, 15 g; tap water, 1,000 ml; pH 6.8 (Waksman, 1950).

Nutrient broth. Peptone, 5 g; beef extract, 3 g; distilled water, 1,000 ml; pH 7.0.

Nutrient agar. Nutrient broth with 1.5 per cent agar.

Glycerol broth. Nutrient broth with 7 per cent (by volume) glycerol.

Soil extract agar. Peptone, 5 g; beef extract, 3 g; agar, 15 g; soil extract, 1,000 ml; pH 7.0. Soil extract was prepared by autoclaving 1,000 g of air-dried soil, sifted through a no. 9 mesh screen, with 2,400 ml of tap water at 121 C for 1 hour; decanting; and filtering through paper.

Glycerol agar. Soil extract agar with 7 per cent glycerol.

Microscopic examination. Cultures on glycerol agar, incubated at 37 C for 48 hours, were smeared on a clean slide, air-dried, and stained for 2 minutes with aqueous fuchsin (0.5 ml of a saturated alcoholic solution of basic fuchsin in 100 ml of distilled water). The vegetative rods (20 per culture) were measured with an ocular micrometer.

Acid fastness of the cultures was determined by the Ziehl-Neelsen method after 5 days' incubation at 28 C on glycerol agar. The cultures were smeared on clean slides, air-dried, and immersed for 5 minutes in a boiling solution of carbol fuchsin (saturated alcoholic solution of basic fuchsin, 10 ml; 5 per cent aqueous solution of phenol, 90 ml). The slides were washed, dipped once in acid alcohol (concentrated HCl, 3 ml; 95 per cent alcohol, 97 ml), quickly washed, and counterstained for 1 minute with methylene blue (saturated alcoholic solution of methylene blue, 30 ml; 0.01 per cent aqueous solution of KOH, 100 ml).

Colonial morphology was observed after inoculating tubes of soil extract agar and Bennett's agar that had been melted and cooled to 47 C. A few drops of each agar were pipetted aseptically to a sterile glass slide and incubated at 28 C inside a sterile, moist chamber. At 3 to 7 days, depending on the rapidity of growth, the colonies were examined under the microscope.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Microbiology. This investigation was supported in part by a research grant, no. E 157, from the National Institutes of Health, Public Health Service.

[2] The writers wish to express their thanks to the investigators who kindly supplied cultures for this study.

Temperatures of growth. Glycerol agar slants were inoculated and immediately placed in a water bath at the desired temperature. After the temperature of the cultures had reached that of the water bath, they were transferred to another water bath inside a constant temperature incubator, previously adjusted to maintain the proper temperature in the bath. Growth was observed after 7 days' incubation at temperatures of 37 C or above and after 14 days at temperatures below 20 C.

Survival of 60 C. Subcultures were prepared on glycerol agar slants, quickly preheated to 60 C, and placed in a water bath at the same temperature. After 4 hours they were removed, immediately cooled, stored at 28 C for 2 weeks, and observed for growth.

Hydrolysis of starch. Duplicate plates of nutrient agar with 1 per cent potato starch[3] were streaked and incubated at 28 C. Hydrolysis of starch was determined by flooding one plate at 5 days and the other at 10 days with 95 per cent alcohol (Kellerman and McBeth, 1912). The amount of hydrolysis was indicated by a clear zone underneath and bordering the growth; unchanged starch became white and opaque.

Acid production from carbohydrates. One-half ml amounts of a 10 per cent aqueous solution of the carbohydrate to be tested, sterilized by autoclaving, were added aseptically to tubes containing 5 ml of sterile, inorganic nitrogen base, a modification of one proposed by Ayers, Rupp, and Johnson (1919). The inorganic nitrogen base had the following composition: $(NH_4)_2HPO_4$, 1 g; KCl, 0.2 g; $MgSO_4$, 0.2 g; agar, 15 g; distilled water, 1,000 ml. The pH of the medium was adjusted to 7.0 before the addition of 15 ml of a 0.04 per cent solution of brom cresol purple. Cultures on this carbohydrate agar were observed for acid production after 7 and 28 days' incubation at 28 C.

Hydrolysis of gelatin. The cultures were streaked once across duplicate plates of nutrient agar plus 0.4 per cent gelatin and incubated at 28 C. One plate was examined for hydrolysis of the gelatin at 5 days and the second at 10 days by flooding with 8 to 10 ml of the following solution: $HgCl_2$, 15 g; concentrated HCl, 20 ml; distilled water, 100 ml (Frazier, 1926). A clear zone underneath and around the growth provided a measure of the hydrolysis, while the unchanged gelatin appeared as a white, opaque precipitate.

Hydrolysis of casein. Skim milk or skim milk powder suspended in the proper amount of water was sterilized by autoclaving at 121 C for 20 minutes; cooled to 47 C; mixed with an equal volume of sterile 2 per cent water agar, also at 47 C; and poured into sterile plates. These were streaked, incubated at 28 C, and observed for clearing of the casein at 7 and 14 days (Hastings, 1903).

Utilization of citrate, succinate, and malate. The cultures were inoculated on slants of each of the three following media, modifications of Koser's citrate agar (1924): NaCl, 1 g; $MgSO_4$, 0.2 g; $(NH_4)_2HPO_4$, 1 g; KH_2PO_4, 0.5 g; Na citrate (or Na succinate, or Ca malate), 2 g; agar, 15 g; distilled water, 1,000 ml. The pH of each medium was adjusted to 7.0 before addition of 20 ml of a 0.04 per cent solution of phenol red. Observations for utilization of the citrate, succinate, and malate, indicated by an alkaline reaction of the specific agar, were made after 7 and 28 days' storage at 28 C.

Reduction of nitrate to nitrite. Tubes of nutrient broth plus 0.1 per cent KNO_3 were inoculated and incubated at 28 C. At 5, 10, and 14 days 1 ml of the broth culture was withdrawn with a sterile pipette and mixed with 3 drops of each of the following solutions: (1) sulfanilic acid, 8 g; 5 N acetic acid (1 part of glacial acetic acid to 2.5 parts of water), 1,000 ml; (2) dimethylalphanaphythylamine, 6 ml; 5 N acetic acid, 1,000 ml. The appearance of a heavy, yellowish precipitate or a red color within 10 minutes was considered proof of the presence of nitrites (Conn, 1951). In the absence of nitrite, a qualitative determination for nitrate always was made by adding 4 to 5 mg[4] of zinc dust to the tube previously tested for nitrites. Reduction of nitrate, if present, to nitrite by the zinc resulted in the formation of a red color.

NaCl tolerance. The cultures were inoculated into 3 tubes of glycerol broth containing 0, 5, and 7 per cent NaCl, respectively. Care was taken to avoid transfer of a large amount of inoculum. The growth in the 3 tubes was compared after 14 and 28 days' incubation at 28 C.

[3] The potato starch was suspended in approximately 40 ml of cold water before being mixed with the hot, melted agar. After autoclaving, the agar was shaken thoroughly to ensure proper distribution of the starch in the plates.

[4] The use of an excessive amount of zinc dust should be avoided.

COMPARATIVE STUDY OF CULTURES

In some instances during the assembly of a collection of rapidly growing, acid fast bacteria the same strain was received from different sources. The opportunity thus afforded to observe any spontaneous dissociation that might have occurred was welcomed, and the cultures were given different numbers and considered as separate strains.

At the time of this report 124 strains have been studied. Of these, 70 were received as named strains, representing 17 different species; the remainder were unidentified isolations from a variety of sources. The strains were examined microscopically and macroscopically, and as many of their physiological reactions were determined as time permitted. On the basis of the data accumulated, 77 of the 124, or 62 per cent, could be placed in two distinct groups. Each group was comprised of closely related strains and was considered representative of a recognizable species. Descriptions of each were prepared. The relationships among the remaining 38 per cent of the strains did not allow any conclusions concerning the number of species present.

Mycobacterium phlei Lehmann and Neumann

The Timothy Bacillus, or Grass Bacillus I, of Moëller (1898*a*,*b*) was named *Mycobacterium phlei* by Lehmann and Neumann in 1899. Although the descriptions of Moëller and Lehmann and Neumann did not offer positive identification of the original culture, there was nothing in them at variance with the identity of the species as recognized today. General agreement on the identity of *M. phlei* was evidenced by the fact that most of the strains received bearing this name were labeled correctly and also by the comprehensive characterization of the species by Penso *et al.* (1951). In instances in which the same characters were investigated, the description of Penso *et al.* was corroborated in the main by the one given below. Two exceptions were the liquefaction of gelatin and reduction of nitrate to nitrite, reported as negative by Penso *et al.* Nearly one-third of the strains studied by the writers hydrolyzed gelatin, but this discrepancy undoubtedly was due to different test methods. All strains examined, including one kindly supplied by Dr. Penso, reduced nitrates to nitrites.

The 21 strains identified as *M. phlei* and upon which the following characterization was based are listed in table 1. Among these was the strain (no. 356) received as *Mycobacterium berolinense* Bergey *et al.*, a species considered as belonging in the synonymy of *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann. (See discussion below under the latter species.) For this reason strain no. 356 was regarded as misnamed.

TABLE 1

Strains identified as Mycobacterium phlei Lehmann and Neumann

LABORATORY NUMBER	NAME AND SOURCE
356	*Mycobacterium berolinense* Bergey *et al.*; ATCC; E. G. Hastings (74C); NCTC (524)
W23	*Mycobacterium phlei* Lehmann and Neumann; S. A. Waksman, Rutgers Univ.; Univ. de Liège
5	*Mycobacterium phlei*; J. H. Hanks, Harvard Univ.
8A	*Mycobacterium phlei*; W. A. Hagan, Cornell Univ.
24	*Mycobacterium phlei*; P. Hauduroy, CCTM* (A602); Trudeau Lab.
45	*Mycobacterium phlei*; Doris Wingate, Duke Univ.; J. D. Aronson (HPI)
354	*Mycobacterium phlei*; ATCC; E. G. Hastings (74A); NCTC (54)
M354	*Mycobacterium phlei*; P. A. Hansen, Univ. of Md.; ATCC (354)
10142	*Mycobacterium phlei*; F. Kavanagh, N. Y. Botanical Gardens (17P)
N8151	*Mycobacterium phlei*; NCTC (Timoteo)
34 to 44	*Mycobacterium* spp; Doris Wingate; W. A. Hagan (39, 105, 114, 142, 40, 64, 116, 47, 13, 41, 51)

* Centre de Collection de Types Microbiens, Lausanne, Switzerland.

Microscopic appearance. The average length of the rods, cultivated on glycerol agar at 37 C for 48 hours, ranged from 1.0 μ to 2.0 μ although the rods of a few cultures were longer, averaging 2.5 μ to 3.0 μ. After 5 days' incubation at 28 C, 80 to 100 per cent of the rods of 12 cultures retained the acid fast stain; the remaining 9 cultures exhibited from 5 to 20 per cent acid fastness. The cells varied from coccoid forms to short rods, some with irregularly and some with uniformly stained protoplasm. Filaments were rarely, if ever, seen.

Three types of colonies were ordinarily present, depending somewhat upon the age of the culture. Dense colonies with smooth edges were usually in the majority; dense colonies surrounded by a fringe of filaments and tiny filamentous colonies completed the picture. After further incubation the filaments fragmented into short rods.

Macroscopic appearance. A large majority of the 21 strains grew in the rough stage, and young cultures (2 to 4 days) on glycerol agar, incubated at 28 C, formed a thin, dry, spreading, pale yellow growth. After 10 to 14 days the growth was thick, waxy, coarsely wrinkled, and deep yellow to orange in color. Cultures in the smooth stage were soft, spreading, butyrous, yellow to orange. On yeast extract agar the growth usually was abundant, dry, spreading, raised, finely wrinkled to coarsely folded, deep yellow to orange. Smooth cultures resembled those on glycerol agar. On glucose asparagine agar the growth, if any, was scant to fair, flat and restricted to raised and finely folded, cream colored to yellowish orange.

Temperatures of growth. All strains grew from 28 C to 52 C inclusive; approximately one-half grew scantly at 15 C; none at 10 C.

Survival of 60 C. With one exception (no. M354) all strains survived 4 hours' exposure to 60 C.

Hydrolysis of starch. All strains were positive.

Acid production from carbohydrates. The 21 cultures formed acid from glucose, mannitol, and sorbitol; with one exception (no. 42) all formed acid from mannose; with two exceptions (nos.

TABLE 2

Strains identified as Mycobacterium smegmatis (Trevisan) Lehmann and Neumann

LABORATORY NUMBER	NAME AND SOURCE
W112	*Mycobacterium avium* Chester; S. A. Waksman, Rutgers Univ.; ATCC (7992)
4676	*Mycobacterium avium;* ATCC; P. Kyes; Th. Smith
7992, 9077	*Mycobacterium avium;* ATCC; U. S. Dept. of Agr.
20	*Mycobacterium berolinense* Bergey *et al.*; ATCC; Hyg. Lab. (181)
29	*Mycobacterium berolinense;* P. Hauduroy, CCTM (A907); G. Penso
280	*Mycobacterium berolinense;* ATCC; E. O. Jordan
30	*Mycobacterium butyricum* Bergey *et al.*; P. Hauduroy (A172); NCTC (334)
W111	*Mycobacterium butyricum;* S. A. Waksman; ATCC (359)
358 to 360	*Mycobacterium butyricum;* ATCC; E. G. Hastings (74E, 74F, 74G); NCTC (335, 334, 333)
362	*Mycobacterium butyricum;* ATCC; E. G. Hastings
32	*Mycobacterium friburgensis* (Korn) Chester; P. Hauduroy (A908); G. Penso
114	*Mycobacterium friedmannii* Holland; ATCC; Hyg. Lab. (169); Friedmann
169	*Mycobacterium friedmannii;* ATCC; Hyg. Lab. (169); Friedmann
26	*Mycobacterium graminis* Chester; P. Hauduroy (A60); Pasteur Inst.
N8150, N8153	*Mycobacterium lacticola* Lehmann and Neumann; NCTC
355	*Mycobacterium phlei* Lehmann and Neumann; ATCC; E. G. Hastings (74B); NCTC (525)
375 to 377, 380	*Mycobacterium phlei;* G. P. Mueller, Univ. of Tenn. (M, 10, 13–47, 77–4–1)
W105	*Mycobacterium ranae* (Küster) Bergey *et al.*; S. A. Waksman; Raybrook Sanitorium
W106	*Mycobacterium ranae;* S. A. Waksman; Trudeau Lab.
W108	*Mycobacterium ranae;* S. A. Waksman; ATCC (110); Hyg. Lab. (393)
110	*Mycobacterium ranae;* ATCC; Hyg. Lab. (393)
W113	*Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann; S. A. Waksman; ATCC (101); Hyg. Lab. (178)
3	*Mycobacterium smegmatis;* W. A. Hagan, Cornell Univ.
25	*Mycobacterium smegmatis;* P. Hauduroy (A909); G. Penso
101	*Mycobacterium smegmatis;* ATCC; Hyg. Lab. (178)
278	*Mycobacterium smegmatis;* ATCC; E. O. Jordan
361	*Mycobacterium smegmatis;* ATCC; E. G. Hastings
10143	*Mycobacterium smegmatis;* ATCC; N. Y. Botanical Gardens (17S)
N523, N8152	*Mycobacterium smegmatis;* NCTC (New York, G. W.)

TABLE 2—*Continued*

LABORATORY NUMBER	NAME AND SOURCE
31	*Mycobacterium stercoris* Bergey *et al.*; P. Hauduroy (A182); NCTC (3821)
77	*Mycobacterium stercoris;* ATCC; Hyg. Lab. (179)
281	*Mycobacterium stercoris;* ATCC; E. O. Jordan
599	*Mycobacterium tuberculosis* var. *bovis* Lehmann and Neumann; ATCC; E. J. Lynch; AMNH (69)
8420	*Mycobacterium tuberculosis* var. *bovis*; ATCC; H. J. Corper (C-33-W)
W24	*Mycobacterium tuberculosis* var. *hominis* Lehmann and Neumann; S. A. Waksman; H. B. Woodruff; ATCC (607)
W114	*Mycobacterium tuberculosis* var. *hominis*; S. A. Waksman (streptomycin resistant mutant of W24)
279	*Mycobacterium tuberculosis* var. *hominis*; ATCC
607	*Mycobacterium tuberculosis* var. *hominis*; ATCC; John McCormick Inst.; F. J. Novy (Koch's strain of the tubercle bacillus)
33	*Mycobacterium* sp; Doris Wingate, Duke Univ.; W. A. Hagan (59)
N334	*Mycobacterium* sp; NCTC (G. P. 61)
66	*Mycobacterium* sp; ATCC; Hyg. Lab. (367, rat #2, associated with leprous lesions)
69	*Mycobacterium* sp; ATCC; Army Med. School (366, associated with leprous lesions of rat)
283	*Mycobacterium* sp; ATCC; E. O. Jordan (associated with leprous lesions)
4236	*Mycobacterium* sp; ATCC; C. W. Duval (108, Brinkerhoff, associated with leprous lesions)
8157	*Mycobacterium* sp; ATCC; Inst. Oswaldo Cruz (sputum of leper)
393	*Mycobacterium* sp; P. Stuart, Ministry of Agr. and Fisheries, England (Bloomfield Sc. 3.51, bovine mastitis)
391, 392	*Nocardia* or *Proactinomyces* spp; C. Møller, Novo Terapeutisk Lab., Denmark (bovine abortion)

37 and 42), from arabinose; and approximately one-half, from xylose and galactose. Rhamnose, inositol, dulcitol, lactose, and raffinose were not attacked.

Hydrolysis of gelatin. Six of the 21 strains hydrolyzed gelatin to a limited extent.

Hydrolysis of casein. All cultures were negative.

Utilization of citrate, succinate, and malate. Nineteen strains (exceptions, nos. 42 and M354) decomposed citrate; 19 (exceptions, nos. 35 and 37) utilized succinate; and 18 (exceptions, nos. 35, 37, and 42), malate.

Reduction of nitrate to nitrite. All cultures were positive.

NaCl tolerance. The cultures grew scantily, if at all, in broth containing 5 per cent NaCl.

Mycobacterium smegmatis (Trevisan) Lehmann and Neumann

The 56 strains listed in table 2 and forming the second distinct group encountered in this study represented, as received, 12 different species. The usual difficulties involved in the selection of the proper species' name for the group (Buchanan *et al.*, 1948) were complicated further by the fact that continued cultivation *in vitro* of pathogenic, acid fast strains produces rapidly growing cultures that cannot be distinguished from acid fast saprophytes present in soil, water, plants, etc. (Moëller, 1899). Strains with histories of long cultivation *in vitro* and labeled *Mycobacterium tuberculosis* var. *hominis* Lehmann and Neumann, *Mycobacterium tuberculosis* var. *bovis* Lehmann and Neumann, or *Mycobacterium avium* Chester belonged to this group. For practical reasons alone, however, it was not feasible to apply the oldest specific epithet, *tuberculosis* (Schroeter, 1886), to this species of rapidly growing, acid fast bacteria. The next oldest epithet borne by strains of this group was *smegmatis* (*Bacillus smegmatis*), given by Trevisan in 1889 to the Smegma Bacillus of Alvarez and Tavel (1885). The name, *M. smegmatis*, first used by Lehmann and Neumann (1899), therefore was proposed for this species. The early descriptions of *M. smegmatis* provided

no opposition to this suggestion although they offered little for positive identification of the original culture.

The early descriptions of 7 species, *M. berolinense* Bergey *et al.* (1923) (Rabinowitsch, 1897; Lehmann and Neumann, 1899), *Mycobacterium butyricum* Bergey *et al.* (1923) (Petri, 1898), *Mycobacterium friburgensis* (Korn) Chester (1901) (Korn, 1899), *Mycobacterium friedmannii* Holland (1920) (Friedmann, 1903), *Mycobacterium graminis* Chester (1901) (Moëller, 1899; Lehmann and Neumann, 1899), *Mycobacterium lacticola* Lehmann and Neumann (1899), and *Mycobacterium stercoris* Bergey *et al.* (1923) (Moëller, 1898*a*,*b*), were not at variance with the strains bearing these names. These 7 species, therefore, were considered to be in the synonymy of *M. smegmatis*. All strains received, labeled *Mycobacterium ranae* (Küster) Bergey *et al.* (1923), were identical with *M. smegmatis*. Küster (1905), however, reported that his organism did not grow at 37 C, and for this reason, the 4 strains of *M. ranae* were regarded as misnamed.

Microscopic appearance. Cells grown on glycerol agar for 48 hours at 37 C averaged 3.0 μ to 5.0 μ in length, the larger number of strains ranging from 4.0 μ to 5.0 μ. Fifty to 80 per cent of the cells of three-fourths of the cultures after 5 days' incubation on glycerol agar at 28 C were acid fast. The remaining cultures showed 10 to 20 per cent acid fastness. The organisms were usually slender; of varying lengths; often filamentous; and sometimes branched, curved, and beaded. Cells swollen with oval, deeper staining bodies were occasionally seen.

Colonial morphology resembled that of *M. phlei*. Dense colonies with smooth edges, dense colonies fringed with filaments, and filamentous colonies were observed. The filaments fragmented into short rods upon continued incubation.

Macroscopic appearance. Growth on glycerol agar at 2 to 3 days was good, spreading, finely wrinkled, and creamy white. At 2 weeks the cultures were abundant, spreading, finely wrinkled, waxy, creamy yellow to pale orange. On yeast extract agar they were abundant, spreading, finely wrinkled to coarsely folded, waxy, creamy yellow to orange. Cultures in the smooth stage were abundant, glistening, butyrous, and sometimes nodular. Growth on glucose asparagine agar was fair to abundant, spreading, flat to finely wrinkled to coarsely folded, smooth and butyrous to dry and waxy, creamy white to yellow to beige to orange.

Temperatures of growth. All strains grew from 28 C to 45 C inclusive; a few grew meagerly at 50 C; none, at 52 C. Growth at 10 C, if any, was scant.

Survival of 60 C. None of the cultures grew after 4 hours in the water bath at 60 C.

Hydrolysis of starch. All cultures were positive.

Acid production from carbohydrates. The 56 cultures formed acid from glucose, rhamnose, xylose, arabinose, sorbitol, inositol, mannose, and galactose. In the very few cases in which a culture failed to yield acid from one of these carbohydrates, a transfer from the negative, 28 day old slant to a fresh tube of the same carbohydrate agar resulted in a positive culture. Acid usually was produced from dulcitol although more slowly than from the other carbohydrates. Lactose was not hydrolyzed, and with one exception (no. 391), raffinose was negative.

Hydrolysis of gelatin. Only one strain (no. 4236) hydrolyzed gelatin to a limited extent.

Hydrolysis of casein. All cultures were negative.

Utilization of citrate, succinate, and malate. Only one strain (no. 4236) failed to utilize citrate; all cultures utilized succinate and malate.

Reduction of nitrate to nitrite. All but 2 strains (nos. 391 and 392) reduced nitrates to nitrites.

NaCl tolerance. Growth was usually positive in 5 per cent NaCl and usually negative in 7 per cent.

Mycobacterium spp

The 47 strains that did not belong to the species *M. phlei* and *M. smegmatis* included those labeled *Mycobacterium enteritidis* Lehmann and Neumann, *Mycobacterium fortuitum* Cruz, *Mycobacterium marinum* Aronson, *Mycobacterium platypoecili* Baker and Hagan, and *Mycobacterium thamnopheos* Aronson. The 47 cultures failed to grow at 45 C, but otherwise, on the basis of the tests applied, exhibited little similarity among themselves. The presence of two additional species was indicated merely by an aggregate of 9 strains and by one of 6, numbers too small for the delineation of species. The remaining strains appeared unrelated or resembled only one or two others.

DISCUSSION

The progress made in this study on the separation of the rapidly growing, acid fast bacteria

into recognizable species admittedly was limited. The compact groups Ia and IIIa of Gordon (1937) and Gordon and Hagan (1938) had their counterparts in the present collection and were described here as *M. smegmatis* (Trevisan) Lehmann and Neumann and *M. phlei* Lehmann and Neumann, respectively.

Only after the study of many more strains can the remaining species of this group be characterized. The various tests and observations employed are not considered adequate for the delineation of the remainder of the species, and the search for additional, correlating characters for their identification must be continued.

Application of additional tests in the study of acid fast strains will result undoubtedly in enlargement and possibly modification of the descriptions of *M. phlei* and *M. smegmatis* as given here. These two species, however, appeared as such distinct units that they are believed to be separable and identifiable by more than one combination of characters.

SUMMARY

A collection of 124 strains of rapidly growing, acid fast bacteria was examined. Sixty-two per cent of these were found to belong to two species, *Mycobacterium phlei* Lehmann and Neumann and *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann. Descriptions of each species were presented. *Mycobacterium berolinense* Bergey *et al.*, *Mycobacterium butyricum* Bergey *et al.*, *Mycobacterium friburgensis* (Korn) Chester, *Mycobacterium friedmannii* Holland, *Mycobacterium graminis* Chester, *Mycobacterium lacticola* Lehmann and Neumann, and *Mycobacterium stercoris* Bergey *et al.* were listed in the synonymy of *Mycobacterium smegmatis*.

REFERENCES

Alvarez, and Tavel 1885 Recherches sur le bacille de Lustgarten. Arch. de physiologie normal et pathologique, **6**, 303–321.

Ayers, S. H., Rupp, R., and Johnson, W. T. 1919 A study of the alkali-forming bacteria found in milk. U. S. Dept. Agr., Bull. 782.

Bergey, D. H. et al. 1923 *Bergey's manual of determinative bacteriology*. 1st ed., p. 377. The Williams & Wilkins Co., Baltimore, Md.

Buchanan, R. E., St. John-Brooks, R., and Breed, R. S. 1948 International bacteriological code of nomenclature. J. Bact., **55**, 287–306.

Chester, F. D. 1901 *Manual of determinative bacteriology*, p. 357. The Macmillan Co., New York and London.

Conn, H. J. 1921 The use of various culture media in characterizing actinomycetes. N. Y. Agr. Exptl. Sta., Tech. Bull. 83.

Conn, H. J. 1951 Routine tests for the descriptive chart. Manual of methods for pure culture study of bacteria. Biotech. Pub., Geneva, N. Y., Leaflet 5, 12th ed., 10–11.

Frazier, W. C. 1926 A method for the detection of changes in gelatin due to bacteria. J. Infectious Diseases, **39**, 302–309.

Friedmann, F. F. 1903 Der Schildkrötentuberkelbacillus, seine Züchtung, Biologie, und Pathogenetät. Zentr. Bakteriol. Parasitenk. Orig., I, **34**, 647–658.

Gordon, R. E. 1937 The classification of acid-fast bacteria. J. Bact., **34**, 617–630.

Gordon, R. E., and Hagan, W. A. 1938 The classification of acid-fast bacteria, II. J. Bact., **36**, 39–46.

Hastings, E. G. 1903 Milchagar als Medium zur Demonstration der Erzeugung proteolytischer Enzyme. Zentr. Bakteriol. Parasitenk., II, **10**, 384.

Holland, D. 1920 Final report of the committee of the Society of American Bacteriologists on characterization and classification of bacterial types. J. Bact., **5**, 224.

Jones, K. L. 1949 Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. J. Bact., **57**, 141–145.

Kellerman, K. K., and McBeth, I. G. 1912 The fermentation of cellulose. Zentr. Bakteriol. Parasitenk., II, **34**, 485–494.

Korn, O. 1899 Zur Kenntnis der säuerfesten Bakterien. Zentr. Bakteriol. Parasitenk., I, **25**, 532–541.

Koser, S. A. 1924 Correlation of citrate utilization by members of the colon-aerogenes groups with other differential characteristics and with habitat. J. Bact., **9**, 59–77.

Küster, E. 1905 Ueber Kaltblütertuberkulose. Münch. med. Wochschr., **52**, 57–59.

Lehmann, K. B., and Neumann, R. O. 1899 Bakteriologische Diagnostik. L. F. Lehmanns Verlg./München, 2. Auf., II. Band, 408–413.

Moëller, A. 1898*a* Microorganismen, die den Tuberkelbacillen verwandt sind und bei Thieren eine miliare Tuberkelkrankheit verursachen. Deut. med. Wochschr., **24**, 376–379.

Moëller, A. 1898*b* Ueber dem Tuberkelbacillus verwandte Mikroorganismen. Therap. Monatsh., **12**, 607–613.

MOËLLER, A. 1899 Ein neuer säure-und alkoholfester Bacillus aus der Tuberkelbacillengrüppe, welcher echte Verzweigungsformen bildet. Zentr. Bakteriol. Parasitenk., I, **25**, 369–373.

PENSO, G., ORTALI, V., GAUDIANO, A., PRINCIVALLE, M., VELLA, L., AND ZAMPIERI, A. 1951 Studi e ricerche sui micobatteri VII. II *Mycobacterium phlei*. Rend-dell'Istituto Superiore di Sanità, **14**, 855–908.

PETRI 1898 Zum Nachweis der Tuberkelbazillen in Butter und Milch. Arbeiten aus der Kaiserlich. Gesundheitsamte, **14**, 1–35.

RABINOWITSCH, L. 1897 Zur Frage des Vorkommens von Tuberkelbazillen in der Marktbutter. Z. Hyg. Infektionskrankh., **26**, 90–111.

SCHROETER, J. 1886 In Cohn, Kryptogamen-Flora von Schleisen., **3**, 164.

THOMSON, H. M. 1932 Studies on saprophytic acid-fast bacteria. Am. Rev. Tuberc., **26**, 162–178.

TREVISAN, V. 1889 I generi e la specie dell Batteriacee, p. 14, Zanaboni e Gabuzzi Milano.

WAKSMAN, S. A. 1950 The actinomycetes. Ann. Cryptogamici et Phytopathologici. Chronica Botanica Co., Waltham, Mass., **9**, 196.