RAPIDLY GROWING, ACID FAST BACTERIA¹

II. SPECIES' DESCRIPTION OF *Mycobacterium fortuitum* CRUZ

RUTH E. GORDON AND MILDRED M. SMITH

Institute of Microbiology, Rutgers University, the State University of New Jersey, New Brunswick, New Jersey

Received for publication October 13, 1954

The taxonomic study of the acid fast bacteria capable of comparatively rapid growth on ordinary media, first reported in 1953 by Gordon and Smith, has been continued. Additional strains have been examined and other tests applied to all the strains. A few supplementary characteristics of the two previously delineated species, *Mycobacterium phlei* Lehmann and Neumann and *Mycobacterium smegmatis* (Trevi-
san) Lehmann and Neumann, are presented, and the strains newly assigned to these species are listed.

As the work progressed, a third group of strains emerged. The strains of this taxon seemed closely related to each other and sufficiently distinct from the other strains of the collection to warrant their separation into a species. A description of this third species, *Mycobacterium fortuitum* Cruz, is given and compared with those of *M. phlei* and *M. smegmatis*.

MATERIALS AND METHODS

In addition to the observations and tests used by Gordon and Smith (1953), the following ones were employed:

Decomposition of tyrosine. The cultures were streaked on plates of tyrosine agar and examined after 14 and 21 days' incubation at 28 C for the disappearance of the tyrosine crystals underneath and around the growth. The agar contained peptone, 5 g; beef extract, 3 g; agar, 15 g; tyrosine, 5 g; distilled water, 1,000 ml; pH 7.0. The medium was thoroughly mixed during each step in its preparation to provide a final, even distribution of the insoluble tyrosine throughout the agar.

Utilization of benzoate. The cultures were inoculated and incubated at 28 C for 4 weeks on

the following medium, a modification of Koser's citrate agar (1924): NaCl, 1 g; MgSO₄, 0.2 g; (NH₄)₂HPO₄, 1 g; KH₂PO₄, 0.5 g; Na benzoate, 2 g; agar, 15 g; distilled water, 1,000 ml. The pH of the agar was adjusted to 7.0, and 20 ml of a 0.04 per cent solution of phenol red were added. An alkaline reaction of the medium indicated use of the benzoate.

Acid from carbohydrates. Maltose and trehalose were used in conjunction with the carbohydrates previously listed.

DESCRIPTIONS OF SPECIES

The collection² of mycobacteria forming the basis of this taxonomic study increased from the 124 strains of the first report to 195. The named strains, as received, represented 23 different species; the unnamed ones were isolated from a variety of sources. Of the 195 strains, 68 per cent were assigned to the following three taxa:

Mycobacterium phlei Lehmann and Neumann

The six strains (three received as *M. phlei* and three unnamed isolations) added to the original 21 of this species (Gordon and Smith, 1953, p. 43) are listed in table 1. General agreement on the identity of *M. phlei* was again evidenced by the correct identification of the three named strains and the absence of misnamed strains and of strains bearing specific names in synonymy.

Cultures of the six strains grown on glycerol agar for 5 days at 28 C were 25 to 80 per cent acid fast. The cells varied from coccoid forms to short filaments, and some had granular protoplasm. The colonial morphology, macroscopic appearance, temperature relationships, and

¹ This investigation was supported in part by a research grant, no. E 157-E 157 (C₂), from the National Institutes of Health, Public Health Service.

² The writers wish to express their sincere appreciation to the investigators who kindly supplied the cultures for this study.

TABLE 1

Strains identified as *Mycobacterium phlei* Lehmann and Neumann

Laboratory Number	Name and Source
464	<i>Mycobacterium phlei</i> Lehmann and Neumann; W. Steenken, Jr., Trudeau Lab.
468	<i>Mycobacterium phlei</i> ; G. Penso, Istituto Superiore de Sanità, Rome (Timoteo 2X)
500	<i>Mycobacterium phlei</i> ; A. Abrams, Army Med. Services Grad. School (52-R-1)
451	<i>Mycobacterium</i> sp; D. W. Bruner, Cornell Univ. (57); C. A. Frey (soil)
479	<i>Mycobacterium</i> sp; P. Stuart, Ministry of Agr. and Fisheries, England (MS. 247.52, bovine mastitis)
501	<i>Mycobacterium</i> sp; A. Abrams (52-B-1, associated with leprous lesions)

other reactions agreed with those of the previously described strains of *M. phlei*. All grew from 28 C to 52 C inclusive; none, at 10 C; and all survived 60 C for 4 hours. Only one culture (no. 451) hydrolyzed gelatin to a limited extent; none decomposed casein or grew in broth containing 5 per cent NaCl. The six strains utilized citrate, succinate, and malate; reduced nitrate to nitrite; hydrolyzed starch; and formed acid from glucose, xylose, arabinose, mannose, galactose, mannitol, and sorbitol. Acid formation from rhamnose, inositol, dulcitol, lactose, and raffinose was negative.

The following reactions of the 27 strains in this collection identified as *M. phlei* were determined and used to supplement the description of the species:

Decomposition of tyrosine. All cultures were negative.

Utilization of benzoate. All cultures were negative.

Acid production from carbohydrates. Two cultures (nos. N8151 and M354) of the 27 were able to form acid from maltose; 18 formed acid from trehalose.

Mycobacterium smegmatis (Trevisan) Lehmann and Neumann

Eighteen of the 24 additional strains assigned to this species were unidentified isolations, and the remaining six were received as representatives of four different species (see table 2). Strain

no. 465 of *Mycobacterium butyricum* and strain no. 446 of *Mycobacterium friedmannii* were in agreement with others bearing the same labels and previously assigned to the synonymy of *M. smegmatis*. The three strains received as *Mycobacterium ranæ* also conformed to those examined earlier and were identical with strains

TABLE 2

Strains identified as *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann

Laboratory Number	Name and Source
447	<i>Mycobacterium aquae</i> Maië; CCTM (7)
465	<i>Mycobacterium butyricum</i> Bergey <i>et al.</i> ; W. Steenken, Jr., Trudeau Lab.
446	<i>Mycobacterium friedmannii</i> Holland; CCTM (178)
540 to 542	<i>Mycobacterium ranæ</i> (Küster) Bergey <i>et al.</i> ; J. Hsie, Still College (1, original wild type; 2, isoniazid resistant, slow growing; 5, isoniazid resistant, fast growing)
108, 114M	<i>Mycobacterium</i> spp; E. B. McKinley, George Washington Univ.
422	<i>Mycobacterium</i> sp; Isabel Christison, Duke Univ. (317); D. K. Miller
450	<i>Mycobacterium</i> sp; D. W. Bruner, Cornell Univ. (50); C. A. Frey (soil)
454	<i>Mycobacterium</i> sp; D. W. Bruner (135); W. Willie (soil)
455	<i>Mycobacterium</i> sp; D. W. Bruner (166); Ruth E. Gordon (soil)
470 to 472	<i>Mycobacterium</i> spp; P. Stuart, Ministry of Agr. and Fisheries, England (MS.231.52"S", MS.231.52 "R", MS.104.51"S", bovine mastitis) (Stuart and Harvey, 1951)
474 to 477	<i>Mycobacterium</i> spp; P. Stuart (MS. 302.51"S", MS.302.51"R", MS. 48.52"S", MS.48.52"R", bovine mastitis)
497, 498	<i>Mycobacterium</i> spp; M. Panisset, Univ. of Montreal (7A, 10A); G. Penso
573, 574	<i>Mycobacterium</i> spp; S. R. Khambata, St. Xavier's College, India (O ₁₁ , O ₁₂ , intestinal flora of earthworm)
4242	<i>Mycobacterium</i> sp; ATCC; C. W. Duval (Levy-Kedrowski 115, associated with leprous lesions)

of *M. smegmatis*. Because of their disagreement with the original account of *M. ranae* (Küster, 1905), they had to be regarded as misnamed.

Strain no. 447 was the first obtained under the name *Mycobacterium aquae*. Galli-Valerio (1912) mentioned acid fast organisms present in scrapings from water faucets but gave them no description or name. Later, Maië (1922) described an acid fast strain from the water pipes of Lausanne and referred to it as *M. aquae* Galli-Valerio. In spite of a careful search, no paper by Galli-Valerio naming and characterizing the species has been found to precede 1922. It is tentatively assumed that Maië is the proper authority for the species and that he attributed the specific name to Galli-Valerio as a matter of courtesy. Maië's account and a later one by Galli-Valerio and Bournand (1927) were very general but did not prohibit the acceptance of strain no. 447 as authentic and the listing of *M. aquae* in the synonymy of *M. smegmatis*.

The microscopic aspect, acid fastness, colonial morphology, and macroscopic appearance of the 24 strains conformed to those of the other strains previously classified as *M. smegmatis*. The 24 strains grew from 28 C to 45 C inclusive; few grew at 10 C; none, at 52 C; and none survived 4 hours at 60 C. Two-thirds of the strains grew in 5 per cent NaCl broth. Gelatin and casein were not attacked; nitrates were reduced to nitrites by all strains; starch was hydrolyzed; citrate, succinate, and malate were utilized; acid was formed from glucose, rhamnose, xylose, arabinose, mannose, galactose, mannitol,³ sorbitol, inositol, and dulcitol. Lactose and raffinose were not used as sources of carbon.

The tests used to supplement the species' description of *M. phlei* were also applied to all the strains of *M. smegmatis*.

Decomposition of tyrosine. All cultures were negative.

Utilization of benzoate. All but four cultures (nos. 375 to 377, 380) were positive.

Acid production from carbohydrates. Twenty-four per cent of the cultures formed acid from maltose; 100 per cent, from trehalose.

The addition of the foregoing characters to the species' description of *M. smegmatis* and a careful check of previous examinations resulted

in the separation from the species of two strains, nos. 283 and 4236 (Gordon and Smith, 1953, p. 45), previously assigned to *M. smegmatis*. Variation from the normal in one or two reactions of the species' pattern was considered of no taxonomic significance. Strains nos. 283 and 4236, however, differed in too many respects from the enlarged series of characteristics to be accepted as belonging to the species and were again placed with the unclassified strains of the collection. Except for a few, uncorrelated, minor variations, the remaining 54 strains previously identified as *M. smegmatis* and the 24 listed in table 2 conformed to the proposed species' description.

Mycobacterium fortuitum Cruz

The strains comprising the third taxon of rapidly growing acid fast bacteria were isolated from soil and from infections of humans, cattle, and cold-blooded animals (table 3). The oldest species represented in the group was designated *Mycobacterium enteritidis*, Lehmann and Neumann's name (1927) for the Johne's bacillus. On the basis of a single strain, no conclusion can be drawn on the relationship between the slowly growing, fastidious Johne's bacillus and these rapidly growing strains. For practical reasons alone, however, the application of a specific name in the synonymy of *Mycobacterium paratuberculosis* Bergey *et al.* to a species of the rapidly growing strains would not be feasible.

The next oldest species represented was labeled *M. fortuitum* and described by Cruz in 1938. This name is tentatively adopted for the species. It is difficult to believe, however, that such a widespread organism was not isolated and characterized long before 1938. Should an authentic strain bearing an earlier effectively published name (Buchanan *et al.*, 1948) be found to belong to this species, the name now applied would have to be changed to the earlier one.

Under the conditions of this examination, strains of *Mycobacterium giae* Darzins (1950) and *Mycobacterium minetti* Penso *et al.* (1952) were identical with others of this taxon. Although some discrepancies existed between the original descriptions of the two species and the one given here, they were believed due to differences in methods. It is recommended, therefore, that *M. giae* and *M. minetti* be placed in the synonymy of *M. fortuitum*. The similarity between NCTC strain no. N946 of *M. friedmannii* Holland and

³ Omitted by error from the previous list of carbohydrates hydrolyzed by *M. smegmatis*.

those of *M. fortuitum* casts some doubt on the previous assignment of *M. friedmannii* to the synonymy of *M. smegmatis*. This assignment was made because ATCC and CCTM strains of *M. friedmannii* were identical with those of *M. smegmatis*, and Friedmann's original description (1903) was too general to establish definitely the identity of his strain. Friedmann's account could be applied to a strain of *M. smegmatis* or to a strain of *M. fortuitum*. Until more information can be obtained, it is suggested that *M. friedmannii* be left in the synonymy of *M. smegmatis* and NCTC strain no. N946 be regarded as possibly misnamed.

The following description of *M. fortuitum* was based on the 28 strains listed in table 3:

Microscopic appearance. Cells grown on glycerol agar for 72 hours at 28 C varied from 1.0 μ to 3.0 μ in length, the largest number ranging from 2.0 μ to 2.2 μ . After 5 days' incubation at 28 C on glycerol agar, 70 to 100 per cent of the cells of 17 of the 28 cultures were acid fast; the remaining 9 cultures were 10 to 50 per cent acid fast. The cells varied from coccoid and short rods to long, slender rods, occasionally beaded or swollen by a nonacid fast, oval body at one end.

Colonial morphology was similar to that of *M. phlei* and *M. smegmatis*. After 3 or 4 days' incubation, dense colonies with smooth edges and dense colonies fringed with short filaments and/or filamentous colonies were observed. Upon further incubation the filaments usually fragmented into short rods.

Macroscopic appearance. Growth on glycerol agar after 2 or 3 days at 28 C was scant to fair, soft and butyrous, or waxy and nodular, off-white to cream colored. After 2 weeks, growth of the cultures was good to abundant, spreading, butyrous and glistening or dull, rough, and waxy, often very nodular, off-white to cream to beige in color. On yeast extract agar growth was moderate to heavy, spreading, butyrous or dry and waxy, usually nodular, sometimes finely wrinkled, off-white to cream to beige. Growth on glucose-asparagine agar at 2 weeks was moderate, spreading, soft and glistening or dull and waxy, off-white to cream color.

Temperatures of growth. The 28 strains grew from 28 C to 35 C; all but 6 (nos. 48, 448, 449, 9822, 11440, N946) grew at 40 C; and approxi-

TABLE 3
Strains identified as *Mycobacterium fortuitum* Cruz

Laboratory Number	Name and Source
N6926	<i>Mycobacterium enteritidis</i> Lehmann and Neumann; NCTC (5026)
6841, 6842	<i>Mycobacterium fortuitum</i> Cruz; ATCC; J. C. Cruz (1, 2, human abscess)
N946	<i>Mycobacterium friedmannii</i> Holland; NCTC
11440	<i>Mycobacterium giae</i> Darzins; ATCC; E. Darzins (systemic, nodular infection of gias)
480	<i>Mycobacterium minetti</i> Penso <i>et al.</i> ; G. Penso, Istituto Superiore de Sanità, Rome (C70)
48	<i>Mycobacterium</i> sp; W. A. Hagan, Cornell Univ. (soil)
374	<i>Mycobacterium</i> sp; J. Bentinck-Smith, Cornell Univ.; E. W. Tucker (bovine mastitis)
389	<i>Mycobacterium</i> sp; G. Alexander, Rutgers Univ. (B8, barnyard soil)
394, 395	<i>Mycobacterium</i> spp; W. A. Hagan (52-80, 52-79); P. Stuart (MS. 197.51, MS.144.51, bovine mastitis)
448, 490	<i>Mycobacterium</i> spp; CCTM (Karliniski 58, sable 73)
449	<i>Mycobacterium</i> sp; D. W. Bruner, Cornell Univ. (48, soil)
456 to 461	<i>Mycobacterium</i> spp; E. W. Tucker, Cornell Univ. (1595, 84/2 cow 1705, 21/2 cow 155, 89/3 cow 1351, 72/2 cow 1555, 76/3 cow 1422, bovine mastitis) (Tucker, 1953)
9820	<i>Mycobacterium</i> sp; ATCC; NCTC (2014); Král Collection (fish)
9821	<i>Mycobacterium</i> sp; ATCC; NCTC (2291); A. S. Griffith (roe of halibut)
9822	<i>Mycobacterium</i> sp; ATCC; NCTC (2006); G. S. Wilson (terrapin)
N2291	<i>Mycobacterium</i> sp; NCTC (halibut)
N3630	<i>Mycobacterium</i> sp; NCTC (Cummin's M bacillus)
N8573	<i>Mycobacterium</i> sp; NCTC; E. Aguis (Malta, cervical adenitis)
N8697	<i>Mycobacterium</i> sp; NCTC (human abscess)
435	<i>Nocardia</i> sp; Isabel Christison, Duke Univ. (2229); N. F. Conant; E. Palik (sputum)

mately one-half grew at 10 C. Growth, if any, was very scant at 45 C.

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. All cultures formed acid from glucose, mannose, and trehalose; a few (nos. 48, 449, 490, 11440, N2291) utilized mannitol. Acid production from rhamnose, xylose, arabinose, galactose, sorbitol, inositol, dulcitol, lactose, and raffinose was negative; with one exception (no. 9822), maltose was not hydrolyzed.

Hydrolysis of gelatin. All cultures were negative.

TABLE 4

Comparison of reactions of three species of *Mycobacterium*

Reactions	<i>Mycobacterium</i>		
	<i>phlei</i>	<i>smegmatis</i>	<i>fortuitum</i>
Decomposition of:			
gelatin	±*	—	—
casein	—	—	—
tyrosine	—	—	—
Growth at:			
52 C	+	—	—
45 C	+	+	—
40 C	+	+	±†
Survival 60 C, 4 hr	+	—	—
Nitrite from nitrate	+	+	+
Utilization of:			
citrate	+	+	+
succinate	+	+	+
malate	+	+	+
benzoate	—	+	—
Hydrolysis of starch	+	+	+
Acid from:			
glucose	+	+	+
rhamnose	—	+	—
xylose	±	+	—
arabinose	+	+	—
mannose	+	+	+
galactose	±	+	—
mannitol	+	+	±
sorbitol	+	+	—
inositol	—	+	—
dulcitol	—	+	—
lactose	—	—	—
raffinose	—	—	—
maltose	—	±	—
trehalose	±	+	+

* Majority of strains negative.

† Majority of strains positive.

Hydrolysis of casein. All cultures were negative.

Utilization of citrate, succinate, and malate.

Only one culture (no. 395) failed to utilize citrate; all utilized succinate and malate.

Reduction of nitrate to nitrite. All but four cultures (nos. 395, 448, 9822, N946) reduced nitrate to nitrite.

NaCl tolerance. Growth, if any, was very scant in 7 per cent NaCl and usually positive in 5 per cent.

Decomposition of tyrosine. All cultures were negative.

Utilization of benzoate. All cultures were negative.

Portions of the descriptions of *M. phlei*, *M. smegmatis*, and *M. fortuitum* are arranged for comparative purposes in table 4. Maximum temperatures of growth, survival of 60 C, utilization of benzoate, and acid production from rhamnose, xylose, arabinose, galactose, sorbitol, inositol, and dulcitol provided combinations of reactions separating the three species one from the other. Although the other reactions listed in table 4 did not differentiate the three species, they were, on the whole, stable and of value in the recognition of the three species. Some of them also gave promise of usefulness in the delineation of the other species, at present partially represented by the remaining 32 per cent of the 195 strains in this collection.

SUMMARY

The collection of rapidly growing, acid fast bacteria was increased from 124 to 195 by the examination of 71 more strains. The new strains, identified as *Mycobacterium phlei* Lehmann and Neumann and *Mycobacterium smegmatis* (Trevise) Lehmann and Neumann, were listed and a few supplementary characters were added to the descriptions of these two species.

A third species, *Mycobacterium fortuitum* Cruz, was characterized and compared with *M. phlei* and *M. smegmatis*. *Mycobacterium giae* Darzens and *Mycobacterium minetti* Penso *et al.* were listed in the synonymy of *M. fortuitum*.

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