Mycobacterium fallax sp. nov.

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A new species, *Mycobacterium fallax*, is described. A study of 22 strains showed that they form a homogeneous group with an internal phenotypic similarity value of $94.6 \pm 4.1\%$. The colony morphology resembled that of *Mycobacterium tuberculosis*, with cord formation on solid medium. The characteristic features of these strains were that they were nonchromogenic and grew rapidly at 30°C but slowly at 37°C. Most formed a thermolabile catalase and produced nitrate reductase. A lipid analysis showed that tuberculostearic acid was present and that only α -mycolic acids were formed. These α -mycolic acids were di- and tri-unsaturated acids, a feature that has not been described previously in the mycobacteria. The type strain has been deposited in the Collection Nationale de Cultures de Microorganismes, Paris, France, as strain CIP 8139.

The properties of 22 mycobacterial strains that form a homogeneous cluster distinct from the previously known *Mycobacterium* species are described in this paper. These organisms were characterized for 47 conventional taxonomic characteristics (10, 17). Because lipid analysis has been useful in resolving taxonomic problems in the genus *Mycobacterium* (1, 14), the lipid compositions of these strains were also investigated. The strains which we studied formed characteristic mycolic acids, including a new mycolic acid structure.

MATERIALS AND METHODS

Bacterial strains. A total of 22 strains were studied. Three of these were isolated from sputum, 14 were isolated from water samples from the River Seine between Melun and Conflans-Sainte-Honorine, 2 were isolated from water samples from the River Marne, a tributary of the River Seine, and 1 was isolated from Lake Settons, which is 250 km from Paris, France. The remaining two strains were kindly sent to us by M. Kubín (Institute of Hygiene and Epidemiology, Prague, Czechoslovakia), who isolated them from soil samples in a region of Czechoslovakia where *Mycobacterium kansasii* is common (9). In addition to these 22 isolates, the following strains of species which are on the Approved Lists of Bacterial Names (19) were studied: *Mycobacterium chelonae* NCTC 946^T (type strain); *Mycobacterium chitae* ATCC 19627^T; *Mycobacterium farcinogenes* NCTC 10955^T; *Mycobacterium flavescens* ATCC 14474^T; *Mycobacterium fortuitum* ATCC 6841^T; *Mycobacterium gastri* ATCC 15754^T; *Mycobacterium gordonae* ATCC 14470^T; *Mycobacterium kansasii* ATCC 12478^T; *Mycobacterium marinum* ATCC 927^T; *Mycobacterium senegalense* NCTC 10956^T; *Mycobacterium smegmatis* ATCC 19420^T; *Mycobacterium terrae* ATCC 15755^T; *Mycobacterium triviale* ATCC 23292^T; *Mycobacterium thermoresistibile* ATCC 19527^T; *Mycobacterium tu-*

berculosis ATCC 27294^T; and *Mycobacterium vaccae* ATCC 15483^T. In addition, the frequency distribution of the test results for 608 mycobacterial isolates from human hosts were included for comparative purposes. These isolates included 31 *M. chelonae* strains, 14 *M. flavescens* strains, 63 *M. fortuitum* strains, 43 *M. gastri* strains, 99 *M. gordonae* strains, 58 *M. kansasii* strains, 14 *M. marinum* strains, 47 *M. terrae* strains, and 239 *M. tuberculosis* strains.

Characterization of strains. The niacin test was performed with test strips (Difco Laboratories, Detroit, Mich.). The catalase test was done by the method of Kubica and Pool (11), and the nitrate reductase and arylsulfatase tests were performed as described by Vestal (22). For the Tween hydrolysis test, we used a commercial substrate obtained from Difco. The urease and β -glucosidase tests were performed by the methods of Meyer and David (13) and David and Jahan (5), respectively. The penicillinase and trehalase tests were performed by following the recommendations of David et al. (6). The iron uptake test and the three tests concerning growth on inositol, mannitol, and sodium citrate were done as described by Silcox et al. (18). The medium for testing growth on fructose was prepared as described by Gordon and Smith (8). The medium for testing the growth on hydroxylamine contained 250 μ g of hydroxylamine per ml (21). Growth on the different antibiotics tested was determined by the method of Canetti et al. (4). For the cephalosporinase test, the substrate was prepared by dissolving 5 mg of Nitrocefin in 0.5 ml of dimethyl sulfoxide and adding 9.5 ml of Tris buffer (pH 7.0; Sigma Chemical Co., St. Louis, Mo.). This solution was distributed in 0.5-ml amounts into screw-capped test tubes (16 by 125 mm). About 5 mg of cells scraped from the surface of Löwenstein-Jensen slants was suspended in each substrate solution, and the mixtures were incubated at 37°C for 2 h. The development of a distinct red color indicated a positive reaction.

Cluster analysis. The data obtained from the study of 47 taxonomic properties were converted to the simple

binary form (i.e., 1 or 0). The degree of internal similarity of the 22 strains of *Mycobacterium fallax* sp. nov. was determined by using the simple matching coefficient (20). Using the same method, we compared the *M. fallax* type strain with the type strains of 16 previously described mycobacterial species.

Cultivation of strains. Most of the bacteria were grown in Löwenstein-Jensen medium at 37°C; exceptions were the *M. fallax* strains, which were cultivated at 30°C. For the isolation of lipid components, bacteria were cultivated on Middlebrook 7H9 base (Difco) supplemented with 0.5% (wt/vol) Casitone and 1% (wt/vol) glucose. Cultures were stirred or shaken during growth and harvested by centrifugation after 8 days at 30°C (*M. fallax* strains) or after 15 days at 37°C (*M. triviale* type strain).

Isolation, purification, and analysis of the lipids. The lipid analysis procedure used for the *M. fallax* strains and the *M. triviale* type strain was that recommended by Asselineau et al. (3). Argentation chromatography was performed by the method of Mangold (12), and visualization of the spots was obtained by charring after spraying with sulfuric acid.

Instrumentation. Gas chromatographic profiles were obtained with a Girdel gas chromatograph by using a 3% OV1 stationary phase on Chromosorb W (column dimensions, 3 m by 1/8 in. [3.2 mm]). For pyrolytic gas chromatography, the injector temperature was raised from 220 to 300°C. Mass spectrometric measurements were done with a Varian model MAT 311A instrument. The electron energy was 70 eV, the source temperature was 250°C, and the direct probe temperature was about 280°C.

Phage studies. The *M. fallax* strains were examined by the methods of Rado et al. (15), except that the inoculated plates were incubated at 30°C for 3 h before they were spotted with phages. Cultures were examined for phage lysis 1 and 5 days after spotting. The following phage titers were used: DS6A, 4×10^8 plaque-forming units (PFU); BK1, 2×10^{10} PFU; BG1, 5×10^6 PFU; D-29, 2×10^{10} PFU; and D-33, 3×10^8 PFU. The plate medium used was Rva29 (16), which was used without the addition of albumin.

RESULTS AND DISCUSSION

Cells of the 22 previously undescribed strains studied were acid-fast short rods which formed high-molecular-weight (C_{70} to C_{78}) mycolic acids and were susceptible to mycobacteriophage D-29. These properties were taken as evidence for the inclusion of these strains in the genus *Mycobacterium*.

A phenotypic similarity value of $94.6 \pm 4.1\%$ among the 22 strains showed their high degree of homogeneity and allowed the differentiation of these strains from other mycobacterial species as a distinct group. This group is formally described below as *M. fallax* sp. nov.

Comparisons of *M. fallax* CIP 8139^T with the type strains of 16 mycobacterial species showed degrees of similarity of 72.34% with *M. farcinogenes*, 71.74% with *M. marinum*, and 70.21% with *M. triviale* (Table 1).

The main differences between *M. fallax* and *M. farcinogenes* were the following characteris-

TABLE 1. Similarity between *M. fallax* strain CIP 8139^T and type strains of 16 other mycobacterial species

Species	% Similarity to CIP 8139 ^T
<i>M. farcinogenes</i>	72.34
<i>M. marinum</i> ^a	71.74
<i>M. triviale</i>	70.21
<i>M. gastri</i>	68.08
<i>M. gordonae</i>	68.08
<i>M. kansasii</i>	68.08
<i>M. senegalense</i>	68.08
<i>M. chitae</i>	63.83
<i>M. smegmatis</i>	63.83
<i>M. terrae</i>	63.83
<i>M. tuberculosis</i>	63.83
<i>M. vaccae</i>	63.83
<i>M. fortuitum</i>	57.45
<i>M. flavescens</i>	55.32
<i>M. thermoresistibile</i>	55.32
<i>M. chelonae</i> ^a	50.00

^a Only 46 properties were compared.

tics of *M. fallax*: growth in less than 5 days at 30°C, positive nitrate reductase test, negative β -glucosidase and cephalosporinase tests, utilization of fructose as a sole carbon source, and susceptibility to ethambutol and cycloserine. *M. fallax* strains were easily differentiated from *M. marinum* by the absence of photochromogenicity, a positive nitrate reductase test, negative urease and cephalosporinase tests, and resistance to ethionamide and pyrazinamide. *M. fallax* strains differed from *M. triviale* by producing growth in less than 5 days at 30°C, having a thermolabile catalase, showing negative reactions for arylsulfatase, trehalase, and urease, utilizing fructose as a sole carbon source, and being unable to grow on 5% NaCl.

The *M. fallax* strains could be confused with *M. tuberculosis* on the basis of colonial morphology. In addition, they were weakly catalase positive, their catalase was thermolabile, and they were nitrate reductase positive, as *M. tuberculosis* is. However, the *M. fallax* strains were niacin and β -glucosidase negative.

Phage studies. *M. fallax* strains were lysed by phage D-29 after 1 day of incubation. Concentrated phage D-29 produced either complete lysis or confluent plaques. However, when phage D-29 lysate was diluted 10 times, isolated plaques were not obtained. This could be attributed to the extreme roughness of the *M. fallax* cultures, which caused difficulties in reading. All of the strains except one were resistant to the other phages tested; the exception was 1 of the 22 strains which was susceptible to phage DS6A.

Results after 5 days of incubation generally confirmed the observations described above. Lysis caused by phage D-29 was more apparent, but only two strains showed isolated plaques

with the 10-fold-diluted lysate. No lysis occurred with the remaining phages, except for two strains which were susceptible to phage DS6A, one strain which was susceptible to phage BK1, and another one strain that was susceptible to phage BG1.

Lipid chromatographic profiles. The lipid fraction obtained after saponification of *M. fallax* whole cells was separated by thin-layer chromatography before and after methyl ester formation. In addition to common fatty acid esters, α -mycolic acid esters were observed. β - and γ -mycolic and dicarboxylic mycolic acid esters were not detected. A gas chromatographic analysis of the total fatty acid methyl esters indicated the presence of myristic acid ($C_{14:0}$), palmitoleic acid ($C_{16:1}$), palmitic acid ($C_{16:0}$, major component), oleic acid ($C_{18:1}$, major component), stearic acid ($C_{18:0}$), and tuberculostearic acid (rC_{19}). An unidentified peak was detected close to the C_{17} acid peak. When pyrolysis gas chromatography was used (injector temperature, 300°C), intense peaks corresponding to C_{22} and C_{24} fatty acid esters were observed, indicating the presence of α -branched, β -hydroxylated esters of the mycolic acid type containing n - C_{20} and n - C_{22} aliphatic α -branching.

Mass spectrometric analysis of the α -mycolic methyl esters. After purification, the methyl esters of the α -mycolic acids were analyzed by mass spectrometry under electron impact. The total numbers of carbon atoms and the numbers of unsaturations or cycles were deduced from the mass/charge values of the $(M-50)^{+}$ ions, which were formed by successive losses of water and methanol from the molecular ion M^{+} . The carbon content ranged between 70 and 78

TABLE 2. Numbers of carbon atoms and unsaturations or cycles of the *M. fallax* α -mycolic acids deduced from the $(M-50)^{+}$ ions

Mass/charge value of $(M-50)^{+}$ ion ^a	No. of carbon atoms and unsaturations
1,100	78:2 ^b
1,098	78:3
1,086	77:2
1,072	76:2
1,070	76:3
1,058	75:2
1,044	74:2
1,042	74:3
1,030	73:2
1,016	72:2
1,002	71:2
998	70:2

^a The $(M-50)^{+}$ ions correspond to the M^{+} ions after successive losses of one methanol molecule and one water molecule.

^b The number to the left of the colon indicates the number of carbon atoms, and the number to the right of the colon indicates the number of double bonds or cyclopropanes.

TABLE 3. Major ions corresponding to the aldehydes obtained after pyrolysis of the *M. fallax* α -mycolic acids

Mass/charge value of $(M-50)^{+}$ ion	No. of carbon atoms and unsaturations
782	55:2 ^a
768	54:2
766	54:3
754	53:2
740	52:2
738	52:3
726	51:2
712	50:2

^a The number to the left of the colon indicates the number of carbon atoms, and the number to the right of the colon indicates the number of double bonds or cycles.

atoms (Table 2). The splitting of the mycolates into aldehydes and esters produced the ions shown in Table 3. Peaks occurring at mass/charge values of 354 and 382 were due to the $C_{22:0}$ and $C_{24:0}$ acid methyl esters ions; this confirmed the gas chromatographic observations. The peak values corresponding to the meroaldehyde ions indicated that all of the unsaturations or cycles were located in the mero-mycolic fragment (Table 3).

Mycolic acid analysis according to the degree of unsaturation. To determine the nature of the meromycolic chain functions (i.e., unsaturations or cycles), the α -mycolate fraction was separated on Silica Gel G impregnated with 5% (wt/wt) silver nitrate (12). Three major spots were observed and compared with authentic samples. A mass spectrometric analysis of each component allowed us to determine hydrogenation content. The following components were observed (with decreasing R_f values): di-unsaturated α -mycolate I, di-unsaturated α -mycolate II, and tri-unsaturated α -mycolate.

The di-unsaturated α -mycolate II possessed the same R_f value on silver nitrate plates as an *M. smegmatis* α -mycolate, in which the two *cis* unsaturations of the meromycolate chain were surrounded only by unbranched polymethylenic chains (7). The behavior of α -mycolate I resembled that of the other α -mycolate acids from *M. smegmatis*, in which one carbon-carbon double bond having a *trans* configuration possessed a methyl branching on an aldehyde position (7). The tri-unsaturated mycolic acid of *M. fallax* represents a new type of mycolic acid. This characteristic proved to be decisive for differentiation of *M. fallax* and *M. triviale* strains. Mycolic acids containing more than two double bonds or two cyclopropanes have not been described previously (2, 14). Tri-unsaturation in hydrocarbons having different chain lengths has been reported in *Nocardia* and *Rhodococcus* species (14).

Since the *M. fallax* strains and *M. triviale* ATCC 23292^T produced similar lipid profiles

FIG. 1. Smears prepared from *M. fallax* grown on Youmans medium, showing cord formation. $\times 40$. Youmans medium was prepared with no Tween 80.

FIG. 2. *M. fallax* CIP 8139^T colonies on solid medium. (A) Löwenstein-Jensen medium. No magnification. (B) Middlebrook 7H10 medium. $\times 6$.

TABLE 4. Properties of *M. fallax* and other mycobacteria

Characteristic	<i>M. fallax</i>		<i>M. chelonae</i>		<i>M. chitae</i> type strain	<i>M. farci-</i> <i>nogenes</i> type strain	<i>M. flavescens</i>		<i>M. fortuitum</i>		<i>M. gastri</i>	
	Type strain	22 Other strains	Type strain	31 Other strains			Type strain	14 Other strains	Type strain	63 Other strains	Type strain	43 Other strains
Rods (>1 µm)	— ^a	0	+	ND	—	+	+	100	+	100	+	100
Coccobacillary (<1 µm)	+	100	—	ND	+	—	—	0	—	0	—	0
Cord formation	+	100	+	ND	—	—	—	ND	—	ND	—	ND
Colonies smooth	—	0	+	ND	+	—	—	ND	+	ND	+	ND
Colonies rough	+	100	—	ND	—	+	+	ND	—	ND	—	ND
Growth at:												
30°C	+	100	+	100	+	+	+	100	+	100	+	100
30°C, <5 days	+	100	+	100	+	—	—	ND	+	100	—	0
37°C	+	77	+	100	+	+	+	100	+	100	+	100
37°C, <5 days	—	18	ND	ND	+	—	—	0	+	100	—	0
45°C	—	0	—	0	—	—	—	0	—	0	—	0
52°C	—	0	—	0	—	—	—	0	—	0	—	0
Growth on nutrient agar at:												
30°C	+	100	+	100	+	—	+	ND	+	ND	—	ND
37°C	—	18	+	ND	+	—	+	100	+	100	—	0
Pigmentation in the dark	—	0	—	0	—	—	+	100	—	0	—	0
Pigmentation in the light	—	0	—	0	—	—	+	100	—	0	—	0
Niacin	—	0	—	0	—	—	—	0	—	0	—	0
Enzymatic activity:												
Arylsulfatase (3 days)	—	0	+	100	—	—	+	86	+	100	—	ND
Catalase (22°C)	+	100	+	100	+	+	+	100	+	100	+	100
Catalase (68°C)	—	27	+	84	+	+	+	100	+	98	—	0
Cephalosporinase (2 h)	—	11 ^b	+	100	+	+	+	33	+	100	+	100
β-Glucosidase (3 h)	—	0	—	0	—	—	—	7	+	100	—	82
Nitrate reductase (2 h)	+	100	—	0	+	—	+	100	+	100	—	0
Penicillinase (5 h)	—	0	+	100	—	—	—	14	—	0	—	3
Trehalase (5 h)	—	9	+	100	—	—	—	7	—	0	—	16
Tween hydrolysis (10 days)	+	100	+	90	—	+	+	100	+	83	+	100
Urease (18 h)	—	4	+	26	+	—	+	14	+	24	+	5
Iron uptake	—	0	+	ND	+	—	—	ND	+	ND	—	ND
Growth on MacConkey agar	—	0	+	ND	—	—	—	ND	+	ND	—	ND
Use as sole carbon source:												
Fructose	+	100	—	0	+	—	—	ND	+	100	—	0
Inositol	—	0	—	ND	+	—	—	ND	—	ND	—	ND
Mannitol	—	0	—	ND	+	—	—	ND	—	ND	—	ND
Sodium citrate	—	0	+	ND	—	—	—	ND	—	ND	—	ND
Growth in the presence of:												
5% NaCl	—	0	—	ND	+	—	+	ND	+	ND	—	ND
NH ₂ OH (250 µg/ml)	+	100	+	100	+	—	—	21	+	100	+	100
Amithiazone (2 µg/ml)	+	100	+	100	+	+	+	100	+	100	—	0
Capreomycin (40 µg/ml)	—	0	+	ND	—	—	—	ND	—	ND	—	ND
D-Cycloserine (30 µg/ml)	—	0	+	ND	+	+	+	ND	+	ND	—	ND

TABLE 4—Continued

<i>M. gordonae</i>		<i>M. kansasii</i>		<i>M. marinum</i>		<i>M. senegal- ense</i> type strain	<i>M. smeg- matis</i> type strain	<i>M. terrae</i>		<i>M. thermo- resistibile</i> type strain	<i>M. trivi- ale</i> type strain	<i>M. tuberculosis</i>		<i>M. vac- cae</i> type strain
Type strain	99 Other strains	Type strain	58 Other strains	Type strain	14 Other strains			Type strain	47 Other strains			Type strain	239 Other strains	
+	100	+	100	+	100	+	+	+	100	+	+	+	100	—
—	0	—	0	—	0	—	—	—	0	—	—	—	0	+
—	ND	+	ND	+	ND	—	—	—	ND	—	+	+	100	—
+	100	—	ND	+	ND	—	—	+	ND	+	—	—	0	+
—	0	+	ND	—	ND	+	+	—	ND	—	+	+	100	—
+	100	+	100	+	100	+	+	+	100	+	+	+	100	+
—	0	—	0	+	100	+	+	—	0	+	—	—	0	+
+	100	+	100	ND	ND	+	+	+	100	+	+	+	100	+
—	0	—	0	—	ND	+	+	—	0	+	—	—	0	+
—	0	—	0	—	0	—	+	—	0	+	—	—	0	—
—	0	—	0	—	0	—	—	—	0	+	—	—	0	—
—	0	—	0	—	0	—	—	—	0	+	—	—	0	—
—	0	—	0	+	ND	+	+	—	0	+	—	—	0	+
—	0	—	0	—	ND	+	+	—	0	+	—	—	0	+
+	100	—	0	—	0	—	—	—	0	+	—	—	0	+
+	100	+	100	+	100	—	—	—	0	+	—	—	0	+
—	0	—	0	—	0	—	—	—	0	—	—	+	100	—
—	ND	—	5	—	ND	+	—	—	ND	—	+	—	ND	—
+	100	+	100	+	100	+	+	+	100	+	+	+	100	+
+	100	+	100	+	100	+	+	+	100	+	+	—	0	+
+	70	+	71	+	100	+	+	+	15	+	—	+	96	+
—	7	+	57	—	7	+	—	—	40	—	—	+	90	+
—	0	+	100	—	0	+	+	+	100	+	+	+	100	+
—	0	—	0	—	0	—	+	—	2	—	—	—	5	—
—	71	+	82	+	50	—	—	+	27	—	+	+	61	—
+	100	+	100	+	100	—	+	+	100	+	+	+	ND	+
—	0	+	12	+	0	+	+	—	0	+	+	+	72	—
—	ND	—	ND	—	ND	—	+	—	ND	—	—	—	ND	+
—	ND	—	ND	—	ND	—	—	—	ND	—	—	—	ND	—
—	ND	—	ND	ND	ND	+	+	—	0	—	—	—	ND	+
—	ND	—	ND	—	ND	—	+	—	ND	—	—	—	ND	—
—	ND	—	ND	—	ND	+	—	—	ND	—	—	—	ND	+
—	ND	—	ND	—	ND	—	—	—	ND	—	—	—	ND	—
—	ND	—	ND	—	ND	—	+	—	ND	+	+	—	ND	—
+	100	+	92	+	80	+	+	+	92	—	+	—	0	+
+	98	—	0	+	100	+	+	+	100	+	+	—	0	+
—	ND	—	ND	—	ND	—	—	+	ND	—	+	—	ND	—
—	ND	—	ND	—	ND	+	+	+	ND	—	—	—	ND	+

TABLE 4—Continued

Characteristic	<i>M. fallax</i>		<i>M. chelonae</i>		<i>M. chitae</i> type strain	<i>M. farci-</i> <i>nogenes</i> type strain	<i>M. flavescens</i>		<i>M. fortuitum</i>		<i>M. gastri</i>	
	Type strain	22 Other strains	Type strain	31 Other strains			Type strain	14 Other strains	Type strain	63 Other strains	Type strain	43 Other strains
Growth in the presence of:												
Ethambutol (2 µg/ml)	—	0	+	100	—	+	—	0	+	97	—	0
Ethionamide (20 µg/ml)	+	94 ^b	ND	ND	+	+	—	ND	—	ND	—	ND
Isoniazid (0.1 µg/ml)	+	100	+	ND	+	+	—	ND	+	ND	+	ND
Kanamycin (20 µg/ml)	—	0	+	ND	—	—	—	ND	—	ND	+	ND
PAS (0.5 µg/ml) ^c	+	100	+	ND	+	+	+	ND	+	ND	+	ND
Pyrazinamide (100 µg/ml)	+	100	+	ND	+	+	+	ND	+	ND	+	ND
Rifampin (40 µg/ml)	—	6 ^b	+	ND	+	—	+	ND	+	ND	—	ND
Rifamycin (40 µg/ml)	—	6 ^b	+	ND	+	+	+	ND	+	ND	—	ND
Streptomycin (4 µg/ml)	+	88 ^b	+	ND	—	+	—	ND	+	ND	—	ND
TCH (2 µg/ml) ^d	+	100	+	100	+	+	+	100	+	100	+	100

^a —, Negative reaction; +, positive reaction; ND, not done. The numbers indicate percentages of strains positive.

^b Only 17 strains were studied.

^c PAS, *p*-Aminosalicylic acid.

^d TCH, Thiophencarboxylic acid hydrazide.

after gas chromatography and thin-layer chromatography, we made a more precise comparison by using the structural features of the α -mycolates. An attempt to isolate α -mycolate from *M. triviale* ATCC 23292^T by argentation chromatography indicated that no unsaturated material was present. The mass spectrometry analysis indicated the presence of two and three unsaturations or cycles. Therefore, it is very likely that in *M. triviale* the cyclopropanes are included in the merochain, as in the mycolic acids of many species. Thus, although strains of *M. fallax* and *M. triviale* ATCC 23292^T had similar lipid compositions and contained only α -type mycolic acids, the *M. triviale* strain had only cyclic mycolic acids containing di- and tricycles (probably *cis*-cyclopropanes), whereas the *M. fallax* strains contained di- and tri-unsaturated α -mycolic acids.

For this distinct group of strains we propose the name *Mycobacterium fallax* sp. nov. (fal'lax. L. adj. *fallax* deceptive; in the sense that the colonies resemble colonies of *M. tuberculosis*).

Morphological, growth, and biochemical characteristics of *M. fallax* sp. nov. The cells of *M. fallax* are short rods 0.5 to 1 µm long; they are acid fast, except for a small number (less than 20%) of cyanophil forms. Smears prepared from

pellicles in Youmans medium (with no Tween 80) show clumps of rods or cords (Fig. 1).

At 30°C, growth occurs within 5 days on Löwenstein-Jensen and Middlebrook 7H10 media and on nutrient agar. At 37°C, however, most strains grow poorly and slowly (12 to 21 days) on the former two media. Some strains (23%) do not grow even after 28 days. Only 18% of the strains grow on nutrient agar at 37°C.

On Löwenstein-Jensen medium at 30°C, colonies are large, eugonic, buff colored, and rough, with a cauliflower-like morphology resembling that of *M. tuberculosis* colonies (Fig. 2A). On Middlebrook 7H10 medium at 30°C, cord formation is evident at the edges of colonies (Fig. 2B). Colonies on both of these media are the same at 37 as at 30°C, except that they are somewhat smaller.

The phenotypic properties of 22 *M. fallax* strains are shown in Table 4. The type strain has been deposited in the Collection Nationale de Cultures de Microorganismes, Paris, France, as strain CIP 8139.

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TABLE 4—Continued

<i>M. gordonae</i>		<i>M. kansasii</i>		<i>M. marinum</i>		<i>M. senegalense</i> type strain	<i>M. smegmatis</i> type strain	<i>M. terrae</i>		<i>M. thermo-resistibile</i> type strain	<i>M. triviale</i> type strain	<i>M. tuberculosis</i>		<i>M. vaccae</i> type strain
Type strain	99 Other strains	Type strain	58 Other strains	Type strain	14 Other strains			Type strain	47 Other strains			Type strain	239 Other strains	
—	2	—	0	—	0	+	—	—	5	—	+	—	0	—
+	ND	—	ND	—	ND	+	+	—	ND	+	—	—	ND	+
+	ND	+	ND	+	ND	+	+	+	ND	+	+	—	ND	—
—	ND	+	ND	—	ND	—	—	+	ND	—	—	—	ND	—
+	ND	+	ND	+	ND	+	+	+	ND	+	+	—	ND	+
+	ND	+	ND	+	ND	+	+	+	ND	—	+	—	ND	+
+	ND	—	ND	+	ND	—	+	+	ND	+	—	—	ND	+
+	ND	+	ND	+	ND	+	+	+	ND	+	—	—	ND	+
+	ND	+	ND	+	ND	+	—	+	ND	—	—	—	ND	—
+	100	+	100	+	100	+	+	+	100	+	+	+	100	+

LITERATURE CITED

- Asselineau, C., and J. Asselineau. 1978. Lipides spécifiques des mycobactéries. Ann. Microbiol. (Paris) 129A:49–69.
- Asselineau, C., and J. Asselineau. 1978. Trehalose-containing glycolipids. Prog. Chem. Fats Other Lipids 16:56–99.
- Asselineau, C., S. Clavel, F. Clément, M. Daffé, H. L. David, M. A. Lanéelle, and J. C. Promé. 1981. Constituants lipidiques de *Mycobacterium leprae* isolé de tatou infecté expérimentalement. Ann. Microbiol. (Paris) 132A:19–30.
- Canetti, G., N. Rist, and J. Grosset. 1963. Mesure de la sensibilité du bacille tuberculeux aux drogues antibacillaires par la méthode des proportions. Rev. Tuberc. Pneumol. 27:217–272.
- David, H. L., and M. T. Jahan. 1977. β -Glucosidase activity in mycobacteria. J. Clin. Microbiol. 13:6–9.
- David, H. L., I. Traore, and A. Feuillet. 1981. Differential identification of *Mycobacterium fortuitum* and *Mycobacterium chelonae*. J. Clin. Microbiol. 5:383–384.
- Etémadi, A. H., F. Pinte, and J. Markovits. 1967. Nouvelle analyse des acides mycoliques de *Mycobacterium smegmatis*. Bull. Soc. Chim. Fr., p. 195–199.
- Gordon, R. E., and M. M. Smith. 1955. Rapidly growing acid-fast bacteria. II. Species description of *Mycobacterium fortuitum* Cruz. J. Bacteriol. 69:502–507.
- Kaustová, J., Ž. Olšovský, M. Kubín, O. Zatloukal, M. Pelikán, and V. Hradil. 1981. Endemic occurrence of *Mycobacterium kansasii* in water supply systems. J. Hyg. Epidemiol. Microbiol. Immunol. 25:24–30.
- Kubica, G. P., I. Baess, R. E. Gordon, P. A. Jenkins, J. B. G. Kwapinski, C. McDermont, S. R. Pattyn, H. Saito, V. Silcox, J. L. Stanford, K. Takeya, and M. Tsukamura. 1972. A co-operative numerical analysis of rapidly growing mycobacteria. J. Gen. Microbiol. 73:55–70.
- Kubica, G. P., and G. L. Pool. 1960. Studies on the catalase activity of acid fast bacilli. I. An attempt to subgroup these organisms on the basis of their catalase activities at different temperatures and pH. Am. Rev. Respir. Dis. 81:387–391.
- Lévy-Frébault, V., J. Grandry, and H. L. David. 1982. Evaluation of rapid tests for the identification of mycobacteria. J. Med. Microbiol. 15:575–577.
- Mangold, H. K. 1969. Aliphatic lipids: argentation chromatography. p. 396–402. In E. Stahl (ed.), Thin-layer chromatography, 2nd ed. Springer-Verlag, Berlin.
- Meyer, L., and H. L. David. 1979. Evaluation de l'activité uréase et de l'activité β -glucosidase pour l'identification pratique des mycobactéries. Ann. Microbiol. (Paris) 130B:323–332.
- Minnikin, D. E., and M. Goodfellow. 1980. Lipid composition in the classification and identification of acid-fast bacteria, p. 189–256. In M. Goodfellow and R. G. Board (ed.), Microbiological classification and identification. Academic Press, Inc., London.
- Rado, T. A., J. H. Bates, H. W. B. Engel, E. Mankiewicz, T. Murohashi, Y. Mizuguchi, and L. Šula. 1975. World Health Organization studies on bacteriophage typing of mycobacteria. Subdivision of the species *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. 111:459–468.
- Redmond, W. B., and D. M. Ward. 1966. Media and methods for phage typing mycobacteria. Bull. W.H.O. 35:563–568.
- Saito, H., R. E. Gordon, I. Juhlin, W. Kappler, J. B. G. Kwapinski, C. McDermont, S. R. Pattyn, E. H. Runyon, J. L. Stanford, I. Tarnok, H. Tasaka, M. Tsukamura, and J. Weiszfeiler. 1977. Cooperative numerical analysis of rapidly growing mycobacteria. Int. J. Syst. Bacteriol. 27:75–85.
- Silcox, V. A., R. C. Good, and M. M. Floyd. 1981. Identification of clinically significant *Mycobacterium fortuitum* complex isolates. J. Clin. Microbiol. 14:686–691.
- Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30:225–420.
- Sokal, R. R., and P. H. A. Sneath. 1963. Principles of numerical taxonomy. W. H. Freeman & Co., San Francisco.
- Tsukamura, M. 1967. Identification of mycobacteria. Tubercle 48:311–338.
- Vestal, A. 1975. Procedures for the isolation and identification of mycobacteria. Publication (CDC) 76-8230. Centers for Disease Control, Atlanta, Ga.