Numerical Classification of Slowly Growing Mycobacteria

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A total of 138 strains of slowly growing mycobacteria, which were received as members of 18 named species, were classified by scoring matching coefficients. Eighty-eight characters were tested for each strain; of these, 48 were useful for differentiating between strains. *Mycobacterium tuberculosis* and *M. bovis* were clearly separated from the other species. *M. kansasii*, *M. marinum*, *M. gastri*, and *M. shimoidei* were regarded as species clearly distinct from each other and from other species in the genus. The other species studied formed a large cluster within which *M. szulgai* and *M. simiae* were distinct. The remaining species could be divided into two large subclusters, one consisting of *M. asiaticum*, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, *M. gordonae*, and *M. xenopi*, and the other consisting of *M. nonchromogenicum*, *M. terrae*, *M. novum*, and *M. triviale*. Within the former cluster, *M. gordonae* was regarded as a distinct species, and *M. asiaticum* and *M. xenopi* as fairly distinct species. In contrast to these, *M. avium*, *M. intracellulare*, and *M. scrofulaceum* were not clearly differentiated from each other and appeared to belong to a single species, for which the name *M. avium* has priority. Within the latter cluster, *M. triviale* appeared to be fairly distinct from the others. *M. nonchromogenicum*, *M. terrae*, and *M. novum* could not be differentiated from each other, and they appear to belong to a single species, for which the name *M. nonchromogenicum* has priority.

The classification of slowly growing mycobacteria entails considerable difficulty, for the organisms show negative matches in many biochemical tests which are useful for differentiating between other microorganisms. Nevertheless, the numerical classification of these microorganisms has been carried out by Bojalil et al. (3), Tsukamura (33, 35, 42, 45), Tsukamura and Tsukamura (56), Bogdanescu and Racotta (2), Wayne (62), Tsukamura and Mizuno (46), Tsukamura et al. (51), and Kubica and Silcox (14). In addition, the numerical classification of a limited number of species of slowly growing mycobacteria was undertaken by Wayne (61), Wayne et al. (64), Tsukamura et al. (50), Tsukamura (41), Wayne et al. (63), Kubica et al. (15), and Meissner et al. (21).

Of the above-mentioned studies, only a few (3, 15, 21, 33, 56, 63) were carried out using an $n \times n$ matrix prepared from large numbers of test strains. The studies by Wayne et al. (63), Meissner et al. (21), and Kubica et al. (15) analyzed one or two groups of Runyon (23). Only three studies (3, 33, 56) covered most of the species of slowly growing mycobacteria available at the time. However, the study by Bojalil et al. (3) in 1962 employed 30 characters, and the studies by Tsukamura (33) and Tsukamura and Tsukamura (56) in 1966 used 94 and 43 characters, respectively, of which 40 to 50 were useful for

differentiating between test strains. These studies were carried out 10 or more years ago. Thereafter, a number of tests were added as useful for differentiating between strains of slowly growing mycobacteria, and several new species were reported. It was considered desirable to make a new numerical analysis of these microorganisms, including representatives of newly described species and using a new set of tests.

MATERIALS AND METHODS

Bacterial strains. A total of 138 strains of slowly growing mycobacteria was employed in this study. The strains and their sources are listed in Table 1. Out of these, strains 24005, 24006, 24008, 24009, 24010, and 24018 of *Mycobacterium novum* showed the same results in all tests. Accordingly, only the data from strain 24018 were included in the analysis. The number of strains used in the $n \times n$ matrix is, therefore, 133.

Tests. Tests useful for differentiating between slowly growing mycobacteria and rapidly growing ones are given in Table 2. Eighty-eight characters were employed in this study (Table 3). These include those useful for differentiating between strains of rapidly growing mycobacteria. The number of characters useful for differentiating between strains of slowly growing mycobacteria is 48. These are as follows: no. 4, 7 to 9, 11 to 31, 36, 38, 39, 41, 43 to 45, 47, 50 to 52, 61, 64, 66, 72 to 77, 85, 86, and 88.

Numerical analysis. The matching coefficient (M

TABLE 1. *Strains studied*

Specific name of strain as received	Strain designation	Source ^a
<i>Mycobacterium tuberculosis</i> (18)	05001	H37Rv; S. Ata
	05002	Aoyama-B; S. Ata
	E7193	M. Tsukamura
<i>Mycobacterium bovis</i> (6)	06001	Ravenel; K. Shoji
	06002	Denken; K. Konno
<i>Mycobacterium kansasii</i> (9)	07001	Forbes84; K. Takeya; E. H. Runyon
	07002	BostrumD35; K. Takeya; E. H. Runyon
	07003	M. Tsukamura
	07005	M. Tsukamura
	07006	P1; K. Takeya; E. H. Runyon
	07007	P16; K. Takeya; E. H. Runyon
	07009	P21; K. Takeya; E. H. Runyon
	07013	M. Tsukamura
	E5412	M. Tsukamura
	E5413	M. Tsukamura
	E5414	M. Tsukamura
<i>Mycobacterium marinum</i> (1)	08002	B916; K. Konno
	08003	B913; K. Konno
	08010	ATCC 927; ATCC
	08011	SN1251; R. Bönicke
	08012	SN1253; R. Bönicke
	08014	SN1256; R. Bönicke
	08015	SN1301; R. Bönicke
	08017	SN1304; R. Bönicke
	08018	SN1305; R. Bönicke
	E4328	ATCC 25034; ATCC ^b
	E4329	ATCC 25035; ATCC ^b
	E4330	ATCC 25036; ATCC ^b
	E4332	ATCC 15038; ATCC ^b
	E4333	ATCC 25039; ATCC ^b
<i>Mycobacterium scrofulaceum</i> (22) (<i>M. marinum</i> [27])	12003	M. Tsukamura
	12320	ATCC 19981; ATCC
	12321	ATCC 15078; ATCC
	12322	ATCC 15080; ATCC
	12340	ATCC 23429; ATCC
	13003	ATCC 19079; ATCC
	36001	ATCC 19275; ATCC
	E7325	M. Tsukamura
<i>Mycobacterium xenopi</i> (25)	28001	ATCC 19156; ATCC
	28002	ATCC 19276; ATCC
	28003	ATCC 19250; ATCC
	28004	ATCC 19970; ATCC
<i>Mycobacterium szulgai</i> (20)	SZ12001	no. 23428; J. Marks
	SZ12002	no. 25932 (= NCTC 10831); J. Marks
	SZ12003	no. 26701; J. Marks
	SZ12004	no. 20886 (= NCTC 10829); J. Marks
	E7145	M. Tsukamura
	E7420	M. Tsukamura
<i>Mycobacterium gordonae</i> (3)	12016	M. Tsukamura
	T12109	ATCC 14470; ATCC
	T12110	ATCC 19277; ATCC
	T12177	ATCC 23283; ATCC
	T12178	ATCC 23284; ATCC
	T12179	ATCC 23285; ATCC
	T12180	ATCC 23286; ATCC
	T12355	ATCC 23398; ATCC
	E6099	M. Tsukamura
	E6100	M. Tsukamura
	E6101	M. Tsukamura
	E6102	M. Tsukamura

TABLE 1—Continued

Specific name of strain as received	Strain designation	Source ^a
<i>Mycobacterium avium</i> (8)	11016	ATCC 15769; ATCC
	11017	ATCC 19075; ATCC
	11018	ATCC 17941; ATCC
	11019	ATCC 17944; ATCC
	11020	ATCC 15773; ATCC
	11021	ATCC 17942; ATCC
	11022	ATCC 17939; ATCC
	11023	ATCC 17938; ATCC
<i>Mycobacterium intracellulare</i> (24)	13082	ATCC 19077; ATCC
	13085	ATCC 15985; ATCC
	E6052	M. Tsukamura
	E6105	M. Tsukamura
	E6106	M. Tsukamura
	E6109	M. Tsukamura
	E7123	M. Tsukamura
	E7301	M. Tsukamura
	E7302	M. Tsukamura
	E7304	M. Tsukamura
	E7306	M. Tsukamura
	E7307	M. Tsukamura
	E7309	M. Tsukamura
	E7328	M. Tsukamura
	E7365	M. Tsukamura
	E7369	M. Tsukamura
	E7371	M. Tsukamura
	E7372	M. Tsukamura
<i>Mycobacterium simiae</i> (66)	E7527	H. Saito; J. Weiszfeiler (no. 51)
	E7528	H. Saito; J. Weiszfeiler (no. 53)
	E7529	H. Saito; J. Weiszfeiler (no. 54)
	E7530	H. Saito; J. Weiszfeiler (no. 55)
	E7531	H. Saito; J. Weiszfeiler (no. 59)
<i>Mycobacterium asiaticum</i> (67)	E7523	H. Saito; J. Weiszfeiler (no. 27)
	E7524	H. Saito; J. Weiszfeiler (no. 32)
	E7525	H. Saito; J. Weiszfeiler (no. 61)
	E7526	H. Saito; J. Weiszfeiler (no. 64)
<i>Mycobacterium shimoidei</i> (52)	Z7367	M. Tsukamura
	E7367	M. Tsukamura
	E7380	M. Tsukamura
<i>Mycobacterium nonchromogenicum</i> (29)	09003	ATCC 19530; M. Tsukamura (E 317)
	09013	ATCC 19531; M. Tsukamura (E1711)
	09015	M. Tsukamura (E1713)
	09016	M. Tsukamura (E1714)
	09017	M. Tsukamura (E1715)
	09020	M. Tsukamura (E1718)
	09023	ATCC 19532; M. Tsukamura (E1721)
	09033	ATCC 19533; M. Tsukamura (E1731)
<i>Mycobacterium terrae</i> (61)	38001	L. G. Wayne (W167)
	38002	L. G. Wayne (W168A)
	38003	L. G. Wayne (W171)
	38004	L. G. Wayne (W527)
	38006	L. G. Wayne (W897)
	38007	L. G. Wayne (W900)
	38008	L. G. Wayne (W923)
	38013	ATCC 15755; L. G. Wayne (W45)
	38014	L. G. Wayne (W511)

TABLE 1—Continued

Specific name of strain as received	Strain designation	Source ^a
<i>Mycobacterium novum</i> (36)	24005	M. Tsukamura
	24006	M. Tsukamura
	24008	M. Tsukamura
	24009	M. Tsukamura
	24010	M. Tsukamura
	24013	M. Tsukamura
	24018	ATCC 19619 (= NCTC 10486); M. Tsukamura
	24019	ATCC 19620 (= NCTC 10496); M. Tsukamura
	24032	M. Tsukamura
<i>Mycobacterium triviale</i> (16)	37001	G. P. Kubica (alb2386)
	37002	G. P. Kubica (DT67-4)
	37003	G. P. Kubica (C1614)
	37004	G. P. Kubica (C5181 = ATCC 19386)
	37005	G. P. Kubica (T20-5)
	37013	G. P. Kubica (T254-3 = ATCC 19387)
<i>Mycobacterium gastri</i> (61)	25001	L. G. Wayne (W417 = ATCC 15754)
	25002	L. G. Wayne (W462)
	25003	L. G. Wayne (W465)
	25004	L. G. Wayne (W471)
	25005	L. G. Wayne (W479)
	25006	L. G. Wayne (W486)
	25007	L. G. Wayne (W487)
	25008	L. G. Wayne (W504)

^a S. Ata, Nagoya University, Nagoya, Japan; K. Shoji, Osaka University, Osaka, Japan; K. Konno, Tohoku University, Sendai, Japan; K. Takeya, Kyushu University, Fukuoka, Japan; E. H. Runyon, Veterans Administration Hospital, Salt Lake City, Utah, U.S.A.; ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; R. Bönicke, Forschungsinstitut Borstel, Borstel, West Germany; J. Marks, Tuberculosis Reference Laboratory, University Hospital of Wales, Cardiff, Great Britain; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; H. Saito, Hiroshima University, Hiroshima, Japan; J. Weiszfeiler, Microbial Research Group, Hungarian Academy of Sciences, Budapest, Hungary; L. G. Wayne, Veterans Administration Hospital, Long Beach, California, U.S.A.

^b These strains were identified in our laboratory.

value) between two strains was calculated by the following equation: $M \text{ value } (\%) = (n_s \times 100)/(n_s + n_d)$, where n_s is the number of characters that show similar code symbols (+ + or - -) and n_d is the number of characters which show different code symbols (+ -). The clustering was made by the single-linkage method (26).

The computer used was the NEAC 2200, model 250B, Nippon Electric Co., Tokyo, Japan, which is located in the Nagoya Factory, the Sumitomo Machinery Co., Obu, Aichi, Japan. The program number is G90001 of the Nagoya Factory, the Sumitomo Machinery Co. The program is written in English (CÖBOL).

RESULTS

The results of this study are shown as dendrograms in Fig. 1 to 3. The data for the original "strains $\times$ characters" matrix have been deposited with the World Data Center for Microorganisms, Brisbane, Australia.

The dendrograms show the following results.

TB complex. Among the slowly growing mycobacteria studied, *M. tuberculosis* and *M. bovis* were differentiated most distinctly from the other species. These two were connected to the others at the 85% level.

Strains O5001 and O5002 of *M. tuberculosis* were connected to each other at the 100% level, and these were connected to strain E7193 of *M. tuberculosis* at the 99% level. Strains O6001 and O6002 of *M. bovis* were connected to each other at 99% level. *M. tuberculosis* and *M. bovis* were connected to each other at the 97% level. Therefore, these two species form a compact cluster, referred to as the "TB complex."

Groups I, II, and III. The organisms that belong to groups I, II, and III of Runyon (23) were joined at the 91% level. Within this large group, *M. kansasii*, *M. marinum*, *M. gastri*, and *M. shimoides* were regarded as distinct species as they were clearly differentiated from each other and from the remaining species,

TABLE 2. Characters useful in differentiating between slowly and rapidly growing mycobacteria

Distinguishing characters ^a	Genus <i>Mycobacterium</i>	
	Slowly growing mycobacteria (A) (subgenus <i>Mycobacterium</i>)	Rapidly growing mycobacteria (B) (subgenus <i>Mycobacterium</i>)
1. Tolerance to 0.2% picric acid (30, 35)	—	+
2. Tolerance to 0.1% NaNO ₂ (at 14 days) (38, 58)	—	+
3. Growth at 3 days (35)	—	+
4. Tolerance to 5% NaCl (11)	—	+
5. Succinate as C source (35) ^b	—	+
6. Malate as C source (35) ^b	—	+
7. Fumarate as C source (35) ^b	—	+
8. Glutamate as N and C sources (35, 37)	—	+
9. Trimethylene diamine as N and C sources (35, 37)	—	+
10. Acid from mannose (35, 45)	—	+

^a There are the following exceptions. Character 1: (A) *M. simiae* shows positive growth on picric acid-Sauton agar; (B) *M. chelonae* subsp. *chelonae* shows negative growth. Character 2: (A) *M. nonchromogenicum* may show positive growth on nitrite-Sauton agar. Character 3: Doubtful growth may occur according to the size of inoculation. Character 5: (A) *M. simiae* shows utilization of succinate; (B) *M. chelonae* subsp. *chelonae* lacks utilization. Character 8: (A) A few strains of *M. intracellulare*, *M. scrofulaceum*, *M. gordonae*, and *M. nonchromogenicum* may show utilization. Character 9: (B) *M. thermoresistibile*, *M. chitae*, *M. chelonae* subsp. *chelonae*, *M. vaccae*, *M. aichiense*, and *M. agari* show negative reaction.

^b In the presence of ammoniacal nitrogen.

which were placed in the "AXGN group" (avium-xenopi-gordonae-nonchromogenicum group).

AGXN group. The members of this group combined into one at the 92% level. Within the group, *M. szulgai* and *M. simiae* were clearly distinct from the other organisms. The others were divided into two subgroups, the "avium-gordonae-xenopi complex" and the "nonchromogenicum complex."

Avium-gordonae-xenopi complex. This complex combined into one at the 94% level. Within the complex, *M. gordonae* was clearly distinct from the others and was regarded as a separate species. *M. xenopi* also seemed to be distinct from the others.

M. avium, *M. intracellulare*, and *M. scrofulaceum* (especially the former two) could not be differentiated from each other on the basis of the tests used. *M. asiaticum* seems to be separate from the three above-mentioned species.

Nonchromogenicum complex. This complex is composed of *M. nonchromogenicum*, *M. terrae*, *M. novum*, and *M. triviale*. These combined into one at the 95% level. Of these four, *M. triviale* was barely distinguishable from the others, but *M. nonchromogenicum*, *M. terrae*, and *M. novum* formed one cluster and could not be differentiated from each other.

"Distinguishing characters." The characters that appeared to be useful for differentiating between slowly growing mycobacterial species are shown in Table 4. The percentages of strains showing positive reactions were based not only on the strains used in the present study but also on strains previously studied in our laboratory but not included in the present study.

DISCUSSION

The results show that, among the species of slowly growing mycobacteria other than *M. tuberculosis* and *M. bovis*, the following species are distinct: *M. kansasii*, *M. marinum*, *M. gastri*, *M. shimoidei*, *M. szulgai*, *M. simiae*, and *M. gordonae*. *M. asiaticum* and *M. xenopi* are regarded as probably distinct species.

M. avium, *M. intracellulare*, and *M. scrofulaceum* were not clearly separated from each other by the tests employed. The majority of the strains of *M. intracellulare*, the strains of *M. avium*, and some of the strains of *M. scrofulaceum* were combined into one cluster at the 97% level, and the majority of the strains of *M. scrofulaceum* were connected to this cluster at the 95% level.

Distinguishing characters between the strains of *M. intracellulare* which failed to grow at 45°C and the strains of *M. scrofulaceum* were only two: pigmentation of colonies in the dark and catalase activity (Table 4).

M. scrofulaceum strains usually did not utilize butanols as carbon sources, and *M. intracellulare* strains that failed to grow at 45°C also did not utilize butanols (47), although *M. intracellulare* strains that were able to grow at 45°C utilized butanols. The utilization of butanols is not a distinguishing character between these two species. Their amidase patterns were also not distinctive, as some strains of *M. intracellulare* showed urease, nicotinamidase, and pyrazinamidase activities, as did some strains of *M. scrofulaceum*. Strains of *M. intracellulare* usually showed nicotinamidase and pyrazinam-

TABLE 3. *List of characters determined on the strains used in this study*^a

1. Strong acid-fastness (35)	45. Growth at 45°C (35)
2. Weak acid-fastness (39)	46. Growth at 52°C (35)
3. Presence of rod form (35)	47. Glucose (dextrose) as C source (35, 59)
4. Cord (35)	48. D-Mannose as C source (35)
5. Fragmenting mycelium (39)	49. Sucrose as C source (35, 59)
6. Permanent mycelium (39)	50. <i>n</i> -Propanol as C source (34)
7. Rough colonies (35)	51. <i>n</i> -Butanol as C source (47)
8. Pigmented colonies in dark (35)	52. <i>iso</i> -Butanol as C source (47)
9. Photochromogenicity (35)	53. Propylene glycol as C source (34)
10. Growth after 3 days (35)	54. D-Galactose as C source (35)
11. Tolerance to 0.1% nitrite (Sauton agar) (38, 58)	55. L-Arabinose as C source (35)
12. Tolerance to 0.2% picric acid (Sauton agar) (30)	56. D-Xylose as C source (35)
13. Resistance to rifampin (25 µg/ml) (43)	57. L-Rhamnose as C source (35)
14. Tolerance to 0.2% sodium <i>p</i> -aminosalicylate (PAS) (32)	58. Trehalose as C source (35)
15. Tolerance to 125 µg of NH ₂ OH.HCl per ml (31)	59. Inositol as C source (35)
16. Tolerance to 250 µg of NH ₂ OH.HCl per ml (31)	60. Mannitol as C source (35)
17. Tolerance to 500 µg of NH ₂ OH.HCl per ml (31)	61. Sorbitol as C source (35)
18. Resistance to 10 µg of thiophene-2-carboxylic acid hydrazide (TCH) per ml (4)	62. Acetate as C source (35, 59)
19. Tolerance to 0.5 mg of sodium salicylate per ml (28)	63. Citrate as C source, (35, 59)
20. Tolerance to 0.5 mg of <i>p</i> -nitrobenzoic acid (PNB) per ml (55)	64. Succinate as C source (35, 59)
21. Niacin (production of nicotinic acid) (12)	65. Malate as C source (35, 59)
22. Tween hydrolysis (at 7 days) (65)	66. Pyruvate as C source (35, 59)
23. Tween hydrolysis (at 14 days) (65)	67. Benzoate as C source (35)
24. α-Esterase (10, 48)	68. Malonate as C source (35, 59)
25. β-Esterase (10, 48)	69. Fumarate as C source (35, 59)
26. Acid phosphatase (simple method, 3 h) (49)	70. Acid from glucose (dextrose) (35)
27. Catalase (semiquantitative) (13)	71. Acid from D-mannose (35)
28. β-Galactosidase (44)	72. Glucose (dextrose) as C source (glutamate-N) (57)
29. Nitrate reduction (60)	73. Acetate as C source (glutamate-N) (57)
30. Three-day arylsulfatase (17)	74. Succinate as C source (glutamate-N) (57)
31. Two-week arylsulfatase (17)	75. Pyruvate as C source (glutamate-N) (57)
32. Salicylate degradation (32)	76. Growth on Sauton agar (35)
33. PAS degradation (32)	77. L-Glutamate as C and N sources (35, 37)
34. Acetamidase (5)	78. L-Serine as C and N sources (35, 37)
35. Benzamidase (5)	79. D-Glucosamine hydrochloride as C and N sources (35, 37)
36. Urease (5)	80. Acetamide as C and N sources (35, 37)
37. Isonicotinamidase (5)	81. Benzamide as C and N sources (35, 37)
38. Nicotinamidase (5)	82. Monoethanolamine as C and N sources (35, 37)
39. Pyrazinamidase (5)	83. Trimethylene diamine as C and N sources (35, 37)
40. Salicylamidase (5)	84. Benzamide as N source (54)
41. Allantoinase (5)	85. Nicotinamide as N source (54)
42. Succinamidase (5)	86. Nitrate as N source (54)
43. Growth at 28°C (35)	87. Nitrite as N source (53)
44. Growth at 37°C (35)	88. Resistance to ethambutol (5 µg/ml) (38, 40)

^a Unless noted otherwise, the tolerance and resistance tests were carried out in Ogawa egg medium (35). Amidase tests (no. 34 to 42) were carried out after incubation of the test organism with amide at 37°C for 24 h. Unless noted otherwise, utilization of carbohydrates was tested in the presence of ammoniacal nitrogen (no. 47 to no. 69) (34, 35, 47, 51).

idase activities, but a few strains of *M. scrofulaceum* also showed these amidase activities.

The results obtained here show that *M. avium*, *M. intracellulare*, and *M. scrofulaceum* should be combined into a single species, for which the name *M. avium* has priority. This suggestion agrees with the results of the numerical taxonomy study carried out previously

by Tsukamura, Mizuno, and Tsukamura (51) and with the results of lipid analysis by Marks, Jenkins, and Schaefer (19). The finding that *M. avium* and *M. intracellulare* are not clearly distinguishable from each other was also obtained by Wayne (62) and by Meissner et al. (21).

In addition, the results of the present study

FIG. 1. Dendrogram showing the relationship of the 138 strains of slowly growing mycobacteria studied.

FIG. 2. Dendrogram showing the relationship of the 138 strains of slowly growing mycobacteria studied.

TABLE 4. Characters useful for differentiating between slowly growing mycobacteria

Character	No. of strains showing positive reaction (%)																
	<i>M. tuberculosis</i>	<i>M. africanum</i>	<i>M. bovis</i>	<i>M. microti</i>	<i>M. kansasii</i>	<i>M. marinum</i>	<i>M. scrofulaceum</i>	<i>M. szulgai</i>	<i>M. goodii</i>	<i>M. avium</i>	<i>M. intracellulare</i>	<i>M. xenopi</i>	<i>M. nonchromogenicum</i>	<i>M. novum</i>	<i>M. terrae</i>	<i>M. triviale</i>	<i>M. gastri</i>
	95 ^a	14	43	6	68	31	46	6	50	26	100	4	93	26	15	22	15
Rough colonies	94	29	37	100	100	84	11	50	4	0	3	0	0	0	100	0	60
Colony pigmentation in dark	0	0	0	0	0	0	0	100	100	0	0	75	0	0	0	0	0
Photochromogenicity	0	0	0	0	97	100	0	0	0	0	0	0	0	0	0	0	100
Growth at 28°C	13	14	18	67	100	100	100	100	100	85	97	0	100	100	100	100	100
Growth at 45°C	0	0	0	0	0	0	4	0	0	77	71	100	0	0	0	0	75
Tolerance to 0.2% PAS	0	0	0	0	0	97	70	0	90	100	99	0	97	100	80	100	100
Tolerance to 0.125 mg of NH ₂ OH per ml	0	0	0	0	96	100	100	100	100	100	100	50	100	100	100	87	100
Tolerance to 0.25 mg of NH ₂ OH per ml	0	0	0	0	22	100	100	100	90	92	100	25	100	100	100	60	100
Tolerance to 0.5 mg of NH ₂ OH per ml	0	0	0	0	0	97	87	100	56	62	97	0	100	100	95	0	100
Growth on Sauton agar	0	0	0	0	100	100	100	100	100	100	100	100	94	0	73	100	100
Tolerance to 0.2% picric acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
Two-week arylsulfatase	0	57	89	100	100	100	85	100	84	8	71	100	80	8	93	100	100
Resistance to TCH (10 µg/ml)	86	0	9	0	100	100	100	100	100	100	100	100	100	100	100	100	100
Tolerance to 0.5 mg of PNB per ml	0	0	0	0	91	90	100	100	100	100	100	100	100	100	100	100	100
Resistance to rifampin (25 µg/ml)	— ^b	0	0	0	0	55	78	0	68	85	96	100	100	100	41	0	100
Resistance to ethambutol (5 µg/ml)	— ^b	0	0	0	24	26	100	0	0	100	100	100	0	0	0	0	100
Niacin	89	79	9	100	0	42	0	0	0	0	0	0	0	0	0	0	100
Tween hydrolysis (7 days)	0	0	0	0	78	23	0	0	80	0	0	0	100	100	100	93	0
Tween hydrolysis (14 days)	0	0	0	0	98	48	11	50	96	0	2	0	100	100	100	100	100
Catalase (foam more than 45 mm)	0	0	0	0	98	5	85	100	96	0	4	0	100	100	87	100	100
α-Esterase	100	86	100	100	6	0	100	83	14	100	97	100	11	8	7	14	0
β-Esterase	100	71	100	100	54	48	100	67	52	100	97	100	84	92	87	100	100
β-Galactosidase	0	0	0	0	0	0	0	0	0	0	0	0	74	100	73	45	0
Acid phosphatase	0	0	0	0	97	90	78	100	24	88	90	0	100	100	100	93	100
Nitrate reduction	100	0	37	0	100	16	0	100	0	0	1	0	29	8	7	100	53
Urease	100	86	93	100	100	100	48	100	54	0	4	0	0	0	9	100	0
Nicotinamidase	96	43	18	100	100	55	7	0	0	35	67	50	48	0	0	0	100
Pyrazinamidase	80	21	0	100	0	29	7	0	0	31	67	50	49	0	0	0	40
Allantoinase	21	7	23	50	15	71	0	0	0	0	0	0	0	0	5	0	60
Glucose as C source (glutamate-N)	0	0	51	33	100	100	91	0	90	15	89	0	0	0	14	100	100
Acetate as C source (glutamate-N)	0	14	58	33	100	100	96	100	90	88	88	100	92	15	13	77	100
Succinate as C source (glutamate-N)	0	0	44	17	57	82	39	0	24	0	56	0	0	0	0	13	100
Pyruvate as C source (glutamate-N)	16	7	83	33	100	100	100	83	88	92	92	100	63	0	7	41	100
Glucose as C source (NH ₃ -N)	0	0	0	0	98	71	70	0	54	0	4	0	0	0	0	67	0
n-Propanol as C source (NH ₃ -N)	0	0	0	0	15	83	76	0	80	58	58	0	26	0	47	5	60
n-Butanol as C source (NH ₃ -N)	0	0	0	0	12	13	15	0	70	27	69	0	42	15	53	14	93
iso-Butanol as C source (NH ₃ -N)	0	0	0	0	12	6	9	0	76	27	64	0	29	0	40	5	93
Acetate as C source (NH ₃ -N)	0	0	0	0	75	100	98	100	98	69	92	100	75	15	33	82	100
Succinate as C source (NH ₃ -N)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80
Pyruvate as C source (NH ₃ -N)	0	0	0	0	100	93	96	100	98	65	90	100	61	0	13	91	100
Tolerance to 0.1% nitrite	0	0	0	0	0	0	0	0	0	0	0	0	60	0	80	41	0

^a Number of strains tested.^b Not counted, as patients treated with these agents may excrete resistant organisms.

FIG. 3. Dendrogram showing the relationship of the 138 strains of slowly growing mycobacteria studied.

showed that *M. nonchromogenicum*, *M. terrae*, and *M. novum* cannot be differentiated from each other and that *M. triviale* is slightly different from these three. Although this finding is similar to that of Kubica, Silcox, and Hall (15), it is, in part, contradictory to that of the International Working Group for Mycobacterial Taxonomy (IWGMT) (21). The IWGMT reported that *M. nonchromogenicum* and *M. terrae* resemble each other but are distinguishable from each other. Cooperative studies benefit from the performance of many tests but have the fault that small differences may be accentuated in importance by the performance of similar tests by several workers. The present study indicates that *M. nonchromogenicum* and *M. terrae* belong to one species rather than to different species. *M. nonchromogenicum*, *M. terrae*, and *M. novum* should be combined into a single species, for which the name *M. nonchromogenicum* has priority. *M. terrae* and *M. novum* thus are subjective synonyms of *M. non-*

chromogenicum. *M. triviale* may be regarded as a different species.

Finally, it is noteworthy that *M. gastri*, *M. shimoides*, *M. szulgai*, and *M. simiae* occupy a distinct position and that *M. asiaticum* is closely related to *M. avium*.

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REPRINT REQUESTS

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