First Report of the Cooperative, Open-Ended Study of Slowly Growing Mycobacteria by the International Working Group on Mycobacterial Taxonomy

L. G. WAYNE,¹ R. C. GOOD,² M. I. KRICHEVSKY,³ R. E. BEAM,² Z. BLACKLOCK,⁴ S. D. CHAPARAS,⁵ D. DAWSON,⁴ S. FROMAN,⁶ W. GROSS,⁷ J. HAWKINS,⁷ P. A. JENKINS,⁸ I. JUHLIN,⁹ W. KÄPPLER,¹⁰ H. H. KLEEBERG,¹¹ I. KRASNOW,¹ M. J. LEFFORD,¹² E. MANKIEWICZ,¹³ C. McDURMONT,¹⁴ G. MEISSNER,¹⁵ P. MORGAN,⁵ E. E. NEL,¹¹ S. R. PATTYN,¹⁶ F. PORTAELS,¹⁶ P. A. RICHARDS,¹ S. RÜSCH,¹⁵ K. H. SCHRÖDER,¹⁵ V. A. SILCOX,² I. SZABO,¹⁷ M. TSUKAMURA,¹⁸ AND B. VERGMANN¹⁹

Veterans Administration Medical Center, Long Beach, California 90822¹; Centers for Disease Control, Atlanta, Georgia 30333²; National Institute of Dental Research, Bethesda, Maryland 20014³; State Health Laboratory, Brisbane, Australia⁴; Food and Drug Administration, Rockville, Maryland 20852⁵; Olive View Medical Center, Sylmar, California 91342⁶; Veterans Administration Hospital, West Haven, Connecticut 06516⁷; University Hospital of Wales, Cardiff, Wales⁸; Institute of Clinical Bacteriology, Malmö, Sweden⁹; Forschungsinstitut für Lungenkrankheiten und Tuberkulose, Berlin-Buch, East Germany¹⁰; South African Medical Research Council, Pretoria 0001, South Africa¹¹; Trudeau Institute, Saranac Lake, New York 12983¹²; Montreal Chest Hospital Centre, Montreal, Canada¹³; State Department of Public Health, Montgomery, Alabama 36130¹⁴; Tuberkulose-Forschungsinstitut, Borstel, West Germany¹⁵; University of Antwerp and Institute of Tropical Medicine, Antwerp, Belgium¹⁶; Koranyi National Institute of Tuberculosis and Pulmonology, Budapest, Hungary¹⁷; Chubu Chest Hospital, Obu, Aichi-Ken, Japan¹⁸; and Statens Seruminstitut, Copenhagen, South Denmark¹⁹

An open-ended study has been initiated by the International Working Group on Mycobacterial Taxonomy to accommodate strains of slow growers that do not belong to well-established or thoroughly characterized species. Numerical taxonomic analysis of the data derived from the first series of cultures examined in this study has permitted preparation of expanded descriptions of *Mycobacterium simiae* and *M. szulgai*, and, to a lesser extent, *M. asiaticum*. The analysis also provides some support for revival of the name "*M. shimoidei*," which is presently without standing. Additional clusters have emerged which may represent new taxa; one of these includes a strain labeled "*M. paraffinicum*," a name which is also now without standing.

Many commonly encountered species of slowly growing mycobacteria have been characterized in a series of cooperative taxonomic studies carried out by the International Working Group on Mycobacterial Taxonomy (IWGMT) (8, 21, 22). The rapid growers were characterized in two additional IWGMT studies (6, 12), as were members of the "rhodochrous" taxon (4) which is now recognized as *Rhodococcus*. In the original numerical taxonomic (NT) studies of the slow growers, some strains failed to aggregate with any of the major clusters and were too few in number to permit assignment to new taxa. To provide a mechanism for continuous introduction of unusual or rarely encountered strains into the mycobacterial taxonomic framework, the IWGMT has initiated a new effort, designated the Open-Ended Study of Slowly Growing Mycobacteria. Accession and detailed study of strains is an ongoing process; as new clusters emerge, their descriptions will be reported and, when appropriate, new species will be proposed. This is the first such report from the IWGMT open-ended study.

MATERIALS AND METHODS

Selection of strains. The data base for this study was derived from cultures meeting one of the following three criteria.

(i) **Reference marker strains.** One culture was selected from a central position in each of the clusters that corresponded to named species in the three prior cooperative studies on slow growers (8, 21, 22). These 13 cultures, which are identified in the NT matrix (see Fig. 1), were not redistributed for further examination; the data previously derived from them were transferred into the computer file reserved for the Open-Ended Study.

(ii) **Unclustered strains from the prior studies.** These strains either appeared in an isolated position or were linked to one or two other strains in one or another of the previously reported studies. These cultures also were not redistributed, but the existing data were simply transferred into the new file.

(iii) **New accessions.** Submission of strains for study was not limited to members of the IWGMT. Anyone wishing to submit a strain for consideration was required to forward it to one of the designated IWGMT transmitters (Z. Blacklock, Australia; H. David, France; H. W. B. Engel, Netherlands; S. Froman, U.S.A.; J. Hawkins, U.S.A.; P. A. Jenkins, U.K.;

W. Kppler, East Germany; H. H. Kleeberg, South Africa; I. Krasnow, U.S.A.; F. Portaels, Belgium; K. H. Schrder, West Germany; I. Szabo, Hungary; M. Tsukamura, Japan; or B. Vergmann, Denmark). Acceptance was limited to cultures which, on subculture, did not develop on Lowenstein-Jensen medium within 7 days at 25C and which exhibited a negative reaction in the iron uptake test. It was further required that the submitting laboratory provide the following information about the culture: (i) a table of reactions from tests for pigmentation, for production of niacin, urease, Tween hydrolysis, β -galactosidase, and catalase, and for resistance to isoniazid (10 μ g/ml), thiophene-2-carboxylic acid hydrazide (1 μ g/ml), hydroxylamine hydrochloride (250 μ g/ml), sodium chloride (50 mg/ml), and *p*-nitrobenzoate (500 μ g/ml) (23, 24); (ii) a brief narrative statement of other properties of the culture which indicated it to be unusual and which justified its inclusion in the study (the cultures were expected to differ from previously described species by at least two of the eleven tests listed or to be accompanied by a strong narrative statement, based on supplementary tests, to support the possibility that it might represent a new taxon); (iii) a history of the culture, indicating its source and whether or not it was associated with disease.

Upon receipt of a culture and the required information, the IWGMT transmitter streaked it for purity and selected a colony for subculture and confirmation of the properties derived from the eleven key tests. If the results were in agreement, the culture and data were forwarded to the distributor (R. C. Good) and to the coordinator (L. G. Wayne) for a decision on entry of the strain into the study. If accepted, the strain was subcultured again, divided, freeze-dried, and distributed to the IWGMT members who had agreed to study the cultures. These strains received code numbers in the 90000 series, and so can be distinguished from the strains in the NT diagram from prior cooperative studies, which range in code numbers from 4243 to 25276.

Editing of data. As in the previous IWGMT permissive studies, all collaborators were free to select the tests they would employ for characterizing the cultures. A deadline of approximately 8 months was established between distribution of cultures and receipt of data by the coordinator. Once NT analysis was started, no new data for the given set of strains were added to the data base.

Because of the permissive nature of the test selections, it became necessary to edit data to eliminate undue weighting of multiple features that resulted from minor differences in test technique or criteria. Thus, when different laboratories reported drug resistance results based on slightly different concentrations of the agents, the concentrations retained in the analysis were those employed by the largest numbers of laboratories. In cases in which comparable numbers of laboratories reported two slightly different concentrations, the one with greatest interlaboratory agreement on a strain by strain basis was retained. In instances in which a single laboratory employed a given concentration, the decision whether or not to retain the data was based on reports of concentrations bracketing that one, in an attempt to avoid transitional concentrations, i.e., those close to a threshold where

reproducibility was likely to be poorest. Similar criteria were applied when slight differences in substrate concentration or reading times were recorded for enzyme-based tests.

A preliminary survey of reports on colonial morphology indicated very poor reproducibility between laboratories, so it was decided to delete these features from the data base used for the NT matrix. Subsequent examination of these reports for the different clusters by individual laboratories was then possible to help determine whether each laboratory achieved consistent subjective interpretation, even if the descriptions could not be adequately verbalized.

As in prior studies, data on thin-layer chromatography (TLC) of lipids, seroagglutination, immunoelectrophoresis, and susceptibility to bacteriophages were reserved for independent correlation with the NT results.

The computer program (18) that was employed in data editing deleted any features that gave the same result for all strains in the study, i.e., any feature that was irrelevant.

Of 363 features that were recorded in the original data base for one or more strains, only 178 remained after editing for use in the NT analysis. After editing, the mean number of features used for the new strains (i.e., those in the 90000 series) was 92, whereas there was an average of only 38 features carried over for the strains examined in the prior cooperative studies on slow growers. Data from six strains examined in the cooperative studies on rapid growers were also used because of interest in the distinction between *Mycobacterium flavescens* and certain slow growers; there was a mean of 108 features available for these strains, most of which were not available for slow growers. The possible problems of interpretation of results based on these different data bases will be considered in the Discussion.

Analysis of data. The features remaining after editing were converted to modal scores, when appropriate (25). The NT analysis was based on simple matching coefficients with sorting by unweighted average linkage (15, 19).

After clusters were defined in an NT matrix, feature frequency tables were generated for each numbered cluster. The distribution of results of the reserved features, i.e., TLC, seroagglutination, immunoelectrophoresis, phage susceptibility, and morphology, were manually tabulated according to cluster.

RESULTS

The NT analysis permitted detection of 10 well-defined clusters of three or more strains each (Fig. 1). The average internal similarity of each group was above 80%. None of these clusters showed significant affinity for the isolated marker strains of *M. terrae*, *M. nonchromogenicum*, *M. kansasii*, *M. xenopi*, *M. marinum*, or *M. gastri*. Furthermore, no strain in this matrix showed a matching score above 60% for marker strains of *M. tuberculosis*, *M. microti*, or *M. bovis* (not shown in Fig. 1).

Of the total of 178 features used to construct the matrix, 41 were identified as being most

definitive for separation of these taxa according to criteria defined previously (22) (Table 1). Feature frequencies based on fewer than three strains in a cluster are omitted from the tabulation but may be commented on in the discussion of individual clusters to follow. In addition to the feature frequencies derived from the strains in clusters 1 and 4-10, Table 1 includes feature frequencies calculated from the data on the slowly growing species examined in prior IWGMT studies. Cluster 4 is poorly described in Table 1 because of much missing data.

Seroagglutination test results (27) were available for all of the new strains in the 90000 series and for 15 of the strains from the earlier studies. These results are presented by phenetic clusters in Table 2, along with the TLC lipid patterns. Phage susceptibility was determined for 17 strains, all in the 90000 series (Table 3).

Cluster 1 consisted of seven strains with a mean internal matching score (MIMS) of 86% and included ATCC 14474, the type strain of *M. flavescens*. Although this species is generally included among the rapid growers, it may be confused with some slow growers, and this cluster was included to permit assessment of the seriousness of the problem when it was compared in terms of tests normally used for slow growers. (Strain 19527 had previously been identified as *M. thermoresistibile* in a cooperative study of rapid growers (12), so it was deleted from the calculation of feature frequencies for Table 1.)

Cluster 2 included the marker for *M. intracellulare* plus three additional strains that linked at an MIMS of 86%. One of these, ATCC 13950, is the type strain, but it had appeared at the periphery of the *M. intracellulare* cluster in the earlier cooperative study (8). The other two, 90004 and 90002, had been introduced as representatives of agglutinating serovars *simiae* 1 and *avium* 18 respectively. All four laboratories reporting serovars confirmed strain 90002 as *avium* 18, but three reported strain 90004 to be *avium* 25, and the fourth reported spontaneous agglutination. Since both strains linked to the *M. intracellulare* marker at a level below 85% and to some members of other clusters at a comparable level, the position of these two strains should be considered provisional. As additional cultures are added to the ongoing study, they may find their home in an as yet unrecognized cluster. The feature list for *M. intracellulare* in Table 1 is based on data from the IWGMT cooperative study of Meissner et al. (8), not on the four strains appearing in cluster 2 in the present study.

Cluster 3 included the marker for *M. scrofulaceum* plus three other strains, from earlier studies, linked at an MIMS of only 84%. The

feature list for *M. scrofulaceum* in Table 1 is based on data from the IWGMT cooperative study of Wayne et al (22) and is not restricted to the four strains in cluster 3.

Cluster 4 was composed of three strains with an MIMS of 87%. Two of these strains, 25224 and 25225, were loosely linked to the *M. scrofulaceum* and *M. intracellulare* clusters, respectively, in the IWGMT cooperative study of Meissner et al (8). The third strain, 90019, was derived from culture 8356 of Tsukamura and Shimoide (16) and was isolated from the same patient as were strains 90016 and 90018 in cluster 9. Since a number of features were lacking for one or another of the three strains in cluster 4, no recommendations can be made at this time concerning the status of this cluster. Seroagglutination was attempted only on strain 90019, and none of the three laboratories was able to type it.

Cluster 5 was composed of 10 strains with an MIMS of 86%. It included ATCC 25275, the type strain of *M. simiae* Karasseva, Weiszfeiler, and Krasznay 1965 (5), and permitted construction of a more complete description of this pathogen than has been heretofore available. The members of the *M. simiae* cluster exhibited mean matching scores of 78% to the members of each of clusters 3, 6, and 7, and 71% or less to all other clusters or marker strains. The major distinctions between *M. simiae* and *M. scrofulaceum* were based on pyruvate utilization, photochromogenicity, tellurite reduction, and, to a lesser extent, some other features shown in Table 1. *M. simiae* differed from *M. asiaticum* in its resistance to capreomycin and its urease and phosphatase activities and from cluster 7 in its urease activity and photochromogenicity.

Although *M. simiae* was originally described as niacin positive (5), the modal result for 80% of the strains described here was negative. However, each strain was reported positive for this feature by some of the reporting laboratories, and it has already been reported that this test, which is very reproducible for other species, gave erratic results with the type strain of *M. simiae* in a highly controlled study (24). Furthermore, although the modal result for 9 of the 10 strains was positive for photochromogenicity, these results also varied from laboratory to laboratory. One investigator noted that pigmentation was more consistent if the cultures were grown in continuous light rather than in light for only 1 to 2 h. The disagreements with modal results for this cluster were the result of failure to detect any pigment rather than of reports of production of pigment in the absence of light.

Four of the strains in cluster 5 were considered to be the cause of human lung disease, three were isolated from sputum but were not consid-

A

[illegible]

FIG. 1. *NT* matrix of 67 strains of slowly growing mycobacteria. (A) Full matrix with matching scores as indicated by symbols. (B) Same as (A) but with symbols that represent matching scores below 80% suppressed to help visualize clusters. Symbols defined in figure.

ered significant, and the remaining three were isolated from monkeys. Of the eight strains tested for seroagglutination, three belonged to the *simiae* 1 serovar and five to *avium* 18. The two strains that gave positive modal results for niacin were of the *simiae* 1 serovar. Four exhibited lipid TLC pattern B, and one exhibited pattern C (Table 2). Four of seven strains tested in laboratory 3 showed susceptibility to at least one phage, but none in laboratory 16 did so.

Cluster 6 consisted of three strains with an MIMS of 88%. As a small cluster, it suffered from the effects of missing data in a manner similar to cluster 4, but, since it included the type strain (ATCC 24276) of the recognized species *M. asiaticum* Weizfeiler, Karasseva, and Karczag 1971 (26), it is included in Table 1, and further discussion of selected features will be presented here. Strains in cluster 6 exhibited a mean matching score of 78% to those in cluster

SIMPLE MATCHING COEFF 80% OR GREATER SIMILARITY

FIG. 1B.

5; distinction from *M. simiae* was presented in the discussion of cluster 5. Strains in cluster 6 exhibited a mean matching score of 75% to clusters 7 and 8, and of 71% or less to all other clusters. *M. asiaticum* was most consistently distinguished from cluster 7 by tests for acid phosphatase and Tween hydrolysis. Furthermore, modal results were positive for photo-

chromogenicity with strains 25274 (of 5 laboratories reporting, 3 reported positive) and 90028 (of 11 laboratories reporting, 7 reported positive). On the other hand, only two of five reporting laboratories noted photochromogenicity of the type strain, 25276. Thus, just as for *M. simiae*, further studies are needed on factors affecting the induction of pigment by light for

TABLE 1. Feature frequencies for 41 properties of selected species and other clusters of slowly growing mycobacteria

Feature	RKC no. ^a	Frequency of features (%) occurring in the following species:																	
		<i>M. flavescentis</i> (1) ^b	<i>M. terrae</i> (P)	<i>M. chromogenicum</i> (P)	<i>M. non-tracellulare</i> (P)	<i>M. in-lare</i> (P)	<i>M. avium</i> (P)	<i>M. scrofulaceum</i> (P)	<i>M. NC^c</i> (4)	<i>M. sim-iae</i> (5)	<i>M. asiaticum</i> (6)	<i>"M. paraffinifum"</i> (7)	<i>M. goodii</i> (P)	<i>M. szulgai</i> (8)	<i>M. kansaii</i> (P)	<i>NC</i> (9)	<i>"M. shimoidei"</i> (10)	<i>M. xenopus</i> (P)	<i>M. marinum</i> (P)
Grows in media containing:																			
Oleic acid (0.025%)	16089	33	67	50	94	83	91	67	25	60	9	0	0	0	17	0			
Picric acid (0.2%)	16259	100	0	18	0	0	0	0	0	0	0	0	0	0	0	0			
Hydroxylamine hydrochloride (125 µg/ml)	16261	0	100	100	100	100	100	100	100	100	100	100	100	97	60	100	86	100	92
Hydroxylamine hydrochloride (500 µg/ml)	16263	0	92	100	81	46	85	0	100	100	100	75	35	0	11	0	0	100	0
Sodium chloride (5%)	18006	100	0	9	3	7	0	0	0	0	0	0	0	0	0	0	0	17	0
p-Nitrobenzoic acid (500 µg/ml)	16264						86	100	100	100	82	100	89	80	100	67	0		
p-Aminosalicylic acid (1 µg/ml)	16253	50	100	100	100	100	14				5					29	100		
Isoniazid (1 µg/ml)	16250	0	100	100	97	100	100	100	100	100	87	11	100	100	0	83	8		
Isoniazid (10 µg/ml)	16251	100	100	100	61	93	86	86	100	20	5	0	15	0	0	100	0		
Thiacetazone (10 µg/ml)	16276	100	100	100	97	100	30	90	67	100	100	9	71	100	100	67	9		
Thiophene-2-carboxylic acid hydrazide (1 µg/ml)	16264	100	100	100	100	100	100	100			100				100	100	100	100	100
Ethambutol (5 µg/ml)	16319	0	0	0	24	100		100	67				0	26	50	0	80	50	0
Capreomycin (10 µg/ml)	40369 ^d							0					100			50			

Capreomycin 71 ($\mu\text{g}/\text{ml}$)	98169 ^d	0	100	100
Growth characteristics				
Grows at 22°C	17036			0
Grows at 25°C	17014			100
Grows at 42°C	17035			0
Grows at 45°C	17017			0
Glucose as sole carbon source	25311			83
1-Propanol as sole carbon source	26524			0
Pyruvate as sole carbon source	28178			100
Glutamate as sole nitrogen and carbon source	29227			25
Acetate as sole carbon source, glutamate as tamate as sole nitrogen source	98091			0
Succinate as sole carbon source, glutamate as tamate as sole nitrogen source	98092			0
Photochromogenic	20008			0
Pigment produced	20018			0
Niacin accumulated	24424			0
Enzymatic properties				
Urease	34143			100
Nicotinamide	30255			100
Pyrazinamide	30258			0

TABLE 1—Continued

Frequency of features (%) occurring in the following species:																			
Feature	RKC no. ^a	<i>M. fla- vescens</i> (1) ^b	<i>M. ter- rae</i> (P)	<i>M. non- chromo- genicum</i> (P)	<i>M. in- tracellu- lare</i> (P)	<i>M. in- avium</i> (P)	<i>M. scrofu- laceum</i> (P)	<i>M. NC^c (4)</i>	<i>M. sim- iae</i> (5)	<i>M. asiati- cum</i> (6)	" <i>M. para- fini- cum</i> " (7)	<i>M. gor- donae</i> (P)	<i>M. szul- gai</i> (8)	<i>M. kansa- sii</i> (P)	NC (9)	" <i>M. shimo- idei</i> " (10)	<i>M. xen- opi</i> (P)	<i>M. mar- inum</i> (P)	<i>M. gas- tri</i> (P)
α-Esterase	34146	60	50	9	97	100	25	100	86	100	100	100	100	9	67		100	0	0
Acid phosphatase	34136	20	100	100	13	0	0	0	0	100	0	35	100	100	33	100	0	100	100
β-Galactosidase	34123		92	100	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Catalase, >45 mm of foam	98009	100	100	100	26	8	100	100	100	100	100	96	100	100	100	0	0	33	0
Catalase re- sists 68°C, 20 min	24425	100	100	100	97	50	100	100	100	100	100	100	100	100	100	100	100	100	0
Peroxidase	34135	0					95				100	58		100	100			100	0
Nitrate reduction	98007	100	73	11	0	0	18	0	0	0	0	0	100	97	0	0	0	0	0
Nitrite reduction >50%, 7 days	98010		83	46	91	23	23	33			0	67		14	0		57	0	8
Tween 80 hydrolysis within 10 days	98030	100	100	100	0	0	0	0	0	100	0	91	50	100	83	100	0	100	100
Tellurite reduced	24175	100	64	100	91	93	95	14				92	0			0	43		20
Arylsulfatase within 10 days	90836	100	60	100	94	29	5	28			67	55	75	94	57	0	100	100	100

^a The RKC number refers to the code number for the features contained in the data file (10).
^b Data within parentheses are cluster designations; numbers are used to designate clusters derived from the pooling of data from previous IWGMT cooperative studies.
^c NC, Species not classified.
^d The RKC method (10) codes for susceptibility to capreomycin; data are therefore inverted for this table.

TABLE 2. *Distribution of agglutinating serovars and TLC patterns of lipids according to cluster*

Cluster	Strain	TLC Pattern	Number of laboratories reporting the following agglutinating serovar:										
			<i>avium</i> 25	<i>avium</i> 3/8	<i>avium</i> 7	<i>avium</i> 18	<i>avium</i> 24B	<i>avium</i> 42	<i>simiae</i> 1	<i>M.</i> <i>szulgai</i>	Baxter	Not typable	Sponta- neous ag- glutination
2	90004 90002		3			4							1
4	90019											2	2
5	25275 90021 90025 90010 90009 90012 90008 90007	 B C B B B				4 3 4 4 3			Type ^a 3 3			1 1 1 1	
6	90028	C										2	1
7	15975 12670 23403			1								1 3	
8	90001 90020 90011	A A A					1			2 2	1 1	1	3
9	25151 90016 90018 25170 15983 23433 23432	 B			1 1			1 1				1 2 2	4
10	90014 90015 90013 90027	C C										1 1 1	3 3 3 4

^a Type of species and reference for this serovar.^b Reporting laboratory indicated uncertainty.

M. asiaticum. In no case was pigment reported for strains that were not exposed to light, so this would also help separate this species from cluster 7. The major distinction between cluster 6 and *M. gordonae* was the resistance to isoniazid. Here again, however, the pigment pattern was significant; whether or not photochromogenicity was observed in a strain of *M. asiaticum*, it would still differ from *M. gordonae*, which is scotochromogenic.

Strain 90028 was isolated from sputum of a patient in Australia, and it was considered to be an agent of lung disease. The other two strains in this cluster were isolated from monkeys.

Cluster 7 consisted of five strains, all of which were carried over from the previous studies. One of them, ATCC 12670, had been the representative culture of "*M. paraffinicum*" Davis, Chase,

and Raymond 1956 (3); it had been noted that it fell into the *M. scrofulaceum* cluster in an earlier study, but the fit was a poor one and its status as a separate species was still open to question (22). Since it was the only known culture bearing that name, and since its status was so uncertain, the name "*M. paraffinicum*" was omitted from the Approved Lists of Bacterial Names (14), and does not have nomenclatural standing. The MIMS of cluster 7, including this strain, was 90% in the present study, and the mean matching score of its members to the most similar marker, *M. gordonae*, was only 80%; therefore, its status must be reconsidered. In the present study, members of cluster 7 were readily differentiated from *M. scrofulaceum* by results of tests for pyruvate utilization; urease, nicotinamidase, and pyrazinamidase activities; and tel-

TABLE 3. Susceptibility of strains to lysis by 14 mycobacteriophages, arranged according to clusters

Cluster	Cluster designation	No. of laboratory	No. of strains tested	No. of strains lysed by phage:													
				Ag1	DS/6A	GS4FB	Bg1	D34	DNA III ₈	PH	Clark	Sedge	Legendre	BK1	33DW	BK4	D11H ₂₇ Rv
4	NC ^a	16	1	0	0	0	0	0	0	0	0	0	0	—	—	—	
5	<i>M. simiae</i>	3	1	— ^b	0	—	—	—	0	—	0	—	0	0	0	0	
		16	7	0	0	0	0	0	0	0	0	0	0	—	—	—	
8	<i>M. szulgai</i>	3	7	—	1	—	—	—	4	—	4	—	—	0	0	0	
		16	3	1	2	0	0	0	0	0	0	0	2	—	—	—	
9	NC	3	3	—	1	—	—	—	0	—	2	—	1	1	0	0	
		16	2	0	0	0	0	0	0	0	0	0	0	—	—	—	
10	<i>"M. shimoides"</i>	3	2	—	0	—	—	—	0	—	1	—	0	0	0	0	
		16	4	0	0	0	0	0	0	0	0	0	0	—	—	—	
		3	—	0	—	—	0	—	0	—	—	0	0	0	0	0	

^a NC, Cluster not classified.^b —, Not determined.

lurite reduction. The distinction from *M. gordonae* was based on pyruvate utilization, Tween hydrolysis, and tellurite reduction. The differences between cluster 7 and *M. simiae* and *M. asiaticum* have been noted above. One strain in cluster 7 was reported to cross-agglutinate with serum for serovars *avium* 3 and 8, and the other two tested strains could not be typed. Two of the cultures in the "*M. paraffinicum*" cluster were isolated from environmental sources; one was isolated from a gastric lavage specimen, but was not considered clinically significant. Information on the sources of the other two strains is no longer available.

Cluster 8 consisted of four strains, had an MIMS of 90%, and conforms to descriptions of *M. szulgai* Marks, Jenkins, and Tsukamura 1972 (7, 13). Members of this cluster exhibited a mean matching score to both the *M. kansasii* marker and the members of cluster 4 of 78% and, to all other clusters, of 71% or less. *M. szulgai* reduced nitrate and exhibited acid phosphatase activity, whereas the converse was true of all three strains in cluster 4. *M. szulgai* differed from *M. kansasii* in pyrazinamidase and α -esterase activities, as well as in susceptibility to thiacetazone and utilization of glucose. The modal results of pigmentation studies on all strains in cluster 8 indicated scotochromogenicity. Analysis of individual reports indicated that 13 of the total of 56 observations on these strains demonstrated photochromogenicity, that only two reports indicated a failure to detect any pigment, and that the balance of 40 reports (71%) indicated scotochromogenicity. It has been reported that *M. szulgai* is photochromogenic only at 25°C, but is scotochromogenic at 37°C (7); however, Selva-Sutter et al. (13) have not found this to be a consistent feature. The present results confirm the need for a careful study of the factors that affect pigment production by *M. szulgai*.

Although the mean matching score of strains in cluster 8 to those in cluster 1 (*M. flavescens*) was only 65%, some further observations on their differentiation is in order, since the application of a small number of tests used in routine clinical microbiology might lead to confusion between these species. That is, both may appear to be scotochromogenic, to reduce nitrate, and to hydrolyze Tween 80. However, the striking differences in their susceptibilities to picrate, hydroxylamine (125 µg/ml), and sodium chloride will permit them to be distinguished from one another.

As seen in Table 2, a unique TLC pattern was recognized for members of cluster 8. Additionally, two strains were recognized as belonging to the *M. szulgai* serovar in two laboratories. The assignment of these two cultures to the Baxter

serovar in a third laboratory suggests that these two serovar designations may be synonymous. Only two of three strains tested in cluster 8 were lysed by any of the phages employed in the two laboratories (Table 3), but no clear pattern was characteristic of *M. szulgai*. Strains 23428 and 90001 were considered to be causes of human lung disease; the clinical significances of the other two strains in this cluster were not known, although one of them, 90011, was isolated from sputum.

Cluster 9 consisted of seven strains with an MIMS of only 82%. They linked to the poorly characterized cluster 4 at a mean matching score of 76%. The next closest matches were *M. goodii* and *M. marinum* at 73% and *M. xenopi* at 72%. Three of these seven strains, 15983, 23432, and 23433, had appeared in a poorly defined cluster of four strains in a previous IWGMT cooperative study (22) in which it was noted that they might represent a new species. However, a proposal to designate them as such was not made at that time. Two strains in the present cluster 9, 90016 and 90018, were derived from Tsukamura's cultures 8159 and 8265, both of which were obtained from the same patient as was strain 90019, which fell into cluster 4 and was mentioned above. These latter three strains were among four isolated from a single patient and described by Tsukamura and Shimoide (16).

The strains in cluster 9 differed from *M. goodii* in their response to tests for nicotinamidase, pyrazinamidase, and pyruvate utilization (only three strains were examined for the latter). They differed from *M. xenopi* in their growth temperatures and susceptibilities to isoniazid and hydroxylamine. The feature frequencies for cluster 9 in Table 1 are based on an average of 5.1 strains examined for each of the 41 tests.

Although scotochromogenic behavior was consistently observed for most of the strains in cluster 9, two of the cultures, of strains 90016 and 90018, were very inconsistent in their pigmentation. Thus, the modal score for these two strains was recorded as negative for pigmentation, but these modal scores were based on 10 out of 17 reporting laboratories failing to detect pigment for strain 90016 and 8 of 14 failing to detect pigment for 90018, i.e., a mean of 42% of the observations on these two strains did reveal pigment. Furthermore, pigment was noted by 8 out of 15 reporting laboratories for strain 90019 (cluster 4). Since strains 90016, 90018, and 90019 were all submitted as having been isolated from the same patient, their distribution into different clusters may be a consequence of a low order of reproducibility of the diagnostic features of a single strain. This variability was also noted in the test for Tween hydrolysis, in which mean

interlaboratory reproducibilities were seen of only 65% for these three strains and of only 69% for cluster 9 as a whole.

The low MIMS for this cluster and the inconsistency of behavior of its members in many features listed in Table 1 lay its validity as a taxonomic entity open to question. However, strains 15983, 23432, and 90016/90018 were considered by their submitters to be responsible for human lung disease; strains 25151, 25170, and 23433 were all isolated from sputum, but information is not available on their clinical significance. Thus, since they may represent a new species of pathogen, further study is especially important.

Cluster 10 was composed of four strains with an MIMS of 85%. Included was strain 90013, which was derived from Tsukamura's culture 4796, the strain that Tsukamura et al. (17) proposed as the type of "*M. shimoidei*." The description included in that proposal was based on seven cultures, all isolated from the same patient over a period of 5 years; "*M. shimoidei*" was not included in the Approved Lists (14) and has no standing at this time. Two of the three additional strains in cluster 10 of the present study are derived from two of the other strains from the single patient described by the original authors (90014 = 7367; 90015 = 4794). However, strain 90027 was derived from Blacklock and Dawson's culture A3830, which was isolated from sputum of a patient in Australia and also considered clinically significant.

The members of cluster 10 have mean matching scores of 77% to *M. xenopi* and of 68% or less to any other cluster or marker in this study. However, Table 1 identifies seven features that provide almost absolute distinctions between them, and there is no reason to believe cluster 10 to be related to *M. xenopi*. None of the strains was lysed by any of the phages tested.

Fused rocket immunoelectrophoresis. Seven of the strains in the 90000 series were selected, before the phenetic analyses were made, to serve as immunological reference systems. Whole-cell sonic extracts were used to immunize rabbits, as described by Chaparas et al. (2). Sonic extracts of 22 of the strains (including the reference cultures) were tested against the seven reference systems by fused rocket immunoelectrophoresis, and the number of precipitin bands seen for each was recorded. The PQS (percent of qualitative sharing) of antigenic components was calculated by dividing the number of bands seen when an extract was run against a reference serum by the number of bands exhibited by the homologous antigen against that serum and multiplying by 100.

As seen in Table 4, only three well-defined

TABLE 4. Percent qualitative sharing of precipitin lines of 22 test strains against 7 reference systems by fused rocket immunoelectrophoresis

Cluster	Test strain	Percent qualitative sharing with reference systems ^a						
		Cluster 5				Cluster 8		Cluster 10
		Strain 90010 (36)	Strain 90009 (27)	Strain 90012 (34)	Strain 90006 (46)	Strain 90020 (38)	Strain 90011 (42)	Strain 90015 (32)
2	90004	19	[48] ^b	29	39	32	29	[47]
	90002	33	70	56	52	53	29	[78]
?	90026	31	[56]	29	41	47	26	31
4	90019	28	30	41	33	[45]	36	16
5	90021	31	37	[41]	[41]	26	24	38
	90025	36	[44]	38	[43]	24	24	25
	90010	H ^c	78	[94]	59	47	81	31
	90009	[75]	H ^c	[76]	65	29	55	41
	90012	64	[93]	H ^c	76	37	74	41
	90008	[75]	67	[74]	50	32	60	31
	90007	31	[41]	35	30	18	33	28
?	90022	58	[74]	64	[74]	55	36	34
	90006	[72]	70	[71]	H ^c	50	57	56
6	90028	22	33	[38]	28	26	24	25
8	90001	22	44	32	[46]	39	40	38
	90020	53	70	[82]	57	H ^c	[83]	44
	90011	61	52	[85]	50	[84]	H ^c	47
9	90016	31	37	[50]	41	26	40	28
	90018	58	[81]	[79]	67	18	50	28
10	90014	33	44	56	41	26	48	[88]
	90015	42	44	53	59	29	52	H ^c
	90013	31	37	76	33	34	60	[94]

^a Strains are reference strains. Numbers within parentheses are the numbers of reference lines.^b Numbers within brackets are the highest PQS values seen for each strain.^c H, Homologous system.

phenetic clusters were represented by the strains chosen as references. The data provide some support for the clustering observed in the NT diagram, but this immunological technique is not yet suitable for use as an independent primary clustering device. Thus, although most of the strains from cluster 5 exhibited their highest PQS values (numbers within brackets in Table 4) against one or more of the three cluster 5 reference systems and the strains in cluster 10 gave maximum PQS values against the cluster 10 reference system, there was some crossing of cluster lines. Four strains in cluster 5 had PQS scores of 74% or higher against the *M. simiae* reference products, with between 27 and 36 bands seen. The other three strains in this cluster had maximum PQS values of only 41 to 44%, representing only 14 to 20 lines, and two of these yielded PQS values within 1 percentage point of the maximum when tested against the undefined

strain 90006 reference serum. Cluster 8 strain antigens cross-reacted to a marked degree with the cluster 5 reference sera, although the converse was not true.

The failure of this technique to provide more definitive classification is probably related to two factors. First, some clusters were not represented by reference sera, so their maximal reactivity could not be determined. Secondly, there is a need for an improved standardization of extracts, as the low maximal PQS values seen within clusters may simply be related to sonic extracts with too low an antigen content.

Reproducibility of tests. In prior IWGMT cooperative studies (23, 24), 11 tests were identified that yielded interlaboratory reproducibility at a level of 90% or higher. The present study is the first one to be initiated after the publication of those reports, and thus it offers an opportunity to extend the observations on those

techniques. The results obtained with the new (90000 series) strains in these tests are analyzed in Table 5, both in terms of overall interlaboratory reproducibility and as a function of cluster. The overall reproducibility values are expressed as both mean and median scores to illustrate examples for which the mean was influenced disproportionately by a few laboratories.

The mean reproducibilities of tests for resistance to thiophene-2-carboxylic acid hydrazide (1 $\mu\text{g/ml}$), to hydroxylamine hydrochloride (250 $\mu\text{g/ml}$), to *p*-nitrobenzoate (500 $\mu\text{g/ml}$), and to sodium chloride (5%) and for accumulation of niacin were all 90% or higher, in agreement with the results of the prior studies.

The poor performance in the test for resistance to 10 μg of isoniazid per ml is not readily explained, since 12 of the 13 participants who performed the test indicated that they had followed the protocol described in the earlier study (24). The technique is not different from that employed successfully for determining susceptibility to other inhibitory agents (Tables 5 and 6). Furthermore, the poor reproducibility is evident among all the clusters, i.e., it is not a result of disproportionate variability among members of a single taxon.

The results of the two pigment-related features are obviously linked. Thus, for cluster 10, there was 100% agreement that no pigment was produced, and so the data on photochromogenicity were also 100% negative. Pigmentation of strains in cluster 8 was noted in 94% of the reports, but a number of laboratories failed to detect an effect of light on induction of that pigment, i.e., the cultures were often recorded as scotochromogens. This was a result different from that which was obtained with clusters 5 and 6, in which a failure to detect an effect of light resulted in recording strains as nonchromogens. In view of the high reproducibility of the test for photochromogenicity reported earlier for *M. kansasii* and *M. marinum* (23), the expression of pigmentation by members of some species included in the present study appears to be influenced by additional factors, the nature of which remain to be clarified.

As seen in Table 5, there is a striking discrepancy between the interlaboratory mean and median scores for agreement with the consensus in the test for urease. Thus, although 11 of 17 laboratories achieved scores of 90% or higher, 4 laboratories scored 61% or lower. Eight laboratories indicated they had used the method based on assay for free ammonia that was evaluated in the earlier cooperative study (23), and 7 indicated use of a method based on a pH change (9, 20). However, these two methods yielded 85 and 86% mean agreement with the consensus, respectively, so the choice of method did not ac-

count for the variability. The discrepancies from the consensus among the four laboratories with the lowest agreement scores were divided as follows. The laboratory using a pH method had false-negative results only. One of the laboratories using the ammonia method had five false-negative and one false-positive results, and the other had seven disagreements in each direction. The fourth laboratory failed to provide information on the method employed.

The agreement between mean and median agreement scores for the test for Tween hydrolysis suggests that problems of technique in individual laboratories do not account for the observation that reproducibility of this test in the present study was lower than in the previous cooperative study (23). It can be seen from the data presented in Table 5 that disagreements are a function of cluster. Thus, clusters 2, 5, and 6 yielded high scores, whereas members of clusters 8, 9, and 10 were less consistent in their behavior in this test. In spite of these problems, 6 of 17 laboratories achieved scores of 90% or higher. The disagreements with the consensus occurred predominantly as false-negative results, and this may be a reflection of failure to appreciate the significance of even a slight and subtle shift in color of the indicator as an indication of a positive reaction in some weakly reactive species.

There is a marked discrepancy between mean and median agreement scores in the test for β -galactosidase. This is because two laboratories had scores below 50%. The mean agreement among the remaining 10 laboratories was 92%. The modal results for all cultures in this series were negative in this test.

The difference between mean and median scores in the semiquantitative catalase test is accounted for by the results of four laboratories, all of which had scores below 70%; the remaining 13 laboratories were above 82%. Of the four laboratories with scores below 70%, three indicated they had followed the previously published protocol (24). Of the 27 instances in which these three laboratories disagreed with the consensus, 25 were in the direction of false-negative results. Two factors that can account for such discrepancies are inadequate ventilation of the cultures and deterioration of the 30% H_2O_2 solution required for this test.

In addition to the 11 features that were evaluated previously and reported to be highly reproducible (23, 24), there are a number of other features listed in Table 1 that provided resolution between clusters. For 10 of these tests there were five or more replications among the 90000 series strains in the present study, and these are evaluated in terms of agreement with the consensus in Table 6.

TABLE 5. *Interlaboratory agreement with the consensus of results obtained for 10 features that had been shown to be highly reproducible in prior cooperative studies (23, 24)*

Agreement with the consensus by labs				Mean agreement with the consensus by cluster																				
Feature	No. of labs	Mean %	Median %	No. of labs in agreement	Cluster 2 (2 strains)		Cluster 4 (1 strain)		Cluster 5 (7 strains)		Cluster 6 (1 strain)		Cluster 8 (3 strains)		Cluster 9 (2 strains)		Cluster 10 (4 strains)							
					No. of replicates	Modal result %	No. of replicates	Modal result %	No. of replicates	Modal result %	No. of replicates	Modal result %	No. of replicates	Modal result %	No. of replicates	Modal result %	No. of replicates	Modal result %	No. of replicates	Modal result %				
Grows in medium containing:																								
Isoniazid (10 µg/ml)	13	67	67	2	12	- ^a	58	12	-	33	88	+	64	6	50	37	-	81	24	-	71	50	-	60
Thiophene-2-carboxylic acid hydrazide (1 µg/ml)	15	97	100	13	28	+	100	14	+	93	102	+	97	9	89	45	+	100	28	+	100	58	+	93
Hydroxylamine (250 µg/ml)	15	90	89	7	28	+	96	14	-	79	102	+	98	9	100	45	+	89	15	- ^b	93	44	- ^d	75
p-Nitrobenzoate (500 µg/ml)	13	91	91	8	24	+	79	12	+	83	88	+	97	8	88	39	+	85	24	+	92	50	+	86
Sodium chloride (5%)	16	96	100	15	30	-	90	15	-	100	109	-	95	10	90	48	-	98	30	-	100	62	-	97
Growth characteristics																								
Pigment produced	17	78	78	4	30	-	73	15	+	53	114	+	71	11	64	51	+	94	31	-	58	64	-	100
Photogenic	17	78	77	6	30	-	80	15	-	73	114	+	71	11	64	51	-	75	31	-	81	64	-	100
Niacin accumulated	17	91	96	11	28	-	100	14	-	100	81	- ^d	79	11	100	46	-	96	29	-	97	62	-	97
Enzymatic properties																								
Urease	17	85	96	11	28	-	86	14	-	71	111	+	80	11	100	51	+	88	30	-	87	62	-	82
Tween hydrolysis (10 day)	17	85	85	6	30	-	100	15	-	53	114	-	90	10	100	16	- ^e	62	31	+	74	64	+	82
β-Galactosidase	12	83	94	7	22	-	86	11	-	91	81	-	86	7	100	36	-	67	22	-	82	46	-	80
Catalase, >45 mm of foam	17	86	91	9	30	-	73	15	+	80	114	+	79	11	100	49	+	88	31	+	97	64	-	94

^a One strain gave a + modal result and the other was -.

^b One strain gave a + modal result and 1 was -.

^c One strain gave a + modal result and 3 were -.

^d Two strains gave + modal results and 5 were -.

^e Two strains gave + modal results and 1 was -.

TABLE 6. *Interlaboratory agreement with consensus of results obtained for 10 features that were not established as highly reproducible in prior cooperative studies*

Agreement with consensus by labs			Mean agreement with the consensus by cluster																						
Feature	No. of labs.	Mean %	Median	No. in ≥ 90% agreement	Cluster 2 (2 strains)		Cluster 4 (1 strain)		Cluster 5 (7 strains)		Cluster 6 (1 strain)		Cluster 8 (3 strains)		Cluster 9 (2 strains)		Cluster 10 (4 strains)								
					No. of replica- tions	Modal result %	Mean %	No. of replica- tions	Modal result %	Mean %	No. of replica- tions	Modal result %	Mean %	No. of replica- tions	Modal result %	Mean %	No. of replica- tions	Modal result %	Mean %	No. of replica- tions	Modal result %	Mean %			
Grows in media containing:																									
Isoniazid (1 µg/ml)	6	93	93	4	12	+	100	6	+	100	41	+	98		18	+/- ^a	50	12	+	100	24	+	96		
Ethambutol (5 µg/ml)	5	87	89	1	8	+	75	4	+	100	33	+	97	4	75	15	-	100	9	-	67	18	-	78	
Growth characteristics																									
Grows at 22°C	6	92	93	3	12	+	100	6	+	100	42	+	95		18	+	100	12	+	100	23	-	65		
Grows at 25°C	7	81	82	2	12	+	83	6	+	83	46	+	87	5	80	21	+	100	13	+	100	26	+/-	58	
Grows at 45°C	12	87	90	6	19	-	95	10	-	100	79	-	87	9	89	36	-	100	21	-	100	42	+/-	58	
Colonies smooth	13	88	89	4	22	+	100	10	-	80	87	+	100	9	100	39	+	85	23	+	78	44	-	64	
Enzymatic properties																									
Nicotinamidase	10	75	71	0	18	+	100	9	+	67	56	- ^b	61	4	-	100	30	+	80	18	+	83	37	+	57
Pyrazinamidase	10	76	78	2	18	+	89	9	+	67	55	- ^b	69	4	-	100	30	+	53	18	+	78	37	+	62
Acid phosphatase	6	89	90	3	12	+	58	6	-	67	39	-	95		18	+	100	11	+	73	24	+	100		
Nitrate reduction	5	94	100	4	10	-	100	5	-	100	33	-	91		15	+	93	10	-	100	19	-	95		

^a +/—,
^b One strain gave a positive modal result and six were negative.

The test for resistance to 1 μ g of isoniazid per ml gave high mean and median scores, with strains in all clusters except cluster 8 exhibiting resistance. The latter cluster yielded extremely variable results, suggesting that this is a transition concentration.

Mean and median agreements for resistance to ethambutol (5 μ g/ml) were just below the 90% threshold of reproducibility defined in the prior studies. However, it is noteworthy that clusters 5 and 8, which were recorded as resistant and as susceptible, respectively, yielded very high agreement scores.

At the lower end of the growth temperature scale, the test at 22°C yielded much more consistent data than at 25°C, although the consensus results for each cluster were the same for both temperatures. These two temperatures were evaluated in different laboratories, and the discrepancies are probably due to differences in proper control of temperature rather than to superiority of one temperature over the other, since the consensus results at both temperatures were in agreement. At the upper end of the growth temperature scale, almost all strains were inhibited at 45°C. Results for cluster 10 showed marked variability at both ends of the scale.

The tests for pyrazinamidase and nicotinamidase show very low mean and median agreement with the consensus; the poor reproducibility is not limited to a few laboratories but is distributed among all. Similarly, poor reproducibility is seen in almost all clusters, so caution must be exercised in interpretation of these tests. The technique used for these features is the same as that used in the ammonia method in the very reproducible urease test (23).

On the other hand, tests for both acid phosphatase and for nitrate reduction yielded good agreement scores, encompassing clusters both with modal-positive and with modal-negative reaction patterns.

Thirteen laboratories reported on colonial morphology. For seven of these laboratories, either so much data was missing as to make analysis impractical, or the preliminary study indicated no clear correlation of morphological type with numerical clustering of the strains. In the other six laboratories, the observers, given multiple choices of descriptive terms in their code sheets, often failed to agree with one another in choice of a term. However, they often did choose consistently within a given laboratory, as reflected by clustering behavior, which was unknown to them at the time the observations were made.

The subjective nature of these descriptions is

evident from the distribution of features, by clusters, as recorded in these six selected laboratories (Table 7). For example, laboratory 6 considered all of cluster 8 and half of the strains in cluster 10 to produce smooth colonies. Laboratory 7 was the only one to code domed and pyramidal separately; most of the strains in clusters 5 and 9 were pyramidal and most of those in clusters 2 and 8 were domed. Laboratory 8 coded for domed strains only, but added the notation "central" opposite the strains that laboratory 7 had recorded as pyramidal. This suggests that both observers recognized the same peculiarity of the dome but coded it differently.

In addition to the broader descriptive categories, some laboratories also coded their observations on colonial morphology according to categories designed "xenopi type" and "kansasii type." Of 146 entries for members of clusters 2, 5, 8, and 9 from 11 laboratories, none were recorded as positive for "xenopi type." On the other hand, observers from six of these laboratories did note similarities to the "xenopi type" among strains of cluster 10. Thus, nine entries from these laboratories in this cluster were positive for this feature, and seven additional entries were accompanied by some verbal notation indicating that something suggestive of *M. xenopi* was seen in these colonies. Only six entries from these six laboratories for members of this cluster failed to record some suggestion of the "xenopi type" of morphology, although 18 entries for the other five laboratories were negative. Of the 16 entries coded as "xenopi type" or with notations suggesting similarity to that type, eight were paralleled by positive coding for filamentous surface, in accord with Runyon's description of the unique colonial morphology of *M. xenopi* (11).

In addition, laboratory 10 recorded "kansasii type" morphology for the three strains in cluster 8 and for one strain in cluster 9, and also noted cording for these strains (Table 7). A second laboratory recorded one strain each in clusters 8 and 9 as the "kansasii type" without also noting cording. The remaining 11 entries for cluster 8 and 6 entries for cluster 9 failed to document similarity to "kansasii type."

Using information in Table 7, it is possible to prepare a verbal description of each cluster, as perceived in each laboratory, which could contribute to distinction of the clusters from one another. However, the description of a given cluster from one laboratory would not agree with, and in fact would often contradict, that from another laboratory. There is evidently an intuitive process at work that permits a well-trained observer to recognize similarities in colony types within a cluster, but verbalization and

TABLE 7. Presence (+) or absence (-) of characteristics of colonial morphology as recorded by six selected laboratories^a

Cluster	Strain	Surface texture ^b																		Density ^c transparent or translucent			
		Rough						Wrinkled or folded		Corded			Filamentous			Elevation ^c domed or pyramidal			Margin ^d spreading; irregular or lobulate				
		6	7	9	10	13	17	7	17	6	10	13	17	6	7	10	13	17	6		7	10	13
2	90004	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	90002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	90021	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	90025	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	90010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	90009	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	90012	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	90008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	90007	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	90001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	90020	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	90011	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	90016	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	90018	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	90014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	90015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	90013	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	90027	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

^a Numbers under the characteristics are numbers of the laboratories.^b Converse is smooth.^c Converse is flat.^d Converse is entire.^e Converse is opaque.

communication of the observations are so inconsistent as to limit the value of these features when applied to the taxonomy of mycobacteria.

DISCUSSION

As noted earlier, we had some concern over the distortions that might be introduced by combining the data from selected strains (old strains) from the previous cooperative studies with data from the 90000 series (new strains) specifically selected for examination in the new open-ended study. This concern was prompted by the marked discrepancy in numbers of features available after editing for NT analysis. Examination of Fig. 1B reveals a number of intercluster crossovers with strains from different clusters exhibiting matching (M) scores to one another of 80% or greater. The high M crossovers almost always involve at least one old strain in the pair. The average number of available features was 38 for old strains and 92 for new strains. Disregarding cluster 1, which was unique in that it was largely based on tests employed for rapid growers, and concentrating only on the remaining numbered clusters in Fig. 1, one observes that there are 24 old strains and 20 new strains represented in these clusters. However, of 64 strain pairs with crossover M scores of 80% or higher, 42 involve 2 old strains, 16 involve 1 old and 1 new strain, and only 6 involve 2 new strains. Nevertheless, clustering behavior is seen that encompasses both groups of strains, and the limited seroagglutination data available for cluster 5 tend to support the validity of that cluster. Retention of the old data in the file appears justified for the immediate future to serve as nuclei around which newly introduced strains may aggregate as they enter the matrix. Furthermore, the use of single, old marker strains of a number of established species that are not represented in the new study did not yield a significant number of high M crossovers with new strains.

M. flavescens is more closely related to rapidly growing mycobacteria than to slow growers. However, as noted earlier, there is some risk of diagnostic error (especially confusion with *M. szulgai*) when a small number of tests is employed in a clinical laboratory, and use of some of the properties noted in Table 1 should permit such errors to be avoided.

Clusters 2 and 3 included several cultures that appear to be peripheral strains of *M. intracellulare* and *M. scrofulaceum*, respectively, and there is presently no compelling reason to propose new taxa to accommodate them.

Any further consideration of the taxonomic status of members of cluster 4 must await en-

largement of the cluster through accession of new strains, for reasons noted in the Results section.

Cluster 5 represented the established species *M. simiae*, which is a human pathogen that is very infrequently encountered in most laboratories. Although it is usually described as positive in the niacin test, the strains were usually recorded as negative in the present study. The data recorded in Table 1 provide an expanded description of this species. On the other hand, this cluster will have to be enlarged considerably with additional strains before it is possible to evaluate the recent proposal of Boisvert and Truffot (1) to further subdivide the species *M. simiae*. Of special concern here is the failure of many laboratories to demonstrate nicotinamidase and pyrazinamidase activities in strains of this cluster.

M. asiaticum is a recognized, although poorly described, species. The two old strains in cluster 6 showed some crossover with *M. simiae*, but the single new strain did not. No change in the taxonomic status of this species is suggested by the present study, but the continued accession of additional strains of this species is clearly needed to provide a satisfactory description.

All of the strains in cluster 7 were from the earlier studies, and a degree of crossover was seen with cluster 5. However, the very high mean intracluster matching score of cluster 7 suggests it to be a unique taxon. Many of the *M. simiae* strains (cluster 5) were clinically significant, but the strains in cluster 7 were derived from environmental sources and were not associated with disease. There are thus practical as well as theoretical reasons for establishing distinctions between the two sets. Although the name "*M. paraffinicum*" is presently without standing, the presence of the original reference strain of that taxon appears to provide some justification for reproposing that species. However, there was an agreement among the members of this study that new species would not be proposed officially in these reports; that would be left to individuals who had introduced the strains.

Cluster 8 did not include a reference or type strain of *M. szulgai*, but the features noted in Table 1 as well as the very distinctive seroagglutination and the unique TLC lipid pattern make it clear that this cluster does represent *M. szulgai*, and the data provide an expanded description of the species.

Cluster 9 was composed of both old and new strains and is very well circumscribed. Several members of the cluster were associated with human disease. The fact that two of the strains were from a single patient, who also yielded a

strain that fell into cluster 4, is unexplained at present. The old strains in this cluster included three pathogens (15983, 23432, and 23433) that were introduced by Wayne into an earlier cooperative study (22) and fell into a small cluster at that time. It was noted then that they "... may constitute a new species. No such designation is proposed at this time, though." Two other strains (25151 and 25170) aggregated in the *M. intracellulare* cluster in a subsequent cooperative study (8) but were reintroduced into the present study because they appeared aberrant when subjected to a new taxonomic probability matrix (24). The two new strains in cluster 9 were described by Tsukamura and Shimoide (16) as associated with disease, but again, no formal proposal was made to assign them to a new species. The aggregation of all of these strains into a single cluster in the present study appears to support proposal of a species to accommodate them.

Cluster 10 included three strains from a single patient in Japan and one strain from a patient in Australia, and these strains were considered to be the cause of the patients' pulmonary diseases. The name "*M. shimoidei*" had been proposed for the cultures from Japan (17), but this name lost standing on omission from the Approved Lists (14). The presence of strains from two distinct geographic areas in cluster 10 plus the unique colonial morphology that was recorded for them suggest that the taxon is distinct and that a proposal to revive the name "*M. shimoidei*" would be in order.

REPRINT REQUESTS

Address reprint requests to: Dr. L. G. Wayne, Veterans Administration Medical Center, Long Beach, CA 90822.

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