Cooperative Numerical Analysis of Rapidly Growing Mycobacteria

The Second Report

H. SAITO, R. E. GORDON, I. JUHLIN, W. KÄPPLER, J. B. G. KWAPINSKI, C. McDURMONT, S. R. PATTYN, E. H. RUNYON, J. L. STANFORD, I. TARNOK, H. TASAKA, M. TSUKAMURA,
AND J. WEISZFEILER

Hiroshima University School of Medicine, Hiroshima, Japan; Shimane Medical College, Izumo, Japan; Rutgers, The State University, New Brunswick, New Jersey; Institute of Clinical Bacteriology, Malmö, Sweden; Forschungsinstitut für Lungenkrankheiten und Tuberkulose, Berlin-Buch, German Democratic Republic; University of Manitoba, Winnipeg, Canada; Department of Public Health, Montgomery, Alabama; Instituut voor Tropische Geneeskunde, Antwerp, Belgium; Microbiology Research, Veterans Administration Hospital, Salt Lake City, Utah; Middlesex Hospital Medical School, London, England; Forschungsinstitut, Borstel über Oldesloe, Federal Republic of Germany; Chubu Chest Hospital, Obu, Aichi-ken, Japan; Hungarian Academy of Sciences, Budapest, Hungary

The second cooperative numerical taxonomic analysis of Runyon group IV rapidly growing mycobacteria was facilitated by combining the results of 12 individual participants, each performing one or more of the selected tests by his or her own choice of method. The 177 characters were coded for analysis and the matching matrix was generated. Eight of nine clusters which were defined in this investigation were of previously named species. The recognized clusters were as follows: *Mycobacterium flavescens*, *M. vaccae*, *M. chitae*, *M. thermoresistibile*, and *M. smegmatis*. One large cluster, designated "*M. parafortuitum* complex" was noted which contained strains representative of taxa earlier identified as *M. parafortuitum*, *M. diernhoferi*, *M. aurum*, *M. neoaurum*, and a cluster of strains of unnamed species. It is suggested that segregation of species or subspecies within this complex not be attempted at this time.

Since the earlier report of a cooperative numerical analysis of rapidly growing mycobacteria (5), several new taxa within this group of acid-fast bacilli have been reported. To determine the distinctiveness and precise definition of these new rapidly growing species, a second cooperative study by the International Working Group on Mycobacterial Taxonomy (IWGMT) was carried out by 12 different laboratories. As in previous cooperative studies (5, 17), the same "permissive" philosophy (17) was used; i.e., each participant was permitted to perform any tests that he or she chose. The only stipulations were that all investigators had to submit their raw data to the study coordinator for collation and numerical analyses, and none was allowed to remove his data once the final numerical analysis was completed. This report presents the results of this study.

MATERIALS AND METHODS

Fifty-nine strains attributed to eight taxa of rapidly growing mycobacteria were examined. These strains are listed in Table 1. There were five strains called *Mycobacterium flavescens* (1), nine called *M. vaccae* (2), five called *M. diernhoferi* (3), and seven called *M. parafortuitum* (14). Representatives of

these four taxa were included in our previous study (5), but problems of their taxonomy remained unsolved. Additionally there were eight strains called *M. thermoresistibile* (10), twelve called *M. aurum* (15), seven called *M. chitae* (11), and six called *M. "lactae."* Since our study began, one strain, ATCC 25795, at first called *M. aurum*, has become the type strain of *M. neoaurum* (12). In most instances, study strains were deposited directly with the American Type Culture Collection (ATCC) by the laboratory of origin. In a few cases, cultures were submitted through the study coordinator to the ATCC. The deposited strains were given code numbers at ATCC, freeze-dried, and sent to 19 members in 10 different countries.

Each investigator performed his series of selected tests on the set of cultures received. In the final analysis, data were submitted to the study coordinator by only 12 of these 19 participants. Data from nine participants were of non-immunoserological nature and were used for numerical analysis, whereas those of the remaining three participants were either of serological character or represented other data not easily coded for computer analysis. Three participants also submitted data on type or neotype strains of *M. smegmatis* (ATCC 14468), *M. phlei* (ATCC 19249), *M. chelonae* subsp. *abscessus* (ATCC 19977), *M. chelonae* subsp. *chelonae* (ATCC 19235), *M. fortuitum* (ATCC 6841) and "*M.*" *thamno* *phoe*s (ATCC 4445).

TABLE 1. List of mycobacteria strains^a

Strain	Species as identified by depositor	Laboratory no.	ATCC no.	Comment
01	<i>M. lactae</i>		25854	
02	<i>M. lactae</i>		25857	
03	<i>M. lactae</i>		25858	
04	<i>M. lactae</i>		25859	
05	<i>M. thermoresistibile</i>	M. Tsukamura 316	19527	Type strain
06	<i>M. thermoresistibile</i>	M. Tsukamura 1002	19528	
07	<i>M. thermoresistibile</i>	M. Tsukamura 1028	19529	
08	<i>M. thermoresistibile</i>	M. Tsukamura 1012	25812	
09	<i>M. thermoresistibile</i>	M. Tsukamura 1013	25813	
10	<i>M. thermoresistibile</i>	M. Tsukamura 1014	25814	
11	<i>M. thermoresistibile</i>	M. Tsukamura 1015	21815	
12	<i>M. thermoresistibile</i>	M. Tsukamura 1016	25816	
13	<i>M. flavescens</i>	L. F. Bojalil D-25	14474	Type strain
14	<i>M. flavescens</i>		23008	
15	<i>M. flavescens</i>		23033	
16	<i>M. flavescens</i>		23035	
17	<i>M. flavescens</i>		23039	
18	<i>M. chitae</i>	M. Tsukamura 2502	19627	Type strain
19	<i>M. chitae</i>	M. Tsukamura 2503	19628	
20	<i>M. chitae</i>	M. Tsukamura 2504	19629	
21	<i>M. chitae</i>	M. Tsukamura 2505	19630	
22	<i>M. chitae</i>	M. Tsukamura 31003	25804	
23	<i>M. chitae</i>	M. Tsukamura 31004	25805	
24	<i>M. chitae</i>	M. Tsukamura 31005	25086	
25	<i>M. vaccae</i>	R. Bönicke SN 920	15483	Type strain
26	<i>M. vaccae</i>		25949	
27	<i>M. vaccae</i>		25950	
28	<i>M. vaccae</i>		25951	
29	<i>M. vaccae</i>		25952	
30	<i>M. vaccae</i>		25953	
31	<i>M. vaccae</i>		25954	
32	<i>M. vaccae</i>		23014	
33	<i>M. vaccae</i>		23027	
34	<i>M. parafortuitum</i>	M. Tsukamura 2590	25808	
35	<i>M. parafortuitum</i>	M. Tsukamura 2593	25811	
36	<i>M. parafortuitum</i>	M. Tsukamura 2592	25810	
37	<i>M. parafortuitum</i>	M. Tsukamura 311	19686	Type strain
38	<i>M. parafortuitum</i>	M. Tsukamura 304	19688	
39	<i>M. parafortuitum</i>	M. Tsukamura 2591	25809	
40	<i>M. parafortuitum</i>	M. Tsukamura 305	25807	
41	<i>M. diernhoferi</i>	R. Bönicke SN 1418	19340	Type strain
42	<i>M. diernhoferi</i>	R. Bönicke SN 1419	19341	
43	<i>M. diernhoferi</i>	R. Bönicke SN 1425	19344	
44	<i>M. diernhoferi</i>		25958	
45	<i>M. diernhoferi</i>		25959	
46	<i>M. aurum</i>	M. Tsukamura 323	25792	
47	<i>M. aurum</i>	M. Tsukamura 3506	25797	
48	<i>M. aurum</i>	M. Tsukamura 340	25793	
49	<i>M. aurum</i>	M. Tsukamura 358	23366	Type strain
50	<i>M. aurum</i>	M. Tsukamura 351	26794	
51	<i>M. aurum</i>	M. Tsukamura 3503	25795	
52	<i>M. aurum</i>	M. Tsukamura 3505	25796	
53	<i>M. aurum</i>	M. Tsukamura 3509	25798	
54	<i>M. aurum</i>	M. Tsukamura 3511	25799	
55	<i>M. aurum</i>	M. Tsukamura 3512	25800	
56	<i>M. aurum</i>	M. Tsukamura 328	25791	
57	<i>M. aurum</i>	M. Tsukamura 309	25790	
58	<i>M. lactae</i>		25855	
59	<i>M. lactae</i>		25856	
60	<i>M. smegmatis</i>		14468	Suggested neotype
61	<i>M. chelonae</i> subsp. <i>abscessus</i>		19977	Type strain
62	<i>M. chelonae</i> subsp. <i>chelonae</i>		19235	
63	<i>M. fortuitum</i>		6841	Suggested neotype
64	" <i>M.</i> " <i>thamnopheos</i>		4445	Type strain
65	<i>M. phlei</i>		19249	

^a ATCC, American Type Culture Collection, Rockville, Md.; L. F. Bojalil, Facultad de Medicina, U.N.A.M., Mexico; R. Bönicke, Institut für experimentelle Biologie und Medizin, Borstel, Germany; M. Tsukamura, National Sanatorium, Chubu Chest Hospital, Obu, Aichi-ken, Japan.

FIG. 1. (a) Matching matrix of 59 strains of rapidly growing mycobacteria based on 177 pooled characters. (b) Evaluation of clusters from (a) at the 85% matching score levels.

(a) Laboratory A, 42 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1	85									
<i>M. thermo-resistibile</i>	2	85	95								
<i>M. flavesceus</i>	3	75	85	90							
<i>M. chitae</i>	4	70	75	70	95						
<i>M. vaccae</i>	5	-	-	-	70	85					
Kanazawa strains	6	85	75	75	75	-	90				
<i>M. parafortuitum-M. diernhoferi</i>	7a	-	-	-	80	75	65	80			
<i>M. aurum</i>	7b	-	-	-	70	75	70	75	85		
<i>M. neoaurum</i>	8	-	-	-	70	70	65	85	50	90	
<i>M. smegmatis</i>	9	-	-	-	-	70	-	70	65	70	85

(b) Laboratory B, 66 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1	95									
<i>M. thermo-resistibile</i>	2	70	95								
<i>M. flavesceus</i>	3	70	85	90							
<i>M. chitae</i>	4	-	65	75	95						
<i>M. vaccae</i>	5	-	70	70	65	90					
Kanazawa strains	6	-	75	85	85	70	95				
<i>M. parafortuitum-M. diernhoferi</i>	7a	-	70	80	85	75	85	90			
<i>M. aurum</i>	7b	-	65	75	75	70	80	80	90		
<i>M. neoaurum</i>	8	-	-	-	80	65	70	75	85	90	
<i>M. smegmatis</i>	9	-	-	-	70	-	70	70	70	85	90

(c) Laboratory C, 51 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1	95									
<i>M. thermo-resistibile</i>	2	85	95								
<i>M. flavesceus</i>	3	75	90	95							
<i>M. chitae</i>	4	75	85	80	100						
<i>M. vaccae</i>	5	-	70	80	75	95					
Kanazawa strains	6	75	75	80	85	75	100				
<i>M. parafortuitum-M. diernhoferi</i>	7a	65	70	80	80	80	85	90			
<i>M. aurum</i>	7b	-	65	75	70	85	65	80	90		
<i>M. neoaurum</i>	8	65	75	80	80	90	75	90	90	95	
<i>M. smegmatis</i>	9	-	-	-	65	70	-	65	75	75	90

(d) Laboratory D, 28 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1	90									
<i>M. thermo-resistibile</i>	2	80	90								
<i>M. flavesceus</i>	3	70	85	85							
<i>M. chitae</i>	4	-	65	75	95						
<i>M. vaccae</i>	5	-	-	-	65	90					
Kanazawa strains	6	75	75	80	80	-	95				
<i>M. parafortuitum-M. diernhoferi</i>	7a	-	65	75	90	75	75	90			
<i>M. aurum</i>	7b	-	-	75	75	70	-	80	85		
<i>M. neoaurum</i>	8	-	-	70	85	75	65	90	80	85	
<i>M. smegmatis</i>	9	-	-	-	-	75	-	-	-	70	100

(e) Laboratory E, 25 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1	95									
<i>M. thermo-resistibile</i>	2	-	100								
<i>M. flavesceus</i>	3	70	80	95							
<i>M. chitae</i>	4	80	75	80	95						
<i>M. vaccae</i>	5	-	65	75	65	95					
Kanazawa strains	6	80	65	75	80	65	95				
<i>M. parafortuitum-M. diernhoferi</i>	7a	-	-	-	-	-	-	80			
<i>M. aurum</i>	7b	-	-	-	-	80	-	65	90		
<i>M. neoaurum</i>	8	-	-	70	-	85	70	-	85	90	
<i>M. smegmatis</i>	9	-	-	65	75	65	65	65	-	-	95

(f) Laboratory F, 71 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1										
<i>M. thermo-resistibile</i>	2		90								
<i>M. flavesceus</i>	3		75	90							
<i>M. chitae</i>	4		75	80	95						
<i>M. vaccae</i>	5		-	75	80	90					
Kanazawa strains	6		75	90	85	80	95				
<i>M. parafortuitum-M. diernhoferi</i>	7a		65	80	80	85	80	90			
<i>M. aurum</i>	7b		-	75	65	80	75	80	90		
<i>M. neoaurum</i>	8		-	75	75	90	75	90	85	90	
<i>M. smegmatis</i>	9		65	65	65	75	70	70	75	70	80

FIG. 2(a)-(i). Analysis of characters by conventional $n \times n$ matrix table of matching scores.

In the analysis of data, characters in which all strains produced the same reaction, i.e., either all positive or all negative, were included for analysis of both the data from each laboratory as well as the pooled data. When the same test was performed by several researchers and a disagreement in test results was observed, the majority opinion was taken as the valid character. Where tests were performed

by only two individuals, and one reported it positive and one reported it negative, the result was recorded as positive. However, if the measurement of the same property was carried out under entirely different test conditions, both sets of results were retained in the event that a procedural difference might yield grossly different results. All data were converted to simply binary (i.e., 1 or 0) form for analysis. The 177

(g) Laboratory G, 22 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1	95									
<i>M. thermo-resistibile</i>	2	-	95								
<i>M. flavescens</i>	3	70	80	95							
<i>M. chitae</i>	4	65	65	85	95						
<i>M. vaccae</i>	5	-	-	-	75	95					
Kanazawa strains	6	70	70	90	95	70	90				
<i>M. parafortuitum-M. diernhoferi</i>	7a	-	-	80	95	80	90	95			
<i>M. aurum</i>	7b	-	-	80	85	70	80	90	90		
<i>M. neoaurum</i>	8	-	-	75	80	70	75	85	100	85	
<i>M. smegmatis</i>	9	-	-	-	-	80	-	65	65	65	90

(n) Laboratory N, 31 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1	95									
<i>M. thermo-resistibile</i>	2	75	95								
<i>M. flavescens</i>	3	65	85	85							
<i>M. chitae</i>	4	-	-	70	90						
<i>M. vaccae</i>	5	-	-	-	-	90					
Kanazawa strains	6	65	65	80	75	-	100				
<i>M. parafortuitum-M. diernhoferi</i>	7a	-	-	65	80	70	80	90			
<i>M. aurum</i>	7b	-	-	65	65	80	70	80	80		
<i>M. neoaurum</i>	8	-	-	-	70	75	-	80	75	90	
<i>M. smegmatis</i>	9	-	-	-	65	70	-	75	70	75	95

(i) Laboratory I, 71 characters

Cluster Name	#	1	2	3	4	5	6	7a	7b	8	9
<i>M. "lactae"</i>	1	95									
<i>M. thermo-resistibile</i>	2	70	95								
<i>M. flavescens</i>	3	70	85	90							
<i>M. chitae</i>	4	65	70	75	95						
<i>M. vaccae</i>	5	-	-	-	-	95					
Kanazawa strains	6	80	75	80	85	-	95				
<i>M. parafortuitum-M. diernhoferi</i>	7a	-	65	75	70	75	70	90			
<i>M. aurum</i>	7b	-	-	65	65	70	70	80	95		
<i>M. neoaurum</i>	8	-	-	70	70	65	70	85	85	90	
<i>M. smegmatis</i>	9	-	-	-	-	70	-	-	-	-	90

FIG. 2.(g)-(i)

characters obtained in this manner were analyzed by the conventional $n \times n$ matrix table of matching scores. To compare clusters obtained from the pooled data with those obtained from analysis of data from individual laboratories, the matrices in Fig. 2 were plotted with the cultures in the same sequence as that resulting from the analysis of the pooled data. After the analysis, selected properties which appeared useful in differential identification were recognized and tabulated. Results of serological data were compared with the numerical analyses.

The full list of characters employed, references to tests used, and the data obtained in the application of these tests have been lodged with the World Data Center for Microorganisms, Department of Microbiology, University of Queensland, Australia, and may be obtained from there or from the author, mailing charges being debited to the addressee.

RESULTS

Figure 1(a) shows the clusters obtained by analyzing the 177 characters generated from the pooled data. Figure 1(b) depicts the computer-generated clusters at 85% matching score level. As can be seen from Fig. 1(b) nine clus-

ters could be recognized at 85% matching score level or higher.

Cluster 1, with 95% internal similarity, contains four strains received as *M. "lactae."* As indicated below, there is no such species.

Cluster 2 contains eight strains having 95% internal similarity. One of these, ATCC 19527, is the type culture of *M. thermoresistibile*, and two additional strains (ATCC 19528 and ATCC 19529) also listed in the ATCC catalog under this name are indicated in this cluster.

Cluster 3 contains five strains having 90% internal similarity. One of these, ATCC 14474, is the type culture of *M. flavescens*.

Cluster 4 comprises seven strains having 95% internal similarity. ATCC 19627, which is the type culture of *M. chitae*, together with three additional strains (ATCC 19628, ATCC 19629, and ATCC 19630) which also bear this name are included.

Cluster 5 has 90% internal similarity and contains nine strains, including ATCC 15483, the type culture of *M. vaccae*.

Cluster 6 consists of three strains having 95%

TABLE 2. Distribution of characters with greatest resolving power of rapidly growing mycobacteria

Tests	No.	Distribution of characters in cluster no. and species:															
		1	2	3	4	5	6	7a		7b	8						
		<i>M.</i> "lactae"	<i>M.</i> <i>thermo-</i> <i>resis-</i> <i>tibile</i>	<i>M.</i> <i>fla-</i> <i>ves-</i> <i>cens</i>	<i>M.</i> <i>chi</i> <i>tae</i>	<i>M.</i> <i>vac</i> <i>cae</i>	Kan- azawa strains	<i>M.</i> <i>para-</i> <i>for-</i> <i>tuitum</i>	<i>M.</i> <i>diern-</i> <i>hoferi</i>	<i>M.</i> <i>aurum</i>	<i>M.</i> <i>neo-</i> <i>aurum</i>	<i>M.</i> <i>smegma-</i> <i>tis</i>	<i>M.</i> <i>chelo-</i> <i>nei</i> (<i>abs-</i> <i>cessus</i>)	<i>M.</i> <i>chelo-</i> <i>nei</i> (<i>chelo-</i> <i>nei</i>)	<i>M.</i> <i>for-</i> <i>tuitum</i>	<i>M.</i> <i>phlei</i>	" <i>M.</i> " <i>tham-</i> <i>nopheos</i>
Pigment																	
In dark	1	0	90	90	0	100	0	100	0	100	100	0	0	0	100	0	0
Photoactive, 1 h	2	0	0	0	0	100	0	25	0	0	0	0	0	0	0	0	0
Photoactive, 1 week	3	100	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0
Enzymatic activity																	
Acid phosphatase	4	100	0	10	100	0	100	0	100	0	0	0	100	100	100	100	0
Acid phosphatase, 70°C/30 min	5	25	0	0	50	0	30	0	0	0	0	0	50	100	0	0	0
Arylsulfatase, 3 day	6	25	0	0	0	0	0	0	0	0	10	100	100	100	0	0	0
Arylsulfatase, 2 week	7	100	0	80	100	100	100	100	100	100	60	100	100	100	50	0	0
Putrescine oxidase	8	0	0	0	100	60	100	100	100	100	100	100	100	100	100	100	0
Hippurate hydroly- sis	9	0	0	80	0	0	0	0	100	80	90	0	80	100	100	100	0
Nitrate reductase	10	100	100	100	100	70	100	100	100	40	80	100	0	17	100	100	0
Tween hydrolysis, 5 day	11	100	100	100	100	100	100	100	60	100	100	100	30	0	55	100	100
Acetamidase	12	0	0	0	100	100	0	100	100	20	100	100	70	80	100	0	100
Benzamidase	13	0	0	0	0	90	0	0	0	0	0	80	0	0	0	0	0
Urease	14	0	100	90	100	100	100	100	100	100	100	100	90	100	100	100	100
Isonicotinamidase	15	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	0
Nicotinamidase	16	0	100	80	100	100	100	90	90	100	100	100	60	100	30	100	100
Pyrazinamidase	17	0	100	90	100	95	100	90	100	100	100	100	90	50	40	100	100
Allantoinase	18	0	0	0	0	100	0	10	0	0	100	70	0	0	90	0	100
Succinamidase	19	0	0	0	0	100	0	0	0	20	0	100	0	0	0	0	0
Growth																	
At 42°C	20	0	100	60	0	90	0	100	0	0	0	100	0	0	100	100	0
At 45°C	21	0	100	0	0	0	0	0	0	0	0	100	0	0	0	100	0
At 52°C	22	0	100	0	0	0	0	0	0	0	0	0	0	0	0	100	0
After 60°C/4 h	23	0	100	0	0	0	0	0	0	0	0	0	0	0	0	100	0
On single carbon source																	
Benzoate	24	0	0	15	100	100	0	0	0	0	0	100	0	0	0	0	0
Fumarate	25	0	0	0	0	100	0	100	40	100	100	100	100	100	100	100	0

Succinate	26	0	0	90	0	100	0	100	100	100	100	100	100	100	100	100	100
Citrate	27	0	0	0	0	90	0	100	100	100	100	20	100	100	100	100	100
Malonate	28	0	0	0	0	90	0	75	0	20	50	100	0	0	0	100	0
Mucate (oxalate)	29	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
Propanol	30	0	0	40	100	0	100	75	0	50	90	100	10	0	100	100	0
Hippurate	31	0	0	25	100	90	100	25	0	0	0	100	0	0	0	0	0
On single nitrogen/ carbon source																	
Acetamide	32	0	0	0	90	0	0	0	0	0	75	100	0	0	100	100	0
Benzamide	33	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
Trimethylenediamine	34	0	0	0	0	100	100	100	100	100	100	100	100	0	100	100	100
In presence of:																	
Hydroxylamine, 250 μg	35	100	0	0	100	0	0	0	0	0	50	0	100	100	100	0	0
Azide, 20 μg	36	0	100	0	100	0	0	0	0	0	0	100	100	0	100	50	60
1% Deoxycholate	37	0	0	0	0	0	0	0	0	0	0	100	100	0	100	100	0
3% NaCl	38	0	100	100	100	50	100	100	0	40	100	100	100	100	100	100	100
0.1% NaNO ₂	39	0	100	60	100	100	100	100	100	100	100	0	0	0	0	0	0
MacConkey agar	40	0	0	0	0	0	0	0	0	0	0	0	100	100	100	0	0
Methylviolet	41	0	0	0	0	0	0	0	0	0	0	80	100	100	100	0	0
0.01% Pyronine	42	0	0	0	0	0	0	0	0	0	0	100	100	100	100	0	0
Degradation of:																	
p-Aminobenzoate	43	0	0	0	25	0	0	0	0	0	0	0	90	100	0	0	0
Salicylate	44	0	0	0	0	0	0	0	0	0	0	0	100	100	30	0	0
Acid production from:																	
Glucose	45	20	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Arabinose	46	0	0	0	0	90	0	80	100	100	80	100	0	0	0	100	0
Dulcitol	47	0	0	0	0	0	0	0	0	20	0	80	0	0	0	0	0
Fructose	48	20	100	75	100	100	100	100	100	100	100	100	0	16	100	100	100
Galactose	49	0	0	0	0	10	0	20	0	40	70	100	0	0	0	100	100
Inositol	50	0	0	0	100	100	0	75	100	100	100	100	0	0	11	0	0
Mannitol	51	0	20	60	100	100	0	100	100	100	100	100	0	0	11	100	100
Rhamnose	52	0	0	0	0	100	0	30	0	80	50	100	0	0	0	0	0
Sorbitol	53	0	0	60	0	100	0	75	0	80	10	100	0	0	0	100	100
Sucrose	54	0	0	0	0	100	0	75	0	80	0	0	0	0	0	0	100
Xylose	55	0	0	0	0	100	0	95	100	100	80	80	0	0	0	100	0
Trehalose	56	0	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Iron uptake	57	0	0	0	0	100	0	80	100	100	100	100	0	0	88	100	100

internal similarity. Although originally identified as *M. parafortuitum*, these three strains appear to be a new taxon which will be discussed later.

Cluster 7 contains two closely related subclusters. Subgroup 7a has 90% internal similarity and contains nine strains. Four strains, including the type culture ATCC 19686, bear the name of *M. parafortuitum*. Five strains, including type culture ATCC 19340, were earlier called *M. diernhoferi*. Subcluster 7b has 90% internal similarity and contains four strains. One of these, ATCC 23366, is the type culture of *M. aurum*.

Cluster 8 has 90% internal similarity and contains eight strains. One of these, ATCC 25795, is the type culture of *M. neoaurum*.

Cluster 9 has 90% internal similarity and contains two strains which were identical with the neotype strain of *M. smegmatis*. Both of these strains were earlier identified as *M. "lactae"*.

The number of characters derived from the tests employed at each of the participating laboratories ranged from 22 to 71. Figures 2(a) to (i) show for each individual laboratory the average internal similarity obtained for each group and also the intergroup similarities (16). The figures indicate matching scores to the lowest 5%. A dash in the square indicates that the matching score is less than 65%. As can be seen from this figure, the results from individual laboratories indicate general agreement with the master matrix in Fig. 1. However, as was observed in earlier cooperative studies, data from some individuals did not always segregate as many clusters as evolved from pooled data. With the exception of data from laboratories A, E, and H (see Fig. 2), all intracuster scores exceeded 85%. On the other hand, intercluster scores ranged from less than 65% to greater than 85% in most cases; occasionally greater than 90 or 95% intercluster values were indicated by some participants. Scores between subclusters 7a and 7b yielded 80% or less matching scores in eight laboratories and greater than 85% in only one laboratory. Six laboratories yielded greater than 85% matching scores between clusters 7b and 8, and six showed greater than 85% between clusters 7a and 8, thus indicating some individual problems in the taxonomic separation of the three epithets represented in these clusters.

Table 2 is a list of reactions of eight recognized clusters, excluding cluster 9, in the 57 test characters selected from the total of 177 as having greatest resolving power of these rapidly growing mycobacteria. The figures in the table indicate the percentage of strains exhibiting

TABLE 3. Distribution of mycobacteria strains by cytoplasmic antigen serogroup (laboratory J) and phenetic clustering behavior

Cluster no.	Species	Total no. strains	No. of strains falling into cytoplasmic antigen group no.:																					
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	T ₃	T ₅	T ₉	T ₁₀	T ₁₁
1	<i>M. "lactae"</i>	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
2	<i>M. thermoresistibile</i>	5	1	.	.	1	1	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.
3	<i>M. flavescens</i>	4	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	<i>M. chitae</i>	5	1	1	.	2	1	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	<i>M. vaccae</i>	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7a	<i>M. parafortuitum</i>	10	.	1	.	1	2	.	1	1	.	.	.	.	.	.	.	.	.	1	.	.	.	.
	<i>M. diernhoferi</i>		.	.	.	.	1	.	1	1	.	.	.	.	.	.	.	.	.	.	2	.	1	.
7b	<i>M. aurum</i>	2	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	<i>M. neoaurum</i>	3	2	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Totals		35	5 ^a	3	.	6	4	.	3	1	5	1	.	.	.	.	.	.	.	1	5	.	1	.

^a Dot indicates that none of the strains fell into the specific cytoplasmic antigen serogroup.

^b Tentatively classified into group 1.

each character. The characteristics of *M. smegmatis*, *M. phlei*, *M. fortuitum*, *M. chelonae*, and "*M.*" *thamnophaeus* were obtained chiefly from the paper reported by Kubica et al. (5). Cluster 6 contains three strains previously called *M. parafortuitum*. However, the organisms of this group could be distinctly separated from those of cluster 7a by their negative reactions in tests for acetamidase; utilization of succinate, citrate, and malonate; acid production from arabinose, inositol, mannitol, and xylose; and positive utilization of hippurate. Cluster 7 contains two groups of organisms, *M. parafortuitum* and *M. diernhoferi*. As listed in Table 2, these two are distinctly different in pigment production, acid phosphatase, hippurate hydrolysis, growth at 42°C, and growth on medium containing 3% sodium chloride. These characters may be sufficient to segregate these two as subspecies.

None of the isolates were found to be associated with human disease.

Cytoplasmic antigen serogrouping was performed by two laboratories. For these studies the soluble portions of mycobacterial lysates were tested by immunodiffusion with antisera selected from studies on antigenic profiles of all groups of mycobacteria (6, 8, 9). Table 3 shows the distribution of strains by cytoplasmic serogroup and phenetic behavior as determined in laboratory J. Six strains representing five species were present in one of these serogroups, IV, and five strains representative of three to four species appeared in serogroups I, IX, and T₉; there were only one to four cultures in the remaining seven serogroups. Thus, there was no localization of species into any one serogroup. Laboratory K, which also used mycobacterial cytoplasmic extracts in gel diffusion reaction against a battery of antisera, showed fairly good agreement with the phenetic clustering (Table 4). In this study antisera to *M. aurum* and *M. parafortuitum* were not used since previous studies had indicated their serological identity with *M. vaccae*. Thus strains of *M. aurum*, *M. parafortuitum*, and *M. vaccae* were grouped together. Four out of eight strains of *M. neoaurum* were identified with *Mycobacterium* species "Z"—a temporary taxon subsequently recognized as *M. neoaurum*. Except for one strain of *M. "lactae"* identified as *Mycobacterium* species "Z" because of a contaminated culture, the *M. "lactae"* strains could not be identified with any of the species to which antisera were employed. The identity of two other strains called *M. "lactae"* with *M. smegmatis* indicated by the numerical study was confirmed.

None of the laboratories carried out skin

TABLE 4. Distribution of strains by cytoplasmic antigen serogroup (laboratory K) and phenetic clustering behavior

Cluster no.	Species	No. of strains	No. of strains in cytoplasmic antigen serogroup of:												Mycobacterium sp. "Z"
			M. thermoresistibile	M. flavescens	M. chitae	M. vaccae	M. diernhoferi	M. smegmatis	M. phlei	M. ranee	M. chelonae	M. gilvum	M. duvalii	"M" thamnophaeos	
1	M. "lactae"	4													1
2	M. thermoresistibile	8	8												
3	M. flavescens	5		5											
4	M. chitae	7			7										
5	M. vaccae	9				9									
6	Kanazawa strains	3					1								
7a	M. parafortuitum-M. diernhoferi	9				3	5								
7b	M. aurum	4				4									
8	M. neoaurum	8													4
9	M. smegmatis	2							2						

tests, phage typing, lipid analysis, or mouse pathogenicity studies; hence it was not possible to obtain information on the correlation of such properties with computer-generated clusters.

DISCUSSION

The results of the present investigation corroborated the earlier recognition of species *M. flavescens* (1), *M. vaccae* (2), *M. thermoresistibile* (10), and *M. chitae* (11) as being distinct taxa.

The designation *M. "lactae"* has never been proposed as a new species; it has no taxonomic status. Of the six strains carrying this label included in the present study, two were identified as *M. smegmatis*. The others have properties very closely matching those of *M. terrae* (16) and are not rapid growers (Table 5). There appears to be no distinctive taxon corresponding to *M. "lactae."* *M. lactis* Chester 1903 is a validly published legitimate name, but the existence of any strains corresponding to Chester's description is unknown.

In the former cooperative study, Kubica et al. (5) suggested that strains containing representative cultures of both *M. vaccae* (2) and *M. parafortuitum* (14) were a homogeneous assembly. However, they suggested that further study was needed. The present examination of

both pooled and individual results (Fig. 1 and 2) showed a relatively low intertaxon matching score between the two taxa, supporting an earlier report (13) that *M. vaccae* and *M. parafortuitum* are distinct taxa.

A separate cluster, cluster 6, was generated from pooled and six of nine individual results from among strains bearing the name of *M. parafortuitum*. On the other hand, laboratories B, C, and G obtained relatively high intertaxon matching scores between 6 and 7a (see Fig. 2). These strains were isolated from soil and identified as *M. parafortuitum* by Kanazawa et al. (4), and are here referred to as "Kanazawa strains."

Both pooled data and those from six of nine individual laboratories indicated that cluster 7a was a homogeneous assembly of strains containing both *M. parafortuitum* and *M. diernhoferi* (3). However, there are several clearly distinctive characters, 1, 3, 4, 9, 20, and 38 (see Table 2), between *M. parafortuitum* and *M. diernhoferi*. A question of specific epithet arises since both species names appeared in the same year; however, because publication of *M. parafortuitum* was first, we suggest that 7a be referred to as *M. parafortuitum*. In the case of clusters 7a and 7b, fusion occurs at an intertaxon matching score of 85% (see Fig. 1).

Examination of pooled data (Fig. 1) showed cluster 8 to be a rather homogeneous assemblage of strains containing representative cultures of *M. neoaurum* (12). On the other hand, six of nine laboratories yielded greater than 85% matching scores between clusters 8 and 7b, and six showed greater than 85% between 8 and 7a (see Fig. 2).

Thus, there appears to be considerable intertaxon similarity in the one complex which contained *M. parafortuitum*, *M. diernhoferi*, *M. aurum*, *M. neoaurum*, and a cluster of Kanazawa strains. It is felt that such closely related, normally saprophytic, rapidly growing mycobacteria should not be segregated, but rather understood to be a complex, designated the "*M. parafortuitum* complex," until such time as more definitive identification is possible.

The immunodiffusion analyses carried out by laboratory K indicated that *M. vaccae* and at least some members of the *M. parafortuitum* complex, particularly those called *M. aurum*, belong to the same serospecies. On the other hand, *M. diernhoferi* was found to be a distinct serospecies from *M. vaccae* and the other members of the *M. parafortuitum* complex.

ACKNOWLEDGMENTS

We thank Erwin F. Lessel, formerly with the American Type Culture Collection, for preparation and distribution of

TABLE 5. Comparative reactions of *M. lactae* and *M. terrae*^a

Character	Positive (%)	
	<i>M. "lactae"</i>	<i>M. terrae</i>
Enzymatic activity		
Phosphatase	100	100
Tween hydrolysis, 5 day	100	100
α -Esterase	0	25
β -Esterase	50	16
Pyrazinamidase	0	0
Urease	0	16
Nicotinamidase	0	0
Catalase, 68°C	100	100
Catalase >45 mm foam	100	100
Nitrate reduction, high	100	100
Arylsulfatase, 3 day	25	0
Arylsulfatase, 10 day	100	58
Tellurite reduction	50	40
Growth		
At 25°C	100	100
At 42°C	0	0
Resistant to:		
NH ₂ OH-HCl, 250 μ g	100	100
Ethambutol, 1 μ g	0	0
Isoniazid, 1 μ g	100	100
Morphology, smooth colony type	0	33

^a The characteristics of *M. terrae* were obtained from the paper reported by Meissner et al. (7).

freeze-dried strains used in this study, and Masami Munaka, the Research Institute for Nuclear Medicine and Biology, Hiroshima University, Japan, for preparation and performance of the program for computer analyses.

REPRINT REQUESTS

Address reprint requests to: Dr. Hajime Saito, Department of Microbiology and Immunology, Shimane Medical College, 1134 Ohtsu-cho, Izumo 693, Shimane, Japan.

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