Numerical Analysis of Rapidly Growing, Scotochromogenic Mycobacteria, Including *Mycobacterium obuense* sp. nov., nom. rev., *Mycobacterium rhodesiae* sp. nov., nom. rev., *Mycobacterium aichiense* sp. nov., nom. rev., *Mycobacterium chubuense* sp. nov., nom. rev., and *Mycobacterium tokaiense* sp. nov., nom. rev.

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We performed numerical analyses of 155 strains of rapidly growing, scotochromogenic mycobacteria by two different methods; in one method we used 104 characters, and in the other we used 84 characters. The following taxa appeared as distinct clusters: *Mycobacterium thermoresistibile*, *Mycobacterium flavescens*, *Mycobacterium duvalii*, *Mycobacterium phlei*, "*Mycobacterium obuense*," *Mycobacterium parafortuitum*, *Mycobacterium vaccae*, *Mycobacterium sphagni*, "*Mycobacterium aichiense*," "*Mycobacterium rhodesiae*," *Mycobacterium neoaurum*, "*Mycobacterium chubuense*," "*Mycobacterium tokaiense*," and *Mycobacterium komossense* (names in quotation marks are not on the Approved Lists of Bacterial Names). *M. flavescens* strains were divided into two subgroups, one consisting of strains isolated in Japan and the other consisting of strains isolated in Rhodesia and strains received from the American Type Culture Collection, including the type strain of *M. flavescens* (ATCC 14474). We found that there are many species of rapidly growing, scotochromogenic mycobacteria, and we believe that new species should be recognized and named on the basis of at least three strains. The following species appeared to be distinct from all presently named species: "*Mycobacterium gallinarum*," "*Mycobacterium armenum*," "*Mycobacterium perpallidum*," and "*Mycobacterium taurus*." However, each of these species was proposed on the basis of only one or two strains. Because of the small number of strains studied, formal proposals for these species should not be made until more strains of each taxon are studied. Furthermore, we consider "*Mycobacterium magistrae*" and "*Mycobacterium auratum*" to be synonyms of *M. flavescens*, "*Mycobacterium pallidum*" to be a synonym of *M. parafortuitum*, "*Mycobacterium liquefactum*" to be a synonym of *M. vaccae*, and "*Mycobacterium sufflavum*" to be a synonym of *M. neoaurum*. "*M. obuense*," "*M. rhodesiae*," "*M. aichiense*," "*M. chubuense*," and "*M. tokaiense*" are not on the Approved Lists of Bacterial Names, but based on the results of this study, we consider these organisms distinct species. Therefore, these names are revived for the same organisms with which they were originally associated. The type strains of these species are strain 47001 (=ATCC 27023 =NCTC 10778), strain 02002 (=ATCC 27024 =NCTC 10779), strain 49005 (=ATCC 27280 =NCTC 10820), strain 48013 (=ATCC 27278 =NCTC 10819), and strain 47503 (=ATCC 27282 =NCTC 10821), respectively.

Mycobacterium flavescens Bojalil et al. (1) was proposed as a new species in 1962. Bojalil et al. also proposed *Mycobacterium acapulcensis* as a new species, but later Tsukamura (18) showed that this organism is identical to *M. flavescens*. At present, the name *M. flavescens* is used for this organism. Tsukamura et al. (33) proposed "*Mycobacterium gallinarum*" as a new species in 1967 (names in quotation marks

were published before 1 January 1980, were not included in the Approved Lists of Bacterial Names [9], and have not been validly published since 1 January 1980). This organism resembles *M. flavescens*, and this name was considered by Tsukamura (unpublished data) to be a synonym of *M. flavescens*. However, Lechevalier et al. (6) differentiated *M. flavescens* and "*M. gallinarum*" on the basis of their lipid compositions.

Tsukamura and Mizuno (26) reported that thin-layer chromatography of ethyl ether-ethanol cellular extracts after incubation with [^{35}S]methionine was useful in differentiating among mycobacterial species. When this technique was used with these organisms, they were classified into two subgroups; subgroup one consisted of "*M. gallinarum*" and some strains labeled *M. flavescens*, and the other consisted of the remaining strains labeled *M. flavescens* (28). Thus, *M. flavescens* was regarded as consisting of two subgroups.

The purpose of this study was to clarify the taxonomy of *M. flavescens* by using numerical analyses. Furthermore, we studied a number of strains recently received as members of new species, some of which are on the Approved Lists of Bacterial Names (9) and some of which are not, together with strains of older named species.

MATERIALS AND METHODS

Bacterial strains. We used 155 strains of rapidly growing, scotochromogenic mycobacteria in this study (Table 1).

Phenotypic characters. For each strain, we examined 104 characters (Table 2). We tested several strains in duplicate to confirm the reliability of the tests; however, only one set of data was used for each of these strains (Table 3). The methods which we used have been described previously (15, 17, 21).

Media. We used Ogawa egg medium and a modified Sauton agar medium as basal media. The composition of the Ogawa egg medium was as follows: basal solution (1% KH_2PO_4 and 1% sodium glutamate), 100 ml; whole eggs, 200 ml; glycerol, 6 ml; and a 2% aqueous solution of malachite green, 6 ml. This medium was poured in 7-ml quantities into tubes (165 by 16.5 mm), and after sterilization at 90°C for 60 min, the medium was made into slants. The composition of the modified Sauton agar was as follows: glycerol, 30 ml; KH_2PO_4 , 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; citric acid, 2.0 g; ferric ammonium citrate, 0.05 g; sodium glutamate, 4.0 g; purified agar, 20.0 g; and distilled water, 970 ml. Before sterilization, the pH of this medium was adjusted to 7.0 by adding 10% KOH; 7 ml of medium was poured into each tube, which was slanted after sterilization of the medium at 120°C for 20 min. Unless noted otherwise, the tubes were observed for growth after incubation at 37°C for 14 days. Acid formation from carbohydrates was determined after incubation at 28°C for 14 days.

Numerical classification. Matching coefficients were calculated as described previously (30). Clustering was done by the single-linkage method (10). The computer used was an IBM S-370 (model 135), which is located in the Nagoya Factory of the Sumitomo Machinery Co., Obu, Aichi, Japan. The data for the original "strain x characters" matrix have been deposited with the World Data Center for Microorganisms, Brisbane, Australia.

Of the 104 phenotypic characters examined, 93 were used to differentiate among the test strains. The following 11 characters were ineffective (i.e., all strains

gave the same result) for differentiating among the test strains: strong acid fastness; weak or partial acid fastness; glucose as C source (glutamate N); acetate as C source (glutamate N); succinate as C source (glutamate N); pyruvate as C source (glutamate N); permanent mycelium; fragmenting mycelium; cord formation; production of niacin; and benzamide as simultaneous N and C sources. The first six of these characters were positive in all test strains, and the remaining five were negative in all test strains.

We used two systems of numerical classification; in one, we used all 104 characters, and in the other we used 84 characters. The 20 characters omitted in the 84-character system were all of the ineffective characters described above except niacin production and utilization of benzamide as simultaneous N and C sources, four characters for which almost all of the strains showed positive results (growth on 1 μg of thiophene-2-carboxylic acid hydrazide per ml, growth on 0.5 mg of salicylate per ml, growth on 1% Tween 80, and growth on 0.5 mg of *p*-nitrobenzoic acid per ml), three related characters (24) (growth on 1% picric acid, growth on 0.2% NaNO_2 , and nitrate reduction at 6 h), and four tests whose results were regarded as not easily reproducible because of difficulties in reading the results (cross-barring, acetamide as N source, urea as N source, and pyrazinamide as N source). The two ineffective characters which were retained were useful in differentiating the strains of rapidly growing, non-photochromogenic mycobacteria which were included in a subsequent study.

RESULTS

Almost identical results were obtained with the two numerical classification systems used. Therefore, only the data from the 84-character method are shown in Fig. 1 to 3. Nine strains were tested in duplicate. Of these, seven had 95 to 99% matching coefficients, thus showing the reproducibility of the test results. Two strain pairs (strains 57 and 58 and strains 95 and 96) showed 92 or 93% similarity in both systems.

Numerical classification with 104 characters. At a similarity level of 91%, we differentiated 14 clusters, containing the following species: (i) *Mycobacterium thermoresistibile*; (ii) *Mycobacterium duvalii*, *M. flavescens*, and two strains received as "*Mycobacterium magistrael*" and "*Mycobacterium auratum*;" (iii) *Mycobacterium phlei*; (iv) "*Mycobacterium obuense*" (except one strain); (v) *Mycobacterium parafortuitum*; (vi) *Mycobacterium vaccae*; (vii) *Mycobacterium sphagni* (4) (received as Sph group); (viii) "*Mycobacterium aichiense*;" (ix) "*Mycobacterium rhodesiae*;" (x) *Mycobacterium neoaurum*; (xi) *Mycobacterium aurum* (only one strain tested); (xii) "*Mycobacterium chubuense*;" (xiii) "*Mycobacterium tokaiense*;" and (xiv) *Mycobacterium komossense* (5) (received as MA group).

M. flavescens and *M. duvalii* could be sep-

TABLE 1. *Strains used in this study*

Strain no. ^a	Laboratory no.	Received as:	Source ^b	Origin	
				Habitat	Country
1	01002	<i>M. thermoresistibile</i> ATCC 19528	ATCC	Soil	
2	01039	<i>M. thermoresistibile</i> ATCC 19527 (type)	ATCC	Soil	
3	01022	<i>M. thermoresistibile</i>	MT	Soil	
4	01023	<i>M. thermoresistibile</i>	MT	Soil	
5	01024	<i>M. thermoresistibile</i>	MT	Soil	
6	01025	<i>M. thermoresistibile</i>	MT	Soil	
7	01027	<i>M. thermoresistibile</i>	MT	Soil	
8	01028	<i>M. thermoresistibile</i>	MT	Soil	
9	01030	<i>M. thermoresistibile</i>	MT	Soil	
10	01031	<i>M. thermoresistibile</i>	MT	Soil	
11	01026	<i>M. thermoresistibile</i>	MT	Soil	
12	01029	<i>M. thermoresistibile</i>	MT	Soil	
13	01038	<i>M. thermoresistibile</i>	MT	Soil	
14	01004	<i>M. thermoresistibile</i>	MT	Soil	
15	32001	" <i>M. gallinarum</i> " ATCC 19710 (type)	ATCC	Chicken organs	
16	33002	<i>M. flavescens</i> E3362	MT	Sputum, humans	Japan
17	33003	<i>M. flavescens</i> E3363	MT	Sputum, humans	Japan
18	33004	<i>M. flavescens</i> E3364	MT	Sputum, humans	Japan
19	33005	<i>M. flavescens</i> E3365	MT	Sputum, humans	Japan
20	33007	<i>M. flavescens</i> E4292	MT	Sputum, humans	Japan
21	33008	<i>M. flavescens</i> E4377	MT	Sputum, humans	Japan
22	33015	<i>M. flavescens</i> E5644	MT	Sputum, humans	Rhodesia
23	E2616	<i>M. flavescens</i>	MT	Sputum, humans	Japan
24	33006	<i>M. flavescens</i> E3366	MT	Sputum, humans	Japan
25	33012	<i>M. flavescens</i> E5399	MT	Sputum, humans	Rhodesia
26	33014	<i>M. flavescens</i> E5378	MT	Sputum, humans	Rhodesia
27	33016	<i>M. flavescens</i> E5758	MT	Sputum, humans	Rhodesia
28	33018	<i>M. flavescens</i> E7040	MT	Sputum, humans	Rhodesia
29	33019	<i>M. flavescens</i> E7044	MT	Sputum, humans	Rhodesia
30	33020	<i>M. flavescens</i> E7045	MT	Sputum, humans	Rhodesia
31	33024	<i>M. flavescens</i> ATCC 23055	ATCC	Unknown	
32	E8382	<i>M. flavescens</i>	MT	Sputum, humans	Rhodesia
33	33009	<i>M. flavescens</i> E4485	MT	Sputum, humans	Rhodesia
34	33031	<i>M. flavescens</i> ATCC 23025	ATCC	Unknown	
35	33023	<i>M. flavescens</i> ATCC 23033	ATCC	Unknown	
36	33028	<i>M. flavescens</i> ATCC 14474 (type)	ATCC	Guinea pigs	
37	33029	<i>M. flavescens</i> E5575	MT	Sputum, humans	Rhodesia
38	33021	<i>M. flavescens</i> ATCC 23008	ATCC	Unknown	
39	33030	<i>M. flavescens</i> E5578	MT	Unknown	
40	33010	<i>M. flavescens</i> E5383	MT	Sputum, humans	Rhodesia
41	33011	<i>M. flavescens</i> E5384	MT	Sputum, humans	Rhodesia
42	33025	<i>M. flavescens</i> ATCC 23039	ATCC	Unknown	
43	E8479	<i>M. flavescens</i>	MT	Sputum, humans	Rhodesia
44	33032	<i>M. flavescens</i> E5580	MT	Sputum, humans	Rhodesia
45	33033	<i>M. flavescens</i> E7713	MT	Sputum, humans	Japan
46	33026	<i>M. flavescens</i> ATCC 23046	ATCC	Unknown	
47	33022	<i>M. flavescens</i> ATCC 23009	ATCC	Unknown	
48	E7479	<i>M. flavescens</i>	MT	Sputum, humans	Rhodesia
49	E8246	" <i>M. magistrae</i> " INHEM 2231B (type)	JAV	Bovine organs	
50	E8247	" <i>M. auratum</i> " INHEM 2474 (type)	JAV	Bovine organs	
51	E8557	<i>M. duvalii</i> NCTC 358 (type)	JLS	Skin lesion, humans	
52	E8558	<i>M. duvalii</i> NCTC 509	JLS	Skin lesion, humans	
53	E8559	<i>M. duvalii</i> NCTC 514	JLS	Skin lesion, humans	
54	E8560	<i>M. duvalii</i> NCTC 8645	JLS	Skin lesion, humans	
55	33013	<i>M. flavescens</i> E5402	MT	Sputum, humans	Rhodesia
56	03001	<i>M. gadium</i> 1066 (ATCC 27726) (type)	MC	Sputum, humans	Spain
57	44011	Duval Lepra bacillus ATCC 4243	ATCC	Skin lesions, humans	
58	44011B ^c	Duval Lepra bacillus ATCC 4243	ATCC	Skin lesions, humans	
59	E8244	" <i>M. armentum</i> " INHEM 2229 (type)	JAV	Bovine organs	
60	E8441		MT	Sputum, humans	Japan
61	14004a	<i>M. phlei</i> SN104	RB	Unknown	
62	14004b	<i>M. phlei</i> SN104	RB	Unknown	
63	14002a	<i>M. phlei</i> SN102	RB	Unknown	
64	14002b	<i>M. phlei</i> SN102	RB	Unknown	
65	14003a	<i>M. phlei</i> SN103	RB	Unknown	
66	14003b	<i>M. phlei</i> SN103	RB	Unknown	
67	14022	<i>M. phlei</i> ATCC 19249	ATCC	Unknown	

TABLE 1—Continued

Strain no. ^a	Laboratory no.	Received as:	Source ^b	Origin	
				Habitat	Country
68	14001	<i>M. phlei</i> SN101	RB	Unknown	
69	14026	<i>M. phlei</i> E8369	MT	Sputum, humans	Japan
70	E9079	<i>M. phlei</i>	MT	Sputum, humans	Rhodesia
71	E9084	<i>M. phlei</i>	MT	Sputum, humans	Rhodesia
72	E9086	<i>M. phlei</i>	MT	Sputum, humans	Rhodesia
73	E8369	<i>M. phlei</i>	MT	Sputum, humans	Japan
74	47001	" <i>M. obuense</i> " ATCC 27023 (NCTC 10778)(type)	ATCC	Soil	
75	47002	" <i>M. obuense</i> " E5433	MT	Soil	
76	47004	" <i>M. obuense</i> " E5436	MT	Soil	
77	47005	" <i>M. obuense</i> " E5437	MT	Soil	
78	47008	" <i>M. obuense</i> " E5843	MT	Sputum, humans	Japan
79	16001a	<i>M. parafortuitum</i> ATCC 19687(NCTC 10410)	ATCC	Soil	
80	16003a	<i>M. parafortuitum</i> ATCC 19688	ATCC	Soil	
81	16003b	<i>M. parafortuitum</i> ATCC 19688	ATCC	Soil	
82	16004a	<i>M. parafortuitum</i> E305	MT	Soil	
83	16004b	<i>M. parafortuitum</i> E305	MT	Soil	
84	16001b	<i>M. parafortuitum</i> ATCC 19687	ATCC	Soil	
85	16002	<i>M. parafortuitum</i> ATCC 19686 (NCTC 10411)(type)	ATCC	Soil	
86	16002B ^c	<i>M. parafortuitum</i> ATCC 19686	ATCC	Soil	
87	15005	<i>M. parafortuitum</i> E352	MT	Soil	
88	E8240	" <i>M. pallidum</i> " INHEM 1670 (type)	JAV	Bovine organs	
89	E8241	" <i>M. perpallidum</i> " INHEM 2105A (type)	JAV	Bovine organs	
90	16039	<i>M. vaccae</i> (?) B11-25-LD	KS	Bovine thelitis	
91	16040	<i>M. vaccae</i> (?) B11-25-OD	KS	Bovine thelitis	
92	E8243	" <i>M. liquefactum</i> " INHEM 2203 (type)	JAV	Bovine organs	
93	21008	<i>M. vaccae</i> ATCC 23002	ATCC	Unknown	
94	21011	<i>M. vaccae</i> ATCC 23014	ATCC	Unknown	
95	21007	<i>M. vaccae</i> ATCC 15483 (type)	ATCC	Environment of cows	
96	21007B ^c	<i>M. vaccae</i> ATCC 15483 (type)	ATCC	Environment of cows	
97	E8760	<i>M. sphagni</i> Sph 16	JK	Sphagnum vegetation	
98	E8761	<i>M. sphagni</i> Sph 38 (ATCC 33027)(type)	JK	Sphagnum vegetation	
99	E8762	<i>M. sphagni</i> Sph 43	JK	Sphagnum vegetation	
100	49001	" <i>M. aichiense</i> " E5540	MT	Soil	
101	49003	" <i>M. aichiense</i> " E5543	MT	Soil	
102	49004	" <i>M. aichiense</i> " ATCC 27281	ATCC	Soil	
103	49005	" <i>M. aichiense</i> " ATCC 29280 (type)	ATCC	Soil	
104	49008	" <i>M. aichiense</i> " E5555	MT	Soil	
105	02001	" <i>M. rhodesiae</i> " E5294	MT	Sputum, humans	Rhodesia
106	02002	" <i>M. rhodesiae</i> " ATCC 27024 (type)	ATCC	Sputum, humans	Rhodesia
107	02003	" <i>M. rhodesiae</i> " E5296	MT	Sputum, humans	Rhodesia
108	02004	" <i>M. rhodesiae</i> " E5297	MT	Sputum, humans	Rhodesia
109	02005	" <i>M. rhodesiae</i> " E5298	MT	Sputum, humans	Rhodesia
110	E5795	" <i>M. rhodesiae</i> "	MT	Sputum, humans	Rhodesia
111	10004	<i>M. neoaurum</i> ATCC 25798	ATCC	Soil	
112	10001	<i>M. neoaurum</i> ATCC 25791	ATCC	Soil	
113	10005	<i>M. neoaurum</i> ATCC 25799	ATCC	Soil	
114	10002	<i>M. neoaurum</i> ATCC 25795(NCTC 10818)(type)	ATCC	Soil	
115	10003	<i>M. neoaurum</i> ATCC 25796	ATCC	Soil	
116	15035	<i>M. neoaurum</i> ATCC 25803	ATCC	Soil	
117	E8245	" <i>M. sufflavum</i> " INHEM 2231A (type)	JAV	Bovine organs	
118	15001	<i>M. aurum</i> NCTC 10438	NCTC	Soil	
119	48002	" <i>M. chubuense</i> " E5501	MT	Soil	
120	48013	" <i>M. chubuense</i> " ATCC 27278(NCTC 10819)(type)	ATCC	Soil	
121	48012	" <i>M. chubuense</i> " ATCC 27279	ATCC	Soil	
122	48001	" <i>M. chubuense</i> " E5499	MT	Soil	
123	48004	" <i>M. chubuense</i> " E5505	MT	Soil	
124	15006	<i>M. aurum</i> ATCC 23366(NCTC 10437)(type)	ATCC	Soil	
125	E9089		MT	Sputum, humans	Rhodesia
126	E8242	" <i>M. taurus</i> " INHEM 2129A (type)	JAV	Bovine organs	
127	E8114	" <i>M. valentiae</i> " type	JFG	Unknown	
128	E8069	Boisvert 5775	AHB	Unknown	
129	47501	" <i>M. tokaiense</i> " E5548	MT	Soil	

TABLE 1—Continued

Strain no. ^a	Laboratory no.	Received as:	Source ^b	Origin	
				Habitat	Country
130	47502	" <i>M. tokaiense</i> " E5548	MT	Soil	
131	47503	" <i>M. tokaiense</i> " ATCC 27282(NCTC 10821) (type)	ATCC	Soil	
132	E8554	<i>M. gilvum</i> Stanford 132	JLS	Unknown	
133	E8555	<i>M. gilvum</i> Stanford 391	JLS	Unknown	
134	E6194	<i>M. gilvum</i> NCTC 10742 (type)	JLS	Sputum, humans	
135	E9061-1	Rfb	JV	Soil	
136	E9061-2	Rfb	JV	Soil	
137	E9063-1	Rfb 10	JV	Soil	
138	E9063-2	Rfb 10	JV	Soil	
139	E9063-3	Rfb 10	JV	Soil	
140	E9063-4	Rfb 10	JV	Soil	
141	E9062-1	Rfb 9	JV	Soil	
142	E9062-2	Rfb 9	JV	Soil	
143	E9062-3	Rfb 9	JV	Soil	
144	E9062-4	Rfb 9	JV	Soil	
145	44005	<i>Mycobacterium</i> sp. E6594	MT	Sputum, humans	Japan
146	E8360	<i>M. fortuitum</i>	MT	Sputum, humans	Japan
147	E8405	<i>M. fortuitum</i>	MT	Sputum, humans	Japan
148	E8423	<i>M. fortuitum</i>	MT	Sputum, humans	Japan
149	E8068	<i>Mycobacterium</i> sp.	MT	Sputum, humans	Japan
150	E8198	<i>Mycobacterium</i> sp.	MT	Sputum, humans	Japan
151	E8757	<i>Mycobacterium</i> sp. MA 2 (ATCC 33013) (type)	JK	Sphagnum vegetation	
152	E8758	<i>Mycobacterium</i> sp. MA 9	JK	Sphagnum vegetation	
153	E8758	<i>Mycobacterium</i> sp. MA 11	JK	Sphagnum vegetation	
154	E9132	<i>Mycobacterium intracellulare</i> (?)	MT	Sputum, humans	Japan
155	E9080	<i>Mycobacterium</i> sp.	MT	Sputum, humans	Rhodesia

^a Strains 135 to 144 were isolated by a single colony isolation technique from strains Rfb, Rfb9, and Rfb 10, which were isolated by J. Viallier from the soil around plant roots.

^b ATCC, American Type Culture Collection, Rockville, Md; MT, M. Tsukamura, National Chubu Hospital, Obu, Aichi, Japan; JAV, J.A. Valdivia Alvarez, Instituto Nacional de Hygiene, Epidemiologia y Microbiologia, Habana, Cuba; JLS, J. L. Stanford, Middlesex Hospital Medical School, London, England; MC, M. Casal, Universidad Autonoma de Madrid, Madrid, Spain; RB, R. Böncke, Forschungsinstitut Borstel, Borstel, West Germany; KS, K. Shimizu, Obihiro Veterinary College, Obihiro, Japan; JK, J. Kazda, Forschungsinstitut Borstel, Borstel, West Germany; NCTC, National Collection of Type Cultures, London, England; JFG, J. F. Garcia Sabater, Hospital Militar, Valencia, Spain; AHB, A. H. Boisvert, Institut Pasteur, Paris, France; JV, J. Viallier, Hopital Jules Courmont, Pierre-Benite, France.

^c All tests for these strains were performed at 28°C.

arated at a level of 92%. The type strain of "*M. gallinarum*" (strain 15) did not fall into any of the above-mentioned clusters. The strains of *M. flavescens* were divided into two subclusters at a similarity level of 94%. One subcluster (FLA1) consisted mostly of isolates from Rhodesia and strains received from the American Type Culture Collection; the other subcluster (FLA2) consisted of isolates from Japan.

Numerical classification with 84 characters. At a 91% level of similarity, strains of the following 12 species formed distinct clusters: *M. flavescens*, *M. duvalii*, *M. parafortuitum*, "*M. rhodesiae*," "*M. aichiense*," "*M. chubuense*," *M. aurum* (only two strains were studied, and the type strain [strain 124] was distinct at the 91% level), *M. sphagni*, "*M. tokaiense*," *M. komosense*, "*M. obuense*," and *M. thermoresistibile* (Fig. 1 through 3). At this level of similarity, some strains of the following species also formed clusters: *M. vaccae*, *M. neoaurum*, and *M. phlei*. All of the *M. vaccae* and *M. phlei* strains clus-

tered at a level of 90%. *Mycobacterium gilvum* strains 132 through 134 clustered at a level of 89%, and *M. neoaurum* strains clustered at a level of 88%. One *M. aurum* strain (strain 118) was incorporated into the *M. neoaurum* cluster, suggesting that this strain was misidentified. The clusters obtained with the two systems were the same. At the 94% level, two subclusters of *M. flavescens* were evident. The single "*M. gallinarum*" strain also appeared to be distinct in this system.

The following strains appeared to be distinct taxa at similarity levels of less than 90% in both systems: *Mycobacterium gadium* (strain 56) and a strain received as a *Lepra bacillus* (strain 57); "*Mycobacterium armentum*" (strain 59); "*Mycobacterium perpallidum*" (strain 69); laboratory strain E9089 (strain 125); "*Mycobacterium taurus*" (strain 126); "*Mycobacterium valentiae*" (strain 127); laboratory strain E8069 (strain 128); laboratory strains E9061-1 and E9061-2 (strains 135 and 136); laboratory strains E9063-

TABLE 2. Characteristics of type strains of *M. obuense* sp. nov., nom. rev., *M. rhodesiae* sp. nov., nom. rev., *M. aichiense* sp. nov., nom. rev., *M. chubuense* sp. nov., nom. rev., and *M. tokaiense* sp. nov., nom. rev.

Character ^a	<i>M. obuense</i> ATCC 27023	<i>M. rhodesiae</i> ATCC 27024	<i>M. aichiense</i> ATCC 27280	<i>M. chubuense</i> ATCC 27278	<i>M. tokaiense</i> ATCC 27282
Strong acid fastness	+	+	+	+	+
Weak or partial acid fastness	+	+	+	+	+
Permanent mycelium	—	—	—	—	—
Fragmenting mycelium	—	—	—	—	—
Long rods (more than 7 µm long)	—	—	—	—	+
Intermediate rods (3–6 µm long)	+	—	—	—	+
Short rods (less than 2 µm long)	+	+	+	— ^b	+
Cross-barring	—	—	—	—	+
Cord formation	—	—	—	—	—
Rough colonies	—	—	—	—	—
Colony pigmentation in the dark	+	+	+	+	+
Photochromogenicity	—	—	—	—	—
Growth after 3 days	+	+	+	+	+
Growth at 28°C	+	+	+	+	+
Growth at 37°C	+	+	+	+	+
Growth at 45°C	—	—	—	—	—
Growth at 52°C	—	—	—	—	—
Growth on 0.2% <i>p</i> -aminosalicylate ^c	+	+	+	+	+
Degradation of <i>p</i> -aminosalicylate	+	—	—	—	—
Growth on NH ₂ OH·HCl (0.125 mg/ml) ^c	—	+	+	+	+
Growth on NH ₂ OH·HCl (0.25 mg/ml) ^c	—	—	—	—	—
Growth on NH ₂ OH·HCl (0.5 mg/ml) ^c	—	—	—	—	—
Growth on Sauton agar	+	+	+	+	+
Growth on 0.1% salicylate ^d	+	—	+	+	+
Degradation of salicylate	+	—	—	—	—
Growth on 0.1% picric acid ^d	+	+	+	+	+
Growth on 0.2% picric acid ^d	+	+	+	+	+
Three-day arylsulfatase	+	+	+	—	+
Two-week arylsulfatase	+	+	+	+	+
Growth on thiophene-2-carboxylic acid hydrazide (1 µg/ml) ^c	+	+	+	+	+
Growth on salicylate (0.5 mg/ml) ^c	+	+	+	+	+
Resistance to ethambutol (5 µg/ml) ^c	+	—	—	—	—
Growth on 0.1% NaNO ₂ ^d	+	+	+	+	+
Growth on 0.2% NaNO ₂ ^d	+	+	+	+	+
Growth on 1% Tween 80 ^d	+	+	+	+	+
Growth on <i>p</i> -nitrobenzoic acid (0.5 mg/ml) ^c	+	+	+	+	+
Resistance to rifampin (25 µg/ml) ^c	+	+	+	+	+
Niacin production	—	—	—	—	—
Tween hydrolysis (7 days)	—	+	—	—	—
Tween hydrolysis (14 days)	+	+	+	+	+
Catalase (semiquantitative; foam, >45 mm)	—	+	—	—	—
α-Esterase	+	—	—	—	+
β-Esterase	+	—	—	+	+
β-Galactosidase	+	—	+	+	+
Acid phosphatase (3-h method)	—	+	+	—	—
Nitrate reduction (6 h)	—	—	—	+	—
Nitrate reduction (24 h)	—	—	—	+	—
Acetamidase	—	—	—	—	+
Benzamidase	—	—	—	—	+
Urease	+	+	+	+	+
Isonicotinamidase	—	—	—	—	+
Nicotinamidase	+	—	—	+	+
Pyrazinamidase	+	+	—	+	+
Salicylamidase	—	—	—	—	—
Allantoinase	+	—	—	—	+
Succinamidase	—	—	—	—	+
Glutamate as N and C sources	+	+	+	+	+
Serine as N and C sources	—	—	—	—	—
Glucosamine as N and C sources	+	+	+	+	+
Acetamide as N and C sources	—	—	—	—	—
Benzamide as N and C sources	—	—	—	—	—
Monoethanolamine as N and C sources	—	—	—	—	—
Trimethylene diamine as N and C sources	—	+	—	+	+
Glucose as C source (glutamate N)	+	+	+	+	+

TABLE 2—Continued

Character ^a	<i>M. obuense</i> ATCC 27023	<i>M. rhodesiae</i> ATCC 27024	<i>M. ai-chiense</i> ATCC 27280	<i>M. chubuense</i> ATCC 27278	<i>M. tokaiense</i> ATCC 27282
Acetate as C source (glutamate N)	+	+	+	+	+
Succinate as C source (glutamate N)	+	+	+	+	+
Pyruvate as C source (glutamate N)	+	+	+	+	+
Acetate as C source	+	+	+	+	+
Citrate as C source	—	—	—	—	—
Succinate as C source	+	+	+	+	+
Malate as C source	+	+	+	+	+
Pyruvate as C source	+	+	+	+	+
Benzoate as C source	—	—	—	—	—
Malonate as C source	—	—	—	—	—
Fumarate as C source	+	+	+	+	+
Glucose as C source	+	+	+	+	+
Fructose as C source	+	+	+	+	+
Sucrose as C source	+	—	—	+	+
Ethanol as C source	+	+	+	+	+
<i>n</i> -Propanol as C source	+	+	+	+	+
Propylene glycol as C source	+	+	+	+	+
1,3-Butylene glycol as C source	—	+	—	—	—
1,4-Butylene glycol as C source	—	—	—	—	—
2,3-Butylene glycol as C source	+	+	—	—	—
<i>n</i> -Butanol as C source	+	+	+	—	—
Isobutanol as C source	+	+	+	+	—
Acid from glucose	+	+	+	+	+
Acid from mannose	+	+	+	+	+
D-Mannose as C source	+	+	+	+	+
D-Galactose as C source	—	—	—	—	—
L-Arabinose as C source	+	—	—	—	+
D-Xylose as C source	—	+	—	+	+
L-Rhamnose as C source	—	—	—	+	+
Trehalose as C source	+	—	+	—	+
Inositol as C source	—	+	+	—	+
Mannitol as C source	+	+	+	—	+
Sorbitol as C source	+	—	—	—	+
Acetamide as N source	—	+	—	+	—
Benzamide as N source	—	—	—	—	—
Urea as N source	—	+	+	+	+
Pyrazinamide as N source	—	+	+	+	+
Nicotinamide as N source	—	+	—	+	—
Nitrate as N source	—	—	—	+	—
Nitrite as N source	—	—	—	—	—

^a Unless specifically noted, the utilization of a compound as a C source was tested in the presence of ammoniacal nitrogen, and the utilization of a compound as an N source was tested in the presence of glycerol as a carbon source.

^b Only coccoids.

^c Tested in Ogawa egg medium.

^d Tested in Sauton agar.

1 and E9063-2 (strains 137 and 138); laboratory strain E9063-3 (strain 139); laboratory strain E9063-4 (strain 140); laboratory strains E9062-1 and E9062-2 (strains 141 and 142); laboratory strains E9062-3 and E9062-4 (strains 143 and 144); laboratory strain 44005 (strain 145); laboratory strain E8068 (strain 149); laboratory strain E8198 (strain 150); and laboratory strain E9080 (strain 155).

Distinguishing characters of the clusters.

The characters which were useful for distinguishing among the clusters detected in this study are shown in Table 3. The characters which are common to all rapidly growing, scotochromogenic mycobacteria are as follows: acid fastness; mycelium not formed; growth after 3

days on egg media; growth at 28 and 37°C; growth on Ogawa egg medium containing 0.5 mg of *p*-nitrobenzoic acid per ml, 0.5 mg of sodium salicylate per ml, 0.2% sodium *p*-aminosalicylate, or 25 µg of rifampin per ml; growth on Sauton agar media containing 0.2% picric acid (pH 7.0), 0.1% sodium nitrite, or 1% Tween 80; urease production; salicylamidase not produced; glutamate, but not serine or benzamide, used as simultaneous N and C sources; glucose used as C source; and benzoate and benzamide not used as sources of C and N, respectively.

DISCUSSION

Our two different systems of numerical classification gave nearly identical results for the

FIG. 1. Dendrogram, 84-character method: part 1. FLA1, *M. flavescens* subcluster FLA1; FLA2, *M. flavescens* subcluster FLA2; DUV, *M. duvalii*; VAC, *M. vaccae*; PAR, *M. parafortuitum*; M value, matching coefficient. Numbers on the right are strain numbers (see Table 1).

mycobacterial strains studied. The difference between the two systems was that the clusters of *M. flavescens* and *M. neoaurum* strains were more compact when the 84-character method was used. Furthermore, with the 104-character method, strain 49 of "*M. magistrael*" and strain 50 of "*M. auratum*" appeared to be intermediate between *M. flavescens* and *M. duvalii*, whereas these two strains were included in the *M. flavescens* cluster by the 84-character method. Accordingly, these two recently proposed names (34) were considered to be synonyms of *M. flavescens*.

To date, only a few numerical taxonomy studies of rapidly growing, scotochromogenic myco-

bacteria have been reported (8, 22, 27, 30). In these studies, *M. neoaurum* was not clearly separated from *M. parafortuitum* (8), and "*M. tokaiense*" was not clearly distinguished from "*M. chubuense*" (27). In contrast, we differentiated these pairs of species from each other in this study. Our ability to do this was almost certainly due to the use of more characters. In view of the results described above, the following 16 species are considered distinct: *M. phlei* Lehmann and Neumann 1899 (7), *M. flavescens* Bojalil et al. 1962 (1), *M. vaccae* Bönicke and Juhasz 1964 (2), *M. parafortuitum* Tsukamura 1966 (16) (this species was first reported in 1965 in Japanese [31]), *M. thermoresistibile* Tsukamura 1966 (14), *M. aurum* Tsukamura 1966 (15) (this species was published earlier in Japanese [32]), "*M. obuense*" Tsukamura and Mizuno 1971 (25), "*M. rhodesiae*" Tsukamura et al. 1971 (29), *M. duvalii* Stanford and Gunthorpe 1971 (11), *M. neoaurum* Tsukamura 1972 (19), "*M. aichiense*" Tsukamura 1973 (20), "*M. chubuense*" Tsukamura 1973 (20), "*M. tokaiense*" Tsukamura 1973

FIG. 2. Dendrogram, 84-character method: part 2. RHO, *M. rhodesiae*; AIC, *M. aichiense*; CHU, *M. chubuense*; SPH, *M. sphagni*; NEO, *M. neoaurum*; M value, matching coefficient. Numbers on the right are strain numbers (see Table 1).

FIG. 3. Dendrogram, 84-character method: part 3. PHL, *M. phlei*; TOK, *M. tokaiense*; MA, *M. komossense*; OBU, *M. obuense*; THE, *M. thermoresistibile*; M value, matching coefficient. Numbers on the right are strain numbers (see Table 1).

(20), *M. komossense* Kazda and Müller 1979 (5), *M. sphagni* Kazda 1980 (4), and *M. gilvum* Stanford and Gunthorpe 1971 (11).

"*M. gallinarum*" Tsukamura et al. 1967 (33) consists of two strains, and one of these strains (the type strain) was included in this study and was shown to be distinct from all other species. *M. gadium* Casal and Rey Calero 1974 (3) is a species consisting of only one strain. This strain formed a small cluster together with strain ATCC 4243, which is one of the leprosy organisms of Duval. However, the strains of Duval maintained by the National Collection of Type Cultures, London, England, were named *M. duvalii* by Stanford and Gunthorpe (11) and formed another cluster in this study.

Recently, Valdivia Alvarez et al. (34) isolated 16 strains of fast-growing scotochromogenic mycobacteria from bovine organs and placed them in eight new species. These strains were included in this study, and the data for them are summarized in Table 4. Of the eight names, we considered five to be synonyms of names of

previously described species, and we suggest that the other three are names of new species. However, the species proposed by Valdivia Alvarez et al. (34), "*M. gallinarum*" Tsukamura (33), and *M. gadium* Casal and Rey Calero (3) each contain only one or two strains at present. We believe that such possible new taxa consisting of only a few strains should remain unnamed, because, if we accept such species, there are at least 13 new species (17 strains in 13 new species) which may be recognized in this study. We have not named these possible new species of rapidly growing, scotochromogenic mycobacteria in this study. However, it is noteworthy that, of the strains thought to belong to new species, strains 141 to 144 (received from J. Viallier) degrade *p*-aminosalicylate and salicylate. This property is unique for *Mycobacterium fortuitum*, *Mycobacterium chelonae*, and "*M. obuense*" (25) and for some strains of *M. komossense*, as shown in this study.

M. aurum was initially included with *M. parafortuitum* (16) but later was separated from it (32). After *M. phlei*, *M. flavescens*, *M. vaccae*, *M. parafortuitum*, and *M. thermoresistibile*, *M. aurum* is the oldest named species to form brilliant yellowish or orange-pigmented, smooth colonies in the dark. However, *M. phlei* and *M. thermoresistibile* can grow at 52°C, and by this unique property these species are differentiated from all other mycobacteria (8, 16, 17). *M. flavescens* is intermediate between the slowly growing mycobacteria and the rapidly growing mycobacteria (30). *M. vaccae* and *M. parafortuitum* are only slightly pigmented when they are grown in the dark and increase their pigmentation very markedly after exposure to light (Table 3). Therefore, it is convenient to refer to rapidly growing, scotochromogenic mycobacteria which have been found after *M. aurum* as members of the *M. aurum* complex; it is certain that numerous additional species of rapidly growing, scotochromogenic mycobacteria will be found.

Previously, Tsukamura and Mizuno (28) divided strains of *M. flavescens* into two subgroups by using thin-layer chromatography of ethyl ether-ethanol cellular extracts after incubation with [³⁵S]methionine. One subgroup consisted of three strains belonging to group FLA1, and the other consisted of four strains belonging to group FLA2, two strains of the FLA1 group, and the type strain of "*M. gallinarum*." Therefore, there is a discrepancy between the results of thin-layer chromatography and the results of phenetic studies.

Colonial morphology apparently is not useful for differentiating among mycobacterial species.

TABLE 3. Distinguishing characteristics of species of rapidly growing, scotochromogenic mycobacteria

Character	<i>M. thermo-</i> <i>resistibile</i> (14 strains)	<i>M. tokatense</i> (3 strains)	<i>M. obuense</i> (4 strains)	<i>M. rhodesiae</i> (6 strains)	<i>M. alchivense</i> (5 strains)	<i>M. sphagnum</i> (3 strains) ^a	<i>M. vaccae</i> (6 strains)	<i>M. parafortitum</i> (6 strains)	<i>M. duvalii</i> (4 strains)	<i>M. flavescens</i> subgroup I (21 strains)	<i>M. flavescens</i> subgroup II (9 strains)	<i>M. phlei</i> (10 strains)	<i>M. komossense</i> (3 strains) ^b	<i>M. chubuense</i> (5 strains)	<i>M. neoaurum</i> (8 strains)	<i>M. aurum</i> (18 strains) ^c	<i>M. gilvum</i> (3 strains)
Long rods	0 ^d	67	0	0	0	0	0	33	25	0	0	0	0	0	25	0	0
Intermediate rods	93	100	100	33	0	100	17	83	50	19	33	70	100	0	88	39	0
Cross-barring of cells	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pigmented colonies in the dark	100	100	100	100	100	100	17	83	0	100	100	100	100	100	100	100	100
Photochromogenicity	0	0	0	0	0	0	83	83	0	0	0	0	0	0	0	0	0
Growth at 45°C	100	0	0	0	0	0	0	0	0	38	63	100	0	0	0	0	0
Growth at 52°C	100	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
PAS degradation ^e	0	0	100	0	0	33	0	0	0	5	0	0	67	0	0	0	0
Tolerance to NH ₂ OH·HCl (0.25 mg/ml)	0	0	0	17	40	0	0	17	0	5	0	0	0	0	0	100	67
Tolerance to NH ₂ OH·HCl (0.5 mg/ml)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	61	0
Growth on Sauton agar containing 0.1% sodium salicylate	100	100	100	0	100	0	100	83	0	0	0	100	0	60	0	0	100
Salicylate degradation	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Three-day arylsulfatase	0	100	100	100	100	100	33	83	0	67	11	0	0	0	0	58	0
Two-week arylsulfatase	0	100	100	100	100	100	100	100	25	100	100	100	100	100	63	100	67
Resistance to ethambutol	0	0	100	17	0	0	0	17	0	0	0	0	67	0	25	100	67
Tolerance to 0.2% NaNO ₂	100	100	100	50	100	67	100	100	100	90	100	100	100	100	75	100	0
Tween hydrolysis (7 days)	0	0	0	67	20	67	0	33	0	0	0	50	67	0	0	100	67
Catalase (semiquantitative)	100	0	0	83	20	0	33	0	0	43	22	0	0	0	0	0	0
α-Esterase	100	67	25	0	0	0	0	0	0	81	100	100	100	0	25	0	0
β-Esterase	100	100	100	0	40	0	100	100	100	100	100	100	100	100	38	100	67
β-Galactosidase	100	0	50	0	20	0	17	83	0	5	0	90	0	100	13	6	0
Acid phosphatase	100	0	0	100	100	67	0	17	0	0	0	100	33	0	0	0	33
Nitrate reduction (24 h)	100	0	0	0	0	0	0	100	100	100	100	100	0	100	38	67	100
Acetamidase	0	100	0	0	0	0	100	100	0	0	0	60	0	0	88	83	0
Benzamidase	0	100	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
Isonicotinamidase	0	100	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
Nicotinamidase	100	100	100	0	0	0	100	100	100	86	11	100	0	100	100	100	100
Pyrazinamidase	100	100	100	67	20	0	100	100	100	86	22	100	0	100	100	100	100
Allantoinase	0	100	100	0	0	0	100	0	0	10	0	20	0	0	88	72	33
Succinamidase	0	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0	0
Glucosamine as N and C sources	0	100	100	100	100	33	100	83	0	0	0	30	100	100	100	94	67
Acetamide as N and C sources	0	0	0	0	0	0	0	0	0	0	0	100	0	0	75	56	0

TABLE 4. Identification of nine strains received as nine new species

Received as:	Strain no. ^a	Identification
<i>M. gadium</i> 1066	56	Probably a new species
" <i>M. magistralae</i> " INHEM 2231B	49	<i>M. flavescens</i>
" <i>M. auratum</i> " INHEM 2474	50	<i>M. flavescens</i>
" <i>M. armentum</i> " INHEM 2229	59	Probably a new species
" <i>M. pallidum</i> " INHEM 1670	88	<i>M. parafortuitum</i>
" <i>M. perpallidum</i> " INHEM 2105A	89	Probably a new species
" <i>M. liquefactum</i> " INHEM 2203	92	<i>M. vaccae</i>
" <i>M. sufflavum</i> " INHEM 2231A	117	<i>M. neoaurum</i>
" <i>M. taurus</i> " INHEM 2129A	126	Probably a new species

^a See Fig. 1 through 3.

Almost all strains of *M. phlei* received from other laboratories produced rough colonies, whereas all freshly isolated strains showed smooth colonies. A similar phenomenon was observed with *Mycobacterium smegmatis* (23); the majority of laboratory strains had rough or intermediate colonial surfaces, whereas colonies of fresh isolates from soil had mucoid surfaces. The change from smooth to rough colonies is due to smooth-colony-forming bacteria being overgrown by rough-colony-forming cells. This change is not due to a difference in generation times but to the fact that the bacteria which produce rough colonies grow over a longer period of time than do those that form smooth colonies (12, 13).

Supplementary descriptions. In this study, we differentiated the following five species from all other species of rapidly growing, scotochromogenic mycobacteria: "*Mycobacterium obuense*" Tsukamura and Mizuno 1971 (25), "*Mycobacterium rhodesiae*" Tsukamura et al. 1971 (29), "*Mycobacterium aichiense*" Tsukamura 1973 (20), "*Mycobacterium chubuense*" Tsukamura 1973 (20), and "*Mycobacterium tokaiense*" Tsukamura 1973 (20). Furthermore, these five species were differentiated from the rapidly growing, non-photochromogenic mycobacteria described in a previous study (22). Thus, they are now considered to be distinct species of rapidly growing, scotochromogenic mycobacteria, although their names are not on the Approved Lists of Bacterial Names (9). These names are here revived for the same organisms with which they were originally associated (20, 25, 29).

(i) *Mycobacterium obuense* (Tsukamura and Mizuno 1971) Tsukamura and Mizuno

sp. nov., nom. rev. This organism was originally isolated in Obu, Japan, from the sputa of patients with pulmonary disease and from soil. The type strain is strain 47001 (previously, strain 4388; =ATCC 27023 =NCTC 10778).

(ii) *Mycobacterium rhodesiae* (Tsukamura et al. 1971) Tsukamura sp. nov., nom. rev. This organism was originally isolated in Rhodesia from patients with lung tuberculosis. The type strain is strain 02002 (previously, strain 5295; =ATCC 27024 =NCTC 10779).

(iii) *Mycobacterium aichiense* (Tsukamura 1973) Tsukamura sp. nov., nom. rev. This organism was originally isolated in Aichi prefecture, Japan, from soil and from sputa of patients. The type strain is strain 49005 (previously, strain 5545; =ATCC 27280 =NCTC 10820).

(iv) *Mycobacterium chubuense* (Tsukamura 1973) Tsukamura sp. nov., nom. rev. This organism was originally isolated from Chubu Hospital garden soil. The type strain is strain 48013 (previously, strain 5517; =ATCC 27278 =NCTC 10819).

(v) *Mycobacterium tokaiense* (Tsukamura 1973) Tsukamura sp. nov., nom. rev. This organism was originally isolated from soil from the Tokai District of Japan. The type strain is strain 47503 (previously, strain 5553; =ATCC 27282 =NCTC 10821).

REPRINT REQUESTS

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LITERATURE CITED

- Bojalil, L. F., J. Cerbón, and A. Trujillo. 1962. Adansonian classification of mycobacteria. *J. Gen. Microbiol.* 28:333-346.
- Bönicke, R., and S. E. Juhasz. 1964. Beschreibung der neuen Species *Mycobacterium vaccae* n. sp. *Zentralbl. Bakteriologie. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A* 192:133-135.
- Casal, M., and J. Rey Calero. 1974. *Mycobacterium gadium* sp. nov. A new species of rapid-growing scotochromogenic mycobacteria. *Tubercle* 55:299-308.
- Kazda, J. 1980. *Mycobacterium sphagni* sp. nov. *Int. J. Syst. Bacteriol.* 30:77-81.
- Kazda, J., and K. Müller. 1979. *Mycobacterium komossense* sp. nov. *Int. J. Syst. Bacteriol.* 29:361-365.
- Lechevalier, M. P., A. C. Horan, and H. Lechevalier. 1971. Lipid composition in the classification of nocardiae and mycobacteria. *J. Bacteriol.* 105:313-318.
- Lehmann, K. B., and R. O. Neumann. 1899. *Lehmann's Medizinische Handatanten. X. Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik*, vol. 2, Auf. 2.
- Saito, H., R. E. Gordon, I. Juhlin, W. Kämpfer, J. B. G. Kwapiński, C. McDermont, S. R. Pattyn, E. H. Runyon, J. L. Stanford, I. Tarnok, H. Tasaka, M. Tsukamura, and J. Weiszfeiler. 1977. Cooperative numerical analysis of rapidly growing mycobacteria. The second report. *Int. J. Syst. Bacteriol.* 27:75-85.
- Skerman, V. B. D., V. McGowan, and P. H. A. Sneath

- (ed.). 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. **30**:225-420.
10. Sokal, R. R., and P. H. A. Sneath. 1963. Principles of numerical taxonomy. W. H. Freeman, San Francisco.
 11. Stanford, J. L., and W. J. Gunthorpe. 1971. A study of some fast-growing scotochromogenic mycobacteria including species description of *Mycobacterium gilvum* (new species) and *Mycobacterium duvalii* (new species). Br. J. Exp. Pathol. **52**:627-637.
 12. Tsukamura, M. 1959. Mutation to R-type colony morphology in *Mycobacterium* sp. strain Jucho produced by subinhibitory concentrations of kanamycin and ammonium sulfate. Med. Biol. (Tokyo) **53**:187-190.
 13. Tsukamura, M. 1959. Studies on the complex mutation in bacteria. I. Jpn. J. Bacteriol. **14**:271-274.
 14. Tsukamura, M. 1966. *Mycobacterium thermoresistibile*, a new species (preliminary report). Med. Biol. (Tokyo) **72**:187-190.
 15. Tsukamura, M. 1966. Adansonian classification of mycobacteria. J. Gen. Microbiol. **45**:253-273.
 16. Tsukamura, M. 1966. *Mycobacterium parafortuitum*: a new species. J. Gen. Microbiol. **42**:7-12.
 17. Tsukamura, M. 1967. Identification of mycobacteria. Tubercle **48**:311-338.
 18. Tsukamura, M. 1968. Classification of scotochromogenic mycobacteria. Jpn. J. Microbiol. **12**:63-75.
 19. Tsukamura, M. 1972. A new species of rapidly growing, scotochromogenic mycobacteria, *Mycobacterium neoaurum* Tsukamura n. sp. Med. Biol. (Tokyo) **85**:229-233.
 20. Tsukamura, M. 1973. New species of rapidly growing, scotochromogenic mycobacteria, *Mycobacterium chubuense* Tsukamura, *Mycobacterium aichiense* Tsukamura, and *Mycobacterium tokaiense* Tsukamura. Med. Biol. (Tokyo) **86**:13-17.
 21. Tsukamura, M. 1975. Identification of mycobacteria. National Sanatorium Chubu Chest Hospital, Obu, Aichi, Japan.
 22. Tsukamura, M. 1975. Numerical analysis of the relationship between *Mycobacterium*, *Rhodococcus* group, and *Nocardia* by use of hypothetical median organisms. Int. J. Syst. Bacteriol. **25**:329-335.
 23. Tsukamura, M. 1976. Properties of *Mycobacterium smegmatis* freshly isolated from soil. Jpn. J. Microbiol. **20**:355-356.
 24. Tsukamura, M. 1977. Extended numerical taxonomy study of *Nocardia*. Int. J. Syst. Bacteriol. **27**:311-323.
 25. Tsukamura, M., and S. Mizuno. 1971. *Mycobacterium obuense*, a rapidly growing scotochromogenic *Mycobacterium* capable of forming a black product from *p*-aminosalicylate and salicylate. J. Gen. Microbiol. **68**:129-134.
 26. Tsukamura, M., and S. Mizuno. 1975. Differentiation among mycobacterial species by thin-layer chromatography. Int. J. Syst. Bacteriol. **25**:271-280.
 27. Tsukamura, M., and S. Mizuno. 1977. Numerical analysis of relationships among rapidly growing, scotochromogenic mycobacteria. J. Gen. Microbiol. **98**:511-517.
 28. Tsukamura, M., and S. Mizuno. 1979. A further study on the method of identification of mycobacteria by thin-layer chromatography after incubation with ³⁵S-methionine. Kekkaku **54**:15-27.
 29. Tsukamura, M., S. Mizuno, N. F. F. Gane, A. Mills, and L. King. 1971. *Mycobacterium rhodesiae* sp. nov. A new species of rapid-growing scotochromogenic mycobacteria. Jpn. J. Microbiol. **15**:407-416.
 30. Tsukamura, M., S. Mizuno, S. Tsukamura, and J. Tsukamura. 1979. Comprehensive numerical classification of 369 strains of *Mycobacterium*, *Rhodococcus*, and *Nocardia*. Int. J. Syst. Bacteriol. **29**:110-129.
 31. Tsukamura, M., H. Toyama, and S. Mizuno. 1965. *Mycobacterium parafortuitum*, a new species. Med. Biol. (Tokyo) **70**:232-235.
 32. Tsukamura, M., and S. Tsukamura. 1966. *Mycobacterium aurum*, a new species. Med. Biol. (Tokyo) **72**:270-273.
 33. Tsukamura, M., S. Tsukamura, S. Mizuno, and H. Toyama. 1967. Bacteriological studies on atypical mycobacteria isolated in Japan. III. A comparison between pathogenic scotochromogens and soil scotochromogens—origin of pathogenic scotochromogens. Kekkaku **42**:15-21.
 34. Valdivia Alvarez, J. A., C. Ferra Salazar, M. Eche-mendia Font, and S. Dumas Valdivieso. 1975. Estudio de 16 cepas de micobacterias cromogénicas y de crecimiento rápido. Rev. Cubana Med. Trop. **27**:213-223.