Comprehensive Numerical Classification of 369 Strains of *Mycobacterium*, *Rhodococcus*, and *Nocardia*

MICHIO TSUKAMURA,¹ SHOJI MIZUNO,¹ SUMIO TSUKAMURA,² AND JUNKO TSUKAMURA²

The National Chubu Hospital, Obu,¹ and Tsukamura Clinic, Obu (Kyowa),² Aichi 474, Japan

A numerical analysis of 369 strains received as members of the genus *Mycobacterium*, *Rhodococcus* (formerly *Gordona*), *Nocardia*, or *Corynebacterium* was carried out using 88 characters. Of the strains tested, 345 mycobacteria, rhodococci, and nocardiae formed four distinct clusters at a similarity level of 85%. The first cluster was composed of slowly growing mycobacteria; the second cluster, of strains of *Mycobacterium flavescens* and *M. thermoresistibile*; the third cluster, of strains of rapidly growing mycobacteria; and the fourth cluster, of strains of *Rhodococcus* and *Nocardia*. Strains of three species of rapidly growing mycobacteria, *M. agri*, *M. smegmatis*, and *M. vaccae*, were located outside of these clusters. The fourth cluster could be divided into three subclusters, one consisting of rhodococci plus some strains of *Nocardia asteroides*, a second consisting of the remaining strains of *Nocardia*, and a third consisting of strains of *Rhodococcus aurantiacus* (*Gordona aurantiaca*) and *Jensenia canicruria*.

Mycobacterium, *Rhodococcus* (formerly *Gordona*), and *Nocardia* are closely related taxa belonging to the order *Actinomycetales* (21).

In 1966, Tsukamura (13) reported that mycobacteria can be divided into two subgroups, which roughly correspond to slowly growing and rapidly growing mycobacteria, by numerical classification. These two subgroups differed from each other not only in growth rate, but also in several biological characters. Subsequently, Tsukamura and Mizuno (14, 24) proposed the subgeneric names *Mycobacterium* and *Mycomycobacterium* for these subgroups. Later, after having compared mycobacteria and nocardiae, Tsukamura (17) recommended that the subgenera *Mycobacterium* (slowly growing mycobacteria) and *Mycomycobacterium* (rapidly growing mycobacteria) be elevated to genus status, i.e., taxa equivalent in status to the genus *Nocardia*.

In 1971, Tsukamura (18) proposed a new genus, *Gordona*, for slightly acid-fast organisms considered to be intermediate between *Mycobacterium* and *Nocardia* and placed it in the family *Actinomycetaceae*, to which the genus *Nocardia* belongs. It was later shown by Tsukamura that the genus *Gordona* contained strain ATCC 13808 of *Rhodococcus rhodochrous* (19), and it was stated that the generic name *Gordona* should be changed to *Rhodococcus* (20), as this is the first name used for an organism belonging to the genus proposed. Independently, Goodfellow (2) observed that "*Mycobacterium*" *rhodochrous* differs from nocardiae, and Goodfellow et al. (4) reported that it also differs from rapidly growing mycobacteria.

The finding that "*M.*" *rhodochrous* differs from both *Mycobacterium* and *Nocardia* was confirmed by an international cooperative study (5). Goodfellow and Alderson (3) also proposed the name *Rhodococcus* for the "*M.*" *rhodochrous* complex, in which they included the genus *Gordona*. These authors also included several new species in the genus *Rhodococcus*.

The taxonomic relationships of the mycobacteria, rhodococci, and nocardiae were studied by Tsukamura (21), who included hypothetical median organisms of representative species of these taxa in his study. Tsukamura proposed that the slowly growing mycobacteria, the rapidly growing mycobacteria, *Gordona* (now *Rhodococcus*), and *Nocardia* be regarded as distinct taxa. He noted that the slowly growing mycobacteria seemed to be only distantly related to the other three groups, which showed a close relationship with each other.

The purpose of the present study was to conduct a numerical taxonomy analysis of a large number of strains belonging to these taxa. A total of 369 strains of *Mycobacterium*, *Rhodococcus*, and *Nocardia* and 4 strains of *Corynebacterium* were investigated.

MATERIALS AND METHODS

Strains. A total of 365 strains received as members of *Mycobacterium*, *Rhodococcus*, or *Nocardia* and 4 strains received as members of *Corynebacterium* were studied (see Table 1).

Tests. A total of 88 characters were used in the numerical analysis. These characters were selected from 118 characters routinely determined in this

TABLE 1. *Strains used in this study*

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
1	E6383	<i>Mycobacterium tuberculosis</i>	TC1	(1)	Humans
2	E6384	<i>Mycobacterium tuberculosis</i>	TC5	(1)	Humans
3	E6543	<i>Mycobacterium tuberculosis</i>	TC32	(1)	Humans
4	E6548	<i>Mycobacterium tuberculosis</i>	TC40	(1)	Humans
5	05001B	<i>Mycobacterium tuberculosis</i>	H37Rv	(2)	Humans
6	05002	<i>Mycobacterium tuberculosis</i>	Aoyama-B	(2)	Humans
7	E6542	<i>Mycobacterium tuberculosis</i>	TC22	(1)	Humans
8	E7949	<i>Mycobacterium tuberculosis</i>		(3)	Sputa, humans
9	E6165	<i>Mycobacterium tuberculosis</i>		(3)	Sputa, humans
10	E6385	<i>Mycobacterium tuberculosis</i>	TC6	(1)	Humans
11	E6387	<i>Mycobacterium tuberculosis</i>	TC9	(1)	Humans
12	E6401	<i>Mycobacterium tuberculosis</i>	TC29	(1)	Humans
13	E6661	<i>Mycobacterium tuberculosis</i>	TC13	(1)	Humans
14	E6662	<i>Mycobacterium tuberculosis</i>	TC15	(1)	Humans
15	E7193	<i>Mycobacterium tuberculosis</i>		(3)	Sputa, humans
16	E7966	<i>Mycobacterium tuberculosis</i>		(3)	Sputa, humans
17	E7950	<i>Mycobacterium tuberculosis</i>		(3)	Sputa, humans
18	05001A	<i>Mycobacterium tuberculosis</i>	H37Rv	(2)	Humans
19	E6394	<i>Mycobacterium tuberculosis</i>	TC21	(1)	Humans
20	E6549	<i>Mycobacterium africanum</i>	TC41	(1)	Humans
21	E6677	<i>Mycobacterium microti</i>	TC62	(1)	?
22	E6567	<i>Mycobacterium africanum</i>	TC93	(1)	Humans
23	E6689	<i>Mycobacterium africanum</i>	TC84	(1)	Humans
24	E6386	<i>Mycobacterium bovis</i>	TC7	(1)	Milk
25	E6537	<i>Mycobacterium africanum</i>	TC3	(1)	Humans
26	E6412	<i>Mycobacterium bovis</i>	TC92	(1)	Dogs
27	E6558	<i>Mycobacterium africanum</i>	TC67	(1)	Humans
28	E6565	<i>Mycobacterium africanum</i>	TC88	(1)	Humans
29	E6683	<i>Mycobacterium africanum</i>	TC74	(1)	Humans
30	E6544	<i>Mycobacterium bovis</i>	TC34	(1)	Cows
31	E6554	<i>Mycobacterium africanum</i>	TC57	(1)	Humans
32	E6560	<i>Mycobacterium africanum</i>	TC73	(1)	Humans
33	E6664	<i>Mycobacterium africanum</i>	TC37	(1)	Humans
34	E6678	<i>Mycobacterium bovis</i>	TC65	(1)	Deer
35	E6692	<i>Mycobacterium africanum</i>	TC8	(1)	Humans
36	E6393	<i>Mycobacterium africanum</i>	TC20	(1)	Humans
37	E6396	<i>Mycobacterium bovis</i>	TC24	(1)	Cows
38	E6671	<i>Mycobacterium bovis</i>	TC51	(1)	Bison
39	E6402	<i>Mycobacterium bovis</i>	TC30	(1)	Cows
40	06001A	<i>Mycobacterium bovis</i>	Ravenel	(4)	Cows
41	06002	<i>Mycobacterium bovis</i>	Denken	(5)	Cows
42	06001B	<i>Mycobacterium bovis</i>	Ravenel	(4)	Cows
43	E6675	<i>Mycobacterium bovis</i>	TC58	(1)	Cows
44	E6388	<i>Mycobacterium bovis</i>	TC11	(1)	BCG (Czech strain)
45	E6691	<i>Mycobacterium microti</i>	TC89	(1)	Llamas
46	E6392	<i>Mycobacterium microti</i>	TC19	(1)	?
47	E6398	<i>Mycobacterium microti</i>	TC26	(1)	Voles
48	E6400	<i>Mycobacterium microti</i>	TC28	(1)	?
49	E6668	<i>Mycobacterium microti</i>	TC48	(1)	Cats
50	E6688	<i>Mycobacterium bovis</i>	TC83	(1)	Minks
51	E7743	<i>Mycobacterium intracellulare</i>	13444	(6)	Humans
52	E7744	<i>Mycobacterium intracellulare</i>	18730	(6)	Humans
53	E7747	<i>Mycobacterium intracellulare</i>	25356	(6)	Humans
54	E7748	<i>Mycobacterium intracellulare</i>	28659	(6)	Sewage
55	E7741	<i>Mycobacterium intracellulare</i>	4130	(6)	Humans
56	E7745	<i>Mycobacterium intracellulare</i>	23774	(6)	Humans
57	E7749	<i>Mycobacterium intracellulare</i>	28660	(6)	?
58	E7750	<i>Mycobacterium intracellulare</i>	28661	(6)	Birds
59	E7742	<i>Mycobacterium intracellulare</i>	10499	(6)	Humans
60	E7746	<i>Mycobacterium intracellulare</i>	24657	(6)	Humans

TABLE 1 (continued)

Serial no.	Laboratory no.	Received as:		Source"	Habitat
		Name	Strain		
61	13022	<i>Mycobacterium intracellulare</i>	NJ-16	(3)	Humans
62	13039	<i>Mycobacterium intracellulare</i>	NJ-17	(3)	Humans
63	E6111	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
64	E6172	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
65	13014	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
66	13015	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
67	13023	<i>Mycobacterium intracellulare</i>	NJ-7	(3)	Sputa, man
68	E7959	<i>Mycobacterium intracellulare</i>	TS-P	(7)	Swine, lymph nodes
69	E7961	<i>Mycobacterium intracellulare</i>	No. 23	(7)	Swine, lymph nodes
70	13024	<i>Mycobacterium intracellulare</i>	NJ-8	(3)	Humans
71	13032	<i>Mycobacterium intracellulare</i>	NJ-10	(3)	Humans
72	13038	<i>Mycobacterium intracellulare</i>	NJ-6	(3)	Humans
73	13936	<i>Mycobacterium intracellulare</i>		(3)	Humans
74	E7957	<i>Mycobacterium intracellulare</i>	TS-H	(7)	Swine, lymph nodes
75	E7963	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
76	13543	<i>Mycobacterium intracellulare</i>	ATCC 19177	(8)	Hens
77	E7958	<i>Mycobacterium intracellulare</i>	TS-L	(7)	Swine
78	11016	<i>Mycobacterium avium</i>	ATCC 15769	(8)	Hens
79	11021	<i>Mycobacterium avium</i>	ATCC 17942	(8)	?
80	11018	<i>Mycobacterium avium</i>	ATCC 17941	(8)	?
81	11017	<i>Mycobacterium avium</i>	ATCC 19075	(8)	Humans, lymph nodes
82	11019	<i>Mycobacterium avium</i>	ATCC 17944	(8)	?
83	11020	<i>Mycobacterium avium</i>	ATCC 15773	(8)	?
84	E7682	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
85	E7954	<i>Mycobacterium intracellulare</i>	A-1	(7)	Swine, lymph nodes
86	11025	<i>Mycobacterium avium?</i>	ATCC 17940	(8)	?
87	E7960	<i>Mycobacterium intracellulare</i>	No. 6	(7)	Swine, lymph nodes
88	13013	<i>Mycobacterium intracellulare</i>	NJ-1	(3)	Humans
89	13016	<i>Mycobacterium intracellulare</i>	NJ-2	(3)	Humans
90	13019	<i>Mycobacterium intracellulare</i>	NJ-18	(3)	Humans
91	13542	?	ATCC 19076	(8)	Humans
92	13935	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
93	12004	<i>Mycobacterium scrofulaceum</i>	SJ-8	(3)	Humans
94	12008	<i>Mycobacterium scrofulaceum</i>	SJ-9	(3)	Humans
95	12014	<i>Mycobacterium scrofulaceum</i>	SJ-1	(3)	Humans
96	12007A	<i>Mycobacterium scrofulaceum</i>	SJ-5	(3)	Humans
97	12017	<i>Mycobacterium scrofulaceum</i>	SJ-12	(3)	Humans
98	12007B	<i>Mycobacterium scrofulaceum</i>	SJ-5	(3)	Humans
99	12016A	<i>Mycobacterium scrofulaceum</i>	SJ-14	(3)	Humans
100	12012	<i>Mycobacterium scrofulaceum</i>	SJ-3	(3)	Humans
101	12019	<i>Mycobacterium scrofulaceum</i>	SJ-15	(3)	Humans
102	12002	<i>Mycobacterium scrofulaceum</i>	P-6	(9)	Humans
103	12009	<i>Mycobacterium scrofulaceum</i>	SJ-13	(3)	Humans
104	12020	<i>Mycobacterium scrofulaceum</i>	SJ-16	(3)	Humans
105	12321	<i>Mycobacterium scrofulaceum</i>	ATCC 15078	(8)	?
106	12320	<i>Mycobacterium scrofulaceum</i>	ATCC 19981 (type)	(8)	Cervical lymph nodes
107	12107	<i>Mycobacterium scrofulaceum</i>	ATCC 15978	(8)	?
108	12108	<i>Mycobacterium scrofulaceum</i>	ATCC 19073	(8)	Sputa, humans
109	E7814	<i>Mycobacterium scrofulaceum</i>		(3)	Sputa, humans
110	13009	<i>Mycobacterium intracellulare</i>	NJ-9	(3)	Humans
111	13011	<i>Mycobacterium intracellulare</i>	NJ-3	(3)	Humans
112	36001	<i>Mycobacterium scrofulaceum</i> (<i>marianum</i>)	ATCC 19275	(8)	Humans
113	12178	<i>Mycobacterium gordonae</i>	ATCC 23284	(8)	?
114	E6009	<i>Mycobacterium intracellulare?</i>		(3)	Humans
115	E6541	<i>Mycobacterium bovis</i>	TC18	(1)	?
116	12001	<i>Mycobacterium scrofulaceum</i>	P-5	(9)	Humans
117	12109	<i>Mycobacterium gordonae</i>	ATCC 14470 (type)	(8)	Gastric lavage
118	12110	<i>Mycobacterium gordonae</i>	ATCC 19277	(8)	Tap water
119	12016B	<i>Mycobacterium gordonae</i>	SJ-6	(3)	Humans

TABLE 1 (continued)

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
120	12177	<i>Mycobacterium gordonae</i>	ATCC 23283	(8)	?
121	12180	<i>Mycobacterium gordonae</i>	ATCC 23286	(8)	Sputa, humans
122	12179	<i>Mycobacterium gordonae</i>	ATCC 23285	(8)	Tap water
123	44008	<i>Mycobacterium gordonae</i> ?	S-25-2	(7)	Cows, thelitis lesions
124	44009	<i>Mycobacterium gordonae</i> ?	S-25-2B	(7)	Cows, thelitis lesions
125	44007	<i>Mycobacterium gordonae</i> ?	0-25-2	(7)	Cows, thelitis lesions
126	E6117	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
127	E6110	<i>Mycobacterium intracellulare</i>		(3)	Sputa, humans
128	28001	<i>Mycobacterium xenopi</i>	ATCC 19156	(8)	Humans
129	28002	<i>Mycobacterium xenopi</i>	ATCC 19276	(8)	Humans
130	28003	<i>Mycobacterium xenopi</i>	ATCC 19250 (type)	(8)	Adult female toads
131	28004	<i>Mycobacterium xenopi</i>	ATCC 19970	(8)	?
132	E6137	<i>Mycobacterium gordonae</i> ?		(3)	Sputa, humans
133	E7962	?	Ishikari-31	(7)	Swine
134	09023	<i>Mycobacterium nonchromogenicum</i>	ATCC 19532	(8)	Soil
135	09033	<i>Mycobacterium nonchromogenicum</i>	ATCC 19533	(8)	Soil
136	09003	<i>Mycobacterium nonchromogenicum</i>	ATCC 19530 (type)	(8)	Soil
137	E4322	<i>Mycobacterium gastri</i> ^{*b}	ATCC 25028	(8)	?
138	E4324	<i>Mycobacterium gastri</i> [*]	ATCC 25030	(8)	?
139	E4323	<i>Mycobacterium gastri</i> [*]	ATCC 25029	(8)	?
140	E4325	<i>Mycobacterium gastri</i> [*]	ATCC 25031	(8)	?
141	E4321	<i>Mycobacterium gastri</i> [*]	ATCC 25027	(8)	?
142	E4326	<i>Mycobacterium gastri</i> [*]	ATCC 25032	(8)	?
143	E4327	<i>Mycobacterium gastri</i> [*]	ATCC 25033	(8)	?
144	E4335	?	ATCC 25041	(8)	?
145	E4342	<i>Mycobacterium kansasii</i> [*]	ATCC 25048	(8)	?
146	E4336	<i>Mycobacterium kansasii</i> [*]	ATCC 25042	(8)	?
147	E4337	<i>Mycobacterium kansasii</i> [*]	ATCC 25043	(8)	?
148	E4339	<i>Mycobacterium kansasii</i> [*]	ATCC 25045	(8)	?
149	E4340	<i>Mycobacterium kansasii</i> [*]	ATCC 25046	(8)	?
150	E4341	<i>Mycobacterium kansasii</i> [*]	ATCC 25047	(8)	?
151	E4343	<i>Mycobacterium kansasii</i> [*]	ATCC 25049	(8)	?
152	07004	<i>Mycobacterium kansasii</i> [*]	Nagai	(3)	Sputa, humans
153	07012	<i>Mycobacterium kansasii</i>	Chinen	(3)	Sputa, humans
154	E6184	<i>Mycobacterium szulgai</i>		(3)	Sputa, humans
155	Z12001	<i>Mycobacterium szulgai</i>	23428	(6)	Humans
156	Z12002	<i>Mycobacterium szulgai</i>	25932	(6)	Humans
157	Z12003	<i>Mycobacterium szulgai</i>	26701	(6)	Humans
158	Z12004	<i>Mycobacterium szulgai</i>	20886	(6)	Humans
159	E6139	?		(3)	Sputa, humans
160	E4796	<i>Mycobacterium shimoidei</i>	ATCC 27962 (type)	(8)	Sputa, humans
161	E7380	<i>Mycobacterium shimoidei</i>		(3)	Sputa, humans
162	44006	?	L-25-2A	(7)	Cows, thelitis lesions
163	E4328	<i>Mycobacterium marinum</i> [*]	ATCC 25034	(8)	?
164	08001	<i>Mycobacterium marinum</i>	ATCC 927	(8)	Fish
165	E6104	?		(3)	Sputa, humans
166	E6107	?		(3)	Sputa, humans
167	E6152	?		(3)	Sputa, humans
168	33004	<i>Mycobacterium flavescens</i>	E3364	(3)	Sputa, humans
169	33005	<i>Mycobacterium flavescens</i>	E3365	(3)	Sputa, humans
170	33001	<i>Mycobacterium flavescens</i>	ATCC 14474 (type)	(8)	Guinea pigs
171	01002	<i>Mycobacterium thermoresistibile</i>	ATCC 19528	(8)	Soil
172	01021	<i>Mycobacterium thermoresistibile</i>		(3)	Soil
173	01028	<i>Mycobacterium thermoresistibile</i>	ATCC 19529	(8)	Soil

TABLE 1 (continued)

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
174	01039	<i>Mycobacterium thermoresistibile</i>	ATCC 19527 (type)	(8)	Soil
175	01011	<i>Mycobacterium thermoresistibile</i>		(3)	Soil
176	E5750	<i>Mycobacterium fortuitum</i>		(3)	Sputa, humans
177	E6029	<i>Mycobacterium fortuitum</i>		(3)	Sputa, humans
178	E6051	<i>Mycobacterium fortuitum</i>		(3)	Sputa, humans
179	E6035	<i>Mycobacterium fortuitum</i>		(3)	Sputa, humans
180	18007	<i>Mycobacterium fortuitum</i>	E308	(3)	Soil
181	18110	<i>Mycobacterium peregrinum</i>	ATCC 14467 (type)	(8)	Humans
182	18009	<i>Mycobacterium fortuitum</i>	NCTC 8697	(9)	?
183	18001	<i>Mycobacterium fortuitum</i>	ATCC 19709	(8)	Soil
184	18010	<i>Mycobacterium fortuitum</i>	R389	(10)	?
185	18011	<i>Mycobacterium fortuitum</i>	E1701	(3)	Soil
186	18109	<i>Mycobacterium fortuitum</i>	ATCC 6842	(8)	Humans, cold abscesses
187	10001	<i>Mycobacterium fortuitum</i>	E328	(3)	Soil
188	18112	<i>Mycobacterium fortuitum</i>	ATCC 6841 (type)	(8)	Humans, cold abscesses
189	42001	<i>Mycobacterium fortuitum</i> (minetti)	ATCC 19542	(8)	?
190	18021	<i>Mycobacterium fortuitum</i>	E1261	(3)	Soil
191	18024	<i>Mycobacterium fortuitum</i>	E1264	(3)	Soil
192	18022	<i>Mycobacterium fortuitum</i>	E1262	(3)	Soil
193	E7820	?	N10596	(10)	?
194	15502	<i>Mycobacterium aurum</i>	ATCC 25801	(8)	Soil
195	15503	<i>Mycobacterium aurum</i>	ATCC 25802	(8)	Soil
196	22011	<i>Mycobacterium abscessus</i> ^c	ATCC 14472	(8)	?
197	22019	<i>Mycobacterium abscessus</i> ^c	ATCC 23045	(8)	?
198	22021	<i>Mycobacterium abscessus</i> ^c	E2846	(3)	Humans, lungs
199	22012	<i>Mycobacterium abscessus</i> ^c	ATCC 19977 (type)	(8)	Abscesses, humans
200	22016	<i>Mycobacterium abscessus</i> ^c	ATCC 23018	(8)	?
201	E5765	<i>Mycobacterium abscessus</i> ^c		(3)	Sputa, humans
202	19009	<i>Mycobacterium chelonae</i> ^d	ATCC 19235	(8)	Gastric lavage
203	19010	<i>Mycobacterium chelonae</i> ^d	ATCC 19237	(8)	Gastric lavage
204	19040	<i>Mycobacterium chelonae</i> ^d	ATCC 23000	(8)	?
205	19041	<i>Mycobacterium chelonae</i> ^d	ATCC 23013	(8)	?
206	19043	<i>Mycobacterium chelonae</i> ^d	ATCC 23030	(8)	?
207	E7818	?	N34	(11)	?
208	14003	<i>Mycobacterium phlei</i>	SN103	(12)	?
209	14017	<i>Mycobacterium phlei</i>	Trudeau	(13)	?
210	14002	<i>Mycobacterium phlei</i>	SN102	(12)	?
211	14004	<i>Mycobacterium phlei</i>	SN104	(12)	?
212	14022	<i>Mycobacterium phlei</i>	ATCC 19249	(8)	?
213	16001	<i>Mycobacterium parafortuitum</i>	ATCC 19687	(8)	Soil
214	16002	<i>Mycobacterium parafortuitum</i>	ATCC 19686 (type)	(8)	Soil
215	16003	<i>Mycobacterium parafortuitum</i>	ATCC 19688	(8)	Soil
216	48008	<i>Mycobacterium chubuense</i>	E5511	(3)	Soil
217	48012	<i>Mycobacterium chubuense</i>	ATCC 27279	(8)	Soil
218	48013	<i>Mycobacterium chubuense</i>	ATCC 27278 (type)	(8)	Soil
219	49004	<i>Mycobacterium aichiense</i>	ATCC 27281	(8)	Soil
220	49005	<i>Mycobacterium aichiense</i>	ATCC 27280 (type)	(8)	Soil
221	49007	<i>Mycobacterium aichiense</i>	E5547	(3)	Soil
222	E5795	<i>Mycobacterium rhodesiae</i>	ATCC 27024 (type)	(8)	Sputa, humans
223	31002	<i>Mycobacterium chitae</i>	ATCC 19627 (type)	(8)	Soil
224	31003	<i>Mycobacterium chitae</i>	ATCC 19628	(8)	Soil
225	31004	<i>Mycobacterium chitae</i>	ATCC 19629	(8)	Soil
226	E7566	<i>Nocardia rubra</i>	N20	(14)	?
227	E7568	<i>Nocardia rubra</i>	N24	(14)	?
228	E7567	<i>Nocardia rubra</i>	N21	(14)	?

TABLE 1 (continued)

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
229	E7572	<i>Nocardia rubra</i>	N50	(14)	?
230	E7570	<i>Nocardia rubra</i>	N26	(14)	?
231	23002	<i>Rhodococcus lentifragmentus</i> ^e	M-1 (type)	(15)	?
232	23022	<i>Rhodococcus lentifragmentus</i> ^e	M-103	(15)	?
233	23022	<i>Rhodococcus lentifragmentus</i> ^e	M-122	(15)	?
234	23016	<i>Rhodococcus lentifragmentus</i> ^e	C-1	(15)	?
235	23024	<i>Rhodococcus lentifragmentus</i> ^e	M-192	(15)	?
236	E7573	<i>Nocardia rubra</i>	N51	(14)	?
237	40017	<i>Rhodococcus rhodochrous</i> ^f	ATCC 4273	(8)	?
238	40020	<i>Rhodococcus rhodochrous</i> ^f	ATCC 14347	(8)	?
239	40021	<i>Rhodococcus rhodochrous</i> ^f	ATCC 14348	(8)	?
240	40022	<i>Rhodococcus rhodochrous</i> ^f	ATCC 14349	(8)	?
241	E3621	<i>Rhodococcus terrae</i> ^g		(3)	Soil
242	E3622	<i>Rhodococcus terrae</i> ^g		(3)	Soil
243	E3612	<i>Rhodococcus terrae</i> ^g		(3)	Soil
244	70006	<i>Rhodococcus terrae</i> ^g	ATCC 25594 (type)	(8)	Soil
245	E5441	<i>Rhodococcus rubropertinctus</i> ^h		(3)	Soil
246	60016	<i>Rhodococcus rubropertinctus</i> ^h	ATCC 14343 (type)	(8)	Soil
247	60002	<i>Rhodococcus rubropertinctus</i> ^h	E3602	(3)	Soil
248	60003	<i>Rhodococcus rubropertinctus</i> ^h	ATCC 25593	(8)	Soil
249	60017	<i>Rhodococcus rubropertinctus</i> ^h	ATCC 19067	(8)	?
250	65001	<i>Rhodococcus roseus</i> ⁱ	ATCC 271	(8)	?
251	65002	<i>Rhodococcus roseus</i> ⁱ	ATCC 4004 (type)	(8)	?
252	E7569	<i>Nocardia rubra</i>	N25	(14)	?
253	23023	<i>Nocardia coeliaca</i>	M-191	(15)	?
254	E7565	<i>Nocardia rubra</i>	N19	(14)	?
255	E7571	<i>Nocardia rubra</i>	N27	(14)	?
256	40001	<i>Rhodococcus rhodochrous</i> [*]		(3)	?
257	50006	<i>Rhodococcus bronchialis</i> ^j	E3413	(3)	Sputa, humans
258	50019	<i>Rhodococcus bronchialis</i> ^j	E3926	(3)	Sputa, humans
259	50003	<i>Rhodococcus bronchialis</i> ^j	ATCC 25592 (type)	(8)	Sputa, humans
260	50011	<i>Rhodococcus bronchialis</i> ^j	E3885	(3)	Sputa, humans
261	50017	<i>Rhodococcus bronchialis</i> ^j	E3924	(3)	Sputa, humans
262	23088	<i>Nocardia asteroides</i> ^k	M206	(15)	?
263	23044	<i>Nocardia asteroides</i> ^k	M124	(15)	?
264	23095	<i>Nocardia asteroides</i> ^k	R433(1)	(10)	?
265	23009	<i>Nocardia asteroides</i> ^k	M124	(15)	?
266	23032	<i>Nocardia asteroides</i> ^k	M10	(15)	?
267	23038	<i>Nocardia asteroides</i> ^k	M93	(15)	?
268	23097	<i>Nocardia asteroides</i> ^k	R553	(10)	?
269	23103	<i>Nocardia asteroides</i> ^k	R9970	(10)	?
270	23045	<i>Nocardia asteroides</i> ^k	M128	(15)	?
271	23057	<i>Nocardia asteroides</i> ^k	M156	(15)	?
272	23006	<i>Nocardia asteroides</i> ^k	M93	(15)	?
273	23007	<i>Nocardia asteroides</i> ^k	M94	(15)	?
274	23039	<i>Nocardia asteroides</i> ^k	M94	(15)	?
275	23049	<i>Nocardia asteroides</i> ^k	M132	(15)	?
276	23054	<i>Nocardia asteroides</i> ^k	M145	(15)	?
277	23056	<i>Nocardia asteroides</i> ^k	M155	(15)	?
278	23046	<i>Nocardia asteroides</i> ^k	M129	(15)	?
279	23012	<i>Nocardia asteroides</i> ^k	M145	(15)	?
280	23105	<i>Nocardia brasiliensis</i>	R432	(10)	?
281	23106	<i>Nocardia brasiliensis</i>	R887	(10)	?
282	23108	<i>Nocardia brasiliensis</i>	R1117A	(10)	?
283	23083	<i>Nocardia brasiliensis</i>	M199	(15)	?
284	23112	<i>Nocardia caviae</i>	R547	(10)	?
285	23113	<i>Nocardia caviae</i>	R617	(10)	?
286	23114	<i>Nocardia caviae</i>	R1291	(10)	?
287	23033	<i>Nocardia caviae</i>	M54	(15)	?
288	23035	<i>Nocardia caviae</i>	M73	(15)	?

TABLE 1 (continued)

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
289	23078	<i>Nocardia caviae</i>	M185	(15)	?
290	23111	<i>Nocardia caviae</i>	R416	(10)	?
291	23109	<i>Nocardia brasiliensis</i>	R1188	(10)	?
292	23005	<i>Nocardia caviae</i>	M73	(15)	?
293	23086	<i>Nocardia brasiliensis</i>	M204	(15)	?
294	23067	<i>Nocardia farcinica</i> ^b	M164	(15)	?
295	23070	<i>Nocardia farcinica</i> ^b	M169	(15)	?
296	23051	<i>Nocardia farcinica</i> ^b	M138	(15)	?
297	23060	<i>Nocardia farcinica</i> ^b	M127	(15)	?
298	23082	<i>Nocardia farcinica</i> ^b	M198	(15)	?
299	23098	<i>Nocardia farcinica</i> ^b	R784	(10)	?
300	23048	<i>Nocardia farcinica</i> ^b	M131	(15)	?
301	23058	<i>Nocardia farcinica</i> ^b	M158	(15)	?
302	23065	<i>Nocardia farcinica</i> ^b	M162	(15)	?
303	23075	<i>Nocardia farcinica</i> ^b	M179	(15)	?
304	23081	<i>Nocardia farcinica</i> ^b	M197	(15)	?
305	23084	<i>Nocardia farcinica</i> ^b	M201	(15)	?
306	23101	<i>Nocardia farcinica</i> ^b	W3409B	(10)	?
307	23041	<i>Nocardia farcinica</i> ^b	M112	(15)	?
308	23004	<i>Nocardia farcinica</i> ^b	M72	(15)	?
309	23034	<i>Nocardia farcinica</i> ^b	M72	(15)	?
310	23011	<i>Nocardia farcinica</i> ^b	M128	(15)	?
311	23013	<i>Nocardia farcinica</i> ^b	M146	(15)	?
312	23040	<i>Nocardia farcinica</i> ^b	M111	(15)	?
313	23102	<i>Nocardia farcinica</i> ^b	R3318 (type)	(10)	?
314	23053	<i>Nocardia farcinica</i> ^b	M144	(15)	?
315	E7551	<i>Nocardia farcinica</i> [*]	VAC462	(16)	?
316	23001	<i>Nocardia farcinica</i> ^b	M75	(15)	?
317	23027	<i>Nocardia farcinica</i> ^b	C743	(15)	?
318	23087	<i>Nocardia farcinica</i> ^b	M205	(15)	?
319	23055	<i>Nocardia farcinica</i> ^b	M146	(15)	?
320	23018	<i>Nocardia farcinica</i> ^b	C75	(15)	?
321	23059	<i>Nocardia farcinica</i> ^b	M86	(15)	?
322	23062	<i>Nocardia farcinica</i> ^b	M158A	(15)	?
323	23092	<i>Nocardia farcinica</i> ^b	M189	(15)	?
324	23010	<i>Nocardia farcinica</i> ^b	M126	(15)	?
325	23073	<i>Nocardia farcinica</i> ^b	M175	(15)	?
326	E7550	<i>Nocardia farcinica</i> [*]	VAC333	(16)	?
327	23069	<i>Nocardia farcinica</i> ^b	M167	(15)	?
328	23074	<i>Nocardia farcinica</i> ^b	M177	(15)	?
329	23093	<i>Nocardia farcinica</i> ^b	M200	(15)	?
330	E7547	<i>Nocardia farcinica</i> [*]	VAC300	(16)	?
331	E7548	<i>Nocardia farcinica</i> [*]	VAC324	(16)	?
332	E7549	<i>Nocardia farcinica</i> [*]	VAC330	(16)	?
333	23077	<i>Nocardia farcinica</i> ^b	M181	(15)	?
334	E7815	<i>Nocardia farcinica</i> [*]	3318/AL	(11)	?
335	23104	<i>Nocardia brasiliensis</i>	R405	(10)	?
336	E7723	<i>Corynebacterium equi</i>	KO-85	(17)	?
337	E3477	<i>Rhodococcus aurantiacus</i> [†]		(3)	Sputa, humans
338	80001	<i>Rhodococcus aurantiacus</i> [†]	ATCC 25938 (type)	(8)	Sputa, humans
339	80003	<i>Rhodococcus aurantiacus</i> [†]	E4409	(3)	Sputa, humans
340	E4134	<i>Rhodococcus aurantiacus</i> [†]		(3)	Sputa, humans
341	E4259	<i>Rhodococcus aurantiacus</i> [†]		(3)	Sputa, humans
342	E7552	<i>Rhodococcus aurantiacus</i> [†]	25938	(16)	Sputa, humans
343	E6618	<i>Jensenia canicruria</i>	KCCA-0132	(18)	?
344	40014A	<i>Jensenia canicruria</i>	KCCA-0132	(18)	?
345	40014	<i>Jensenia canicruria</i>	KCCA-0132 (type)	(18)	?
346	23066	?	M163	(15)	?
347	23085	<i>Nocardia tenuis-convoluta</i>	M203	(15)	?
348	E6222	<i>Mycobacterium agri</i>		(3)	Soil

TABLE 1 (continued)

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
349	90001	<i>Mycobacterium agri</i>	E6203	(3)	Soil
350	90012	<i>Mycobacterium agri</i>	ATCC 27406 (type)	(8)	Soil
351	90018	<i>Mycobacterium agri</i>	ATCC 27407	(8)	Soil
352	17002	<i>Mycobacterium smegmatis</i>	SN2	(12)	?
353	17005	<i>Mycobacterium smegmatis</i>	SN5	(12)	?
354	17023	<i>Mycobacterium smegmatis</i> *	Jucho	(19)	Hens
355	17027	<i>Mycobacterium smegmatis</i>	ATCC 14468 (neo-type)	(8)	?
356	43001	<i>Mycobacterium smegmatis</i> (butyricum)	ATCC 19979	(8)	?
357	E7817	?	IP740	(11)	?
358	E7819	?	N10955	(11)	?
359	23031	<i>Proactinomyces restrictus</i>	M9	(15)	?
360	E7816	?	IP736	(11)	?
361	E7729	<i>Corynebacterium ulcerans</i>	9304	(17)	?
362	23030	<i>Nocardia polychromogenes</i>	M6	(15)	?
363	E7955	?	B-11-25-LD	(7)	Swine, thelitis lesions
364	E7956	?	B-11-25-OD	(7)	Swine, thelitis lesions
365	E7724	<i>Corynebacterium hoffmanii</i>	39	(17)	?
366	21007	<i>Mycobacterium vaccae</i>	ATCC 15483 (type)	(8)	Cow's milk
367	21011	<i>Mycobacterium vaccae</i>	ATCC 23014	(8)	?
368	21012	<i>Mycobacterium vaccae</i>	ATCC 23027	(8)	?
369	E7728	<i>Corynebacterium diphtheriae</i>	PW8	(17)	?

^a (1) H. H. Kleeberg, Tuberculosis Research Institute, Pretoria, South Africa; (2) S. Ata, Nagoya University, Nagoya, Japan; (3) M. Tsukamura, National Chubu Hospital, Obu, Aichi, Japan; (4) K. Shoji, Osaka University, Osaka, Japan; (5) K. Konno, Tohoku University, Sendai, Japan; (6) J. Marks, Tuberculosis Reference Laboratory, University Hospital of Wales, Cardiff, Wales; (7) K. Shimizu, Obihiro Veterinary College, Hokkaido, Japan; (8) ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; (9) K. Takeya, Kyushu University, Fukuoka, Japan; (10) R. E. Gordon, Rutgers University, New Brunswick, N.J., U.S.A.; (11) M. P. Lechevalier, Rutgers University, New Brunswick, N.J., U.S.A.; (12) R. Bönicke, Forschungsinstitut Borstel, Borstel, West Germany; (13) H. Saito, Hiroshima University, Hiroshima, Japan; (14) M. Riddel, University of Göteborg, Göteborg, Sweden; (15) I. Uesaka, Kyoto University, Kyoto, Japan (originally received from N. M. McClung, University of South Florida, Tampa, Fl., U.S.A.); (16) S. G. Bradley, Virginia Commonwealth University, Richmond, Va., U.S.A.; (17) M. Yoneda, Osaka University, Osaka, Japan; (18) A. Seino, Kaken Chemical Co., Tokyo, Japan; (19) T. Hasegawa, Tokyo University, Tokyo, Japan.

^b Strains marked with an asterisk were identified in this laboratory.

^c *Mycobacterium chelonae* subsp. *abscessus* should be named *M. abscessus* (16).

^d *Mycobacterium chelonae* subsp. *chelonae* should be named *M. chelonae* (16) (*M. borstelense* is a synonym of this organism).

^e Formerly *Gordona lentifragmenta* (synonym: *Nocardia rubra*) (26); see reference 20.

^f Formerly *Gordona rhodochroa* (19); see reference 20.

^g Formerly *Gordona terrae* (18); see reference 20.

^h Formerly *Gordona rubropertincta* (synonym *Gordona rubra*) (18, 19); refer to (20).

ⁱ Formerly *Gordona rosea* (19, 20).

^j Formerly *Gordona bronchialis* (18, 20).

^k Identified in this laboratory (15); for the names as received, see reference 15.

^l Formerly *Gordona aurantiaca* (20, 25).

laboratory, omitting, as far as possible, linked characters, which are considered to give unreliable results (23). The characters were the same as those used previously for a numerical classification of slowly growing mycobacteria (22). Taken as a whole, the 88 characters were effective for differentiating the strains.

Numerical analysis. The matching coefficient (*M* value) between any two strains was calculated by the following equation: *M* value (percent) = ($n_s \times 100\%$) / ($n_s + n_d$), where n_s is the number of characters that show similar code symbols (+ + or - -) and n_d is the

number of characters which show different code symbols (+ -). Clustering was done by the single-linkage method (12).

The computer used was the IBM S-370, model 135, which is located in the Nagoya Factory of the Sumitomo Machinery Co., Obu, Aichi, Japan. The program is written in English (CÔBOL), and the program number is G90002. The data for the original "strain $\times$ characters" matrix have been deposited with the World Data Center for Microorganisms, Brisbane, Australia.

In the numerical classification, two or three replicates of a few strains were included in the study to confirm the reliability of the results.

RESULTS

The results of the study are presented as dendrograms (Fig. 1 to 7). Of the 369 strains studied, 346 strains formed a large cluster at a level of 84%; this cluster was subdivided into four smaller clusters at a level of 85%.

The first cluster (A) corresponded to the group of organisms referred to as slowly growing mycobacteria; the second (B), to intermediates between slowly and rapidly growing mycobacteria; the third (C), to rapidly growing mycobacteria; and the fourth (D), to rhodococci and nocardiae. The remaining 23 strains, including those of *Mycobacterium agri*, *M. smegmatis*, and *M. vaccae*, were located outside of these four clusters. Each of these clusters is discussed below.

Cluster A (strains 1-167). In cluster A (Fig.

1, 2, and 3; slowly growing mycobacteria), the tuberculosis (TB) complex, consisting of *Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*, formed a compact subcluster at a level of 97%. Within this subcluster, strains of *M. tuberculosis* could be distinguished from strains of the other three species, but these could not be distinguished from each other (Fig. 1).

In cluster A, the following 11 subclusters were evident: 1, TB complex; 2, *M. avium*—*M. intracellulare*—*M. scrofulaceum* complex; 3, *M. gordonae*; 4, *M. xenopi*; 5, *M. nonchromogenicum*; 6, *M. gastri*; 7, *M. kansasii*; 8, *M. szulgai*; 9, *M. shimoidei*; 10, *M. marinum*; and 11, strains 165 and 166. These subclusters were formed at levels between 92 and 98%. A close relationship between subclusters 2 (*M. avium*—*M. intracellulare*—*M. scrofulaceum* complex) and 3 (*M. gordonae*) was observed. These were combined into a single cluster at a level of 93%. Subclusters 2 to 11, inclusive, formed a single cluster at a level of 89% and were linked together with subcluster 1 (TB complex) at a level of 85%.

FIG. 1. Dendrogram (part 1). Subcluster 1, TB complex; 1a, *M. tuberculosis*; 1b, *M. africanum* and *M. bovis*; 1c, *M. bovis*; 1d, *M. bovis*; 1e, *M. microti*.

FIG. 2. Dendrogram (part 2). Subcluster 2, *M. avium*—*M. intracellulare*—*M. scrofulaceum* complex; 2a, Marks's strains of *M. intracellulare*; 2b, *M. intracellulare*; 2c, *M. avium*; 2d, *M. intracellulare*; 2e, *M. scrofulaceum*; 3, *M. gordonae*.

Cluster B (strains 168–175). Cluster B (Fig. 3) was composed of strains of only two mycobacterial species, *M. flavescens* (subcluster 12) and *M. thermoresistibile* (subcluster 12). These two subclusters consisted of strains intermediate between the slowly growing and the rapidly growing mycobacteria.

Cluster C (strains 176–225). Cluster C (Fig. 4) was composed of the following subclusters

(14–22, inclusive) of rapidly growing mycobacteria: 14, *M. fortuitum*; 15, *M. aurum*; 16, *M. abscessus* (*M. chelonae* subsp. *abscessus*); 17, *M. chelonae* (*M. chelonae* subsp. *chelonae*); 18, *M. phlei*; 19, *M. parafortuitum*; 20, *M. chubuense*; 21, *M. aichiense*; and 22, *M. chitae*. (*M. rhodesiae* was not shown as a subcluster, because only a single strain was tested in this study.)

Subclusters 14 to 22, inclusive, were distinctly

FIG. 3. *Dendrogram* (part 3). Subcluster 4, *M. xenopi*; 5, *M. nonchromogenicum*; 6, *M. gastri*; 7, *M. kansasii*; 8, *M. szulgai*; 9, *M. shimoidei*; 10, *M. marinum*; 11, strains 165 and 166; 12, *M. flavescentis*; 13, *M. thermoresistibile*.

differentiated from each other at a level of 90% and were combined into a single cluster, C, at a level of 85%.

Cluster D (strains 226–345). Cluster D (Fig. 5 and 6) were composed of subclusters (23–33, inclusive) of strains of the following species of the genera *Rhodococcus* and *Nocardia*: 23, *Rhodococcus lentifragmentus* (formerly *Gordona lentifragmenta* [26]; according to Goodfellow and Alderson [3], *R. ruber*; synonym: *Nocardia rubra*); 24, *R. rhodochrous* (synonym: *Gordona rhodochroa*); 25a, *R. terrae* (synonym: *Gordona terrae*); 25b, *R. rubropertinctus* (synonyms: *Gordona rubropertincta* and *G. rubra*); 25c, *R. roseus* (synonym: *Gordona rosea*); 26, *R. bronchialis* (synonym: *Gordona bronchialis*); 27, *Nocardia asteroides* B (23); 28, *N. asteroides* A (23); 29, *N. brasiliensis*; 30, *N. caviae*; 31, *N. farcinica*; 32, *R. aurantiacus* (synonym: *Gordona aurantiaca*); and 33, *Jensenia canicruria* (three replicates from a single strain).

Cluster D contained two large subclusters, D1 (from 23–27, Fig. 5) and D2 (from 28–31, Fig. 6), the former consisting of *R. lentifragmentus* to

N. asteroides B and the latter consisting of *N. asteroides* A to *N. farcinica*. Subclusters D1 and D2 corresponded to rhodococci and nocardiae, respectively, although some strains of *N. asteroides* were located in the former. Strains of *R. aurantiacus* and *J. canicruria* were located outside of these two large subclusters. The single strain of *Corynebacterium equi* was incorporated into cluster D.

Distinguishing characters. Characters useful for differentiating clusters A, B, C, and D from each other are given in Table 2. Distinctive characters of the species in each cluster are presented in Tables 3 to 5.

DISCUSSION

In the present study, four major clusters of the 369 strains studied were observed (Fig. 7). These clusters were composed of: (A) slowly growing mycobacteria; (B) intermediate mycobacteria; (C) rapidly growing mycobacteria; and (D) rhodococci and nocardiae. Previously, Tsukamura (17, 21) suggested that slowly growing

FIG. 4. Dendrogram (part 4). Subcluster 14, *M. fortuitum*; 15, *M. aurum*; 16, *M. abscessus* (*M. chelonae* subsp. *abscessus*); 17, *M. chelonae* (*M. chelonae* susp. *chelonae*); 18, *M. phlei*; 19, *M. parafortuitum*; 20, *M. chubuense*; 21, *M. aichiense*; 22, *M. chitae*.

FIG. 5. Dendrogram (part 5). Subcluster 23, *R. lentifragmentus*; 24, *R. rhodochrous*; 25a, *R. terrae*; 25b, *R. rubropertinctus*; 25c, *R. roseus*; 26, *R. bronchialis*; 27, *N. asteroides* B.

FIG. 6. *Dendrogram* (part 6). Subcluster 28, *N. asteroides* A; 29, *N. brasiliensis*; 30, *N. caviae*; 31, *N. farcinica*; 32, *R. aurantiacus*; 33, *J. canicruria*; 34, *M. agri*; 35, *M. smegmatis*; 36, mycobacterial strains 363 and 364; 37, *M. vaccae*.

TABLE 2. Characters useful for distinguishing clusters A, B, C, and D

Cluster	Sub-cluster ^a	No. of strains tested	No. of strains showing positive reaction															
			Strong acid fastness	Fragmenting mycelium	Growth after 3 days	Tolerance to nitrite	Tolerance to picric acid	β -Galactosidase	Nitrate reduction	Arylsulfatase (2 weeks)	Mannose as C source	Sucrose as C source	Succinate as C source	Malate as C source	Fumarate as C source	Acid from glucose	Glutamate as N and C source	Trimethylenediamine as N and C source
A	1	50	50	0	0	0	0	0	21	22	0	0	0	0	0	0	0	0
	2	63	63	0	0	0	0	0	0	52	0	0	0	0	0	0	3	0
	3	8	8	0	0	0	0	0	0	8	0	0	0	0	0	0	1	0
	4	4	4	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
	5	3	3	0	0	1	0	3	0	3	0	0	0	0	0	0	0	0
	6	7	7	0	0	0	0	0	7	7	0	0	0	0	0	0	0	0
	7	10	10	0	0	0	0	0	10	10	0	0	0	0	0	0	5	0
	8	5	5	0	0	0	0	0	5	4	0	0	0	0	0	0	0	0
	9	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	2	2	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0
	11	2	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
B	12	3	3	0	3	3	3	0	3	3	3	0	2	0	0	2	3	0
	13	5	5	0	5	5	5	0	5	0	0	0	2	4	5	0	5	0
C	14	18	18	0	18	18	18	0	18	18	18	0	18	18	18	18	18	18
	15	2	2	0	2	2	2	0	2	2	2	0	2	2	2	2	2	2
	16	6	6	0	6	6	6	6	0	6	6	0	6	6	4	4	6	6
	17	5	5	0	5	5	0	5	0	5	5	0	1	0	1	5	5	0
	18	5	5	0	5	5	5	5	5	5	5	1	5	5	5	5	5	5
	19	3	3	0	3	3	3	0	2	3	3	3	3	3	3	3	3	3
	20	3	3	0	3	3	3	0	3	3	3	3	3	3	3	3	3	3
	21	3	3	0	3	3	3	0	0	3	3	0	3	3	3	3	3	0
	22	3	3	0	3	3	3	0	3	3	3	0	0	0	3	3	3	0
D	23	11	0	9	11	11	11	0	10	0	11	5	11	11	11	11	11	0
	24	4	0	1	4	4	4	0	4	0	4	4	3	4	4	4	4	0
	25	11	0	0	11	11	11	0	11	0	11	11	11	11	11	11	11	0
	26	5	0	0	5	5	5	0	5	0	5	5	5	5	5	5	5	0
	27	10	0	10	10	10	10	10	7	4	1	0	8	7	9	6	10	0
	28	7	0	7	7	7	7	7	2	0	2	0	7	7	6	2	7	0
	29	4	0	4	4	4	4	4	0	0	3	0	4	4	4	4	4	0
	30	8	0	8	8	8	8	8	0	0	8	1	8	8	8	8	8	0
	31	39	0	39	39	25	38	39	1	0	38	12	39	37	38	39	39	0
	32	6	0	0	6	6	6	6	1	0	6	6	6	6	6	6	6	0
	33	3	0	0	3	3	3	0	0	0	3	3	3	3	3	3	1	0
	34	4	4	0	4	4	4	4	4	4	4	0	4	4	4	4	4	0
	35	5	5	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	36	2	2	0	2	2	2	0	0	0	2	2	2	2	2	2	2	2
	37	3	3	0	3	3	3	0	0	3	3	0	0	0	0	3	3	3

^a Subclusters: 1, TB complex; 2, *M. avium*-*M. intracellulare*-*M. scrofulaceum* complex; 3, *M. gordonae*; 4, *M. xenopi*; 5, *M. nonchromogenicum*; 6, *M. gastri*; 7, *M. kansasii*; 8, *M. szulgai*; 9, *M. shimoidei*; 10, *M. marinum*; 11, mycobacterial strains 165 and 166; 12, *M. flavescens*; 13, *M. thermoresistibile*; 14, *M. fortuitum*; 15, *M. aurum*; 16, *M. abscessus*; 17, *M. chelonae*; 18, *M. phlei*; 19, *M. parafortuitum*; 20, *M. chubuense*; 21, *M. aichiense*; 22, *M. chitae*; 23, *R. lentifragmentus*; 24, *R. rhodochrous*; 25, *R. terrae*-*R. rubropertinctus*-*R. roseus*; 26, *R. bronchialis*; 27, *N. asteroides* B; 28, *N. asteroides* A; 29, *N. brasiliensis*; 30, *N. caviae*; 31, *N. farcinica*; 32, *R. aurantiacus*; 33, *J. canicruria*; 34, *M. agri*; 35, *M. smegmatis*; 36, mycobacterial strains 363 and 364; 37, *M. vaccae*.

TABLE 3. Distinguishing characters for the species of clusters A and B^a

Character	% of strains showing positive reaction								
	<i>M. tuberculosis</i> (n = 19)	<i>M. bovis-M. africanum-M. microti</i> complex (n = 31)	<i>M. avium-M. intracellulare-M. scrofulaceum</i> complex (n = 63)	<i>M. goodii</i> (n = 8)	<i>M. xenopi</i> (n = 4)	<i>M. gastri</i> (n = 7)	<i>M. kansasii</i> (n = 10)	<i>M. szulgai</i> (n = 5)	<i>M. thermoresistibile</i> (n = 5)
Rough colonies	100	84	0	0	0	0	100	60	100
Photochromogenicity	0	0	2	0	0	0	100	0	0
Resistance to rifampin (25 µg/ml)	0	0	100	50	75	14	0	20	100
Resistance to <i>p</i> -aminosalicylic acid (2 mg/ml)	0	0	81	25	25	0	0	0	100
NH ₂ OH-HCl (0.125 mg/ml)	0	0	100	100	75	100	100	100	100
NH ₂ OH-HCl (0.25 mg/ml)	0	0	100	100	25	100	30	100	0
NH ₂ OH-HCl (0.5 mg/ml)	0	0	91	50	0	0	0	100	0
Resistance to TCH (10 µg/ml)	90	0	100	100	100	100	100	100	100
<i>p</i> -Nitrobenzoic acid medium	0	0	100	100	100	100	100	100	100
Niacin	100	58	0	0	0	0	0	0	0
Tween hydrolysis (14 days)	0	0	2	100	100	100	90	40	100
α -Esterase	100	97	100	88	100	0	0	80	100
β -Esterase	100	94	95	100	100	0	20	80	100
Acid phosphatase (3 h)	0	0	79	0	0	100	90	100	100
Nitrate reduction (24 h)	100	3	0	0	0	100	100	100	100
Arylsulfatase (2 weeks)	0	74	81	100	100	100	100	100	0
Urease	100	94	16	63	0	100	100	100	100
Nicotinamidase	95	36	33	13	50	100	100	0	100
Pyrazinamidase	89	19	33	13	50	0	0	0	100
Growth at 28°C	5	26	100	100	0	100	100	100	100
Growth at 45°C	0	0	65	0	100	0	0	0	100
Growth at 52°C	0	0	0	0	0	0	0	0	100
Glucose as C source (NH ₃ -N)	0	0	56	100	0	100	100	0	100
<i>n</i> -Propanol as C source (NH ₃ -N)	0	0	65	100	0	100	30	0	100
<i>iso</i> -Butanol as C source (NH ₃ -N)	0	0	41	100	0	86	40	0	100
<i>n</i> -Butanol as C source (NH ₃ -N)	0	0	44	88	0	86	40	0	100
Acetate as C source (NH ₃ -N)	0	0	97	100	75	100	50	100	100
Pyruvate as C source (NH ₃ -N)	0	0	94	100	75	100	100	100	100
Fumarate as C source	0	0	0	0	0	0	0	0	100
Glucose as C source (glutamate-N)	0	26	86	100	0	100	100	0	100
Acetate as C source (glutamate-N)	5	26	97	100	100	100	100	100	100
Resistance to ethambutol (5 µg/ml)	0	0	84	0	100	0	10	0	20
Tolerance to 0.1% nitrite	0	0	0	0	0	0	0	0	100
Tolerance to 0.2% picric acid	0	0	0	0	0	0	0	0	100

^a *M. tuberculosis* and the *M. bovis-M. africanum-M. microti* complex are differentiated by resistance to TCH (thiophene-2-carboxylic acid hydrazide), nitrate reduction, and the 2-week arylsulfatase test. These two subgroups form the TB complex. Only species for which more than four strains were studied are shown in the table.

TABLE 4. Distinguishing characters for species of rapidly growing mycobacteria^a

Character	% of strains showing positive reaction						
	<i>M. thermoresistibile</i> (n = 5)	<i>M. fortuitum</i> (n = 18)	<i>M. abscessus</i> (<i>M. chelonae</i> subsp. <i>abscessus</i>) (n = 6)	<i>M. chelonae</i> (<i>M. chelonae</i> subsp. <i>chelonae</i>) (n = 5)	<i>M. phlei</i> (n = 5)	<i>M. agri</i> (n = 4)	<i>M. smegmatis</i> (n = 5)
Pigmentation of colonies	100	0	0	0	100	0	0
Tolerance to 0.2% picric acid (Sauton agar)	100	100	100	0	100	100	100
Resistance to NH ₂ OH-HCl (0.25 mg/ml)	0	100	100	100	0	0	60
Tween hydrolysis (7 days)	100	0	0	0	100	100	100
α -Esterase	100	0	0	100	100	100	0
β -Esterase	100	100	100	100	100	100	0
Acid phosphatase	100	100	17	100	100	0	0
β -Galactosidase	0	6	100	100	100	100	100
Nitrate reduction (24 h)	100	100	0	0	100	100	100
Arylsulfatase (3 days)	0	100	100	100	0	0	0
Arylsulfatase (2 weeks)	0	100	100	100	100	100	100
Salicylate degradation	0	89	100	50	0	0	0
<i>p</i> -Aminosalicylic acid degradation	0	89	100	100	0	0	0
Acetamidase	0	100	0	100	0	100	100
Benzamidase	0	0	0	0	0	0	100
Isonicotinamidase	0	0	0	0	0	0	100
Allantoinase	0	100	0	0	0	100	60
Growth at 45°C	100	17	0	0	100	100	100
Growth at 52°C	100	0	0	0	100	0	0
<i>n</i> -Propanol as C source	100	100	0	0	100	100	100
<i>n</i> -Butanol as C source	100	100	0	100	100	100	100
<i>iso</i> -Butanol as C source	100	100	0	0	100	25	100
Propylene glycol as C source	0	100	0	20	40	25	100
Galactose as C source	0	0	0	0	80	0	100
Arabinose as C source	0	0	0	0	100	0	100
Xylose as C source	0	0	0	0	40	100	100
Rhamnose as C source	0	0	0	0	0	0	100
Trehalose as C source	0	56	67	100	80	0	100
Inositol as C source	0	0	0	0	0	100	100
Mannitol as C source	0	56	0	0	100	0	100
Sorbitol as C source	0	0	0	0	100	0	100
Citrate as C source	0	44	0	100	0	0	100
Succinate as C source	0	100	100	20	100	100	100
Malate as C source	80	100	100	0	100	100	100
Benzoate as C source	0	0	0	0	0	0	100
Malonate as C source	0	0	0	0	100	0	100
Serine as N and C source	0	44	100	100	0	0	100
Glucosamine as N and C source	0	100	33	0	20	100	100
Acetamide as N and C source	0	61	0	0	100	0	100
Benzamide as N and C source	0	0	0	0	0	0	100
Monoethanolamine as N and C source	0	100	100	100	20	0	100
Trimethylenediamine as N and C source	0	100	100	0	100	0	100
Nitrite as N source	0	89	100	0	100	0	100
Resistance to ethambutol (5 µg/ml)	20	100	100	100	100	0	100

^a Only species for which more than four strains were studied are shown in the table. Tests for the utilization of sole carbon sources included ammoniacal nitrogen in the medium.

TABLE 5. *Distinguishing characters for species of cluster D^a*

Character	% of strains showing positive reaction											
	<i>R. bronchialis</i> (n = 5)	<i>R. rhodochrous</i> (n = 4)	<i>R. terrae</i> (n = 4)	<i>R. rubropertinctus</i> (n = 5)	<i>R. roseus</i> (n = 2)	<i>R. lentifragmentus</i> (n = 11)	<i>R. aurantiacus</i> (n = 6)	<i>N. asteroides</i> A (n = 7)	<i>N. asteroides</i> B (n = 10)	<i>N. brasiliensis</i> (n = 4)	<i>N. caviae</i> (n = 8)	<i>N. farcinica</i> (n = 39)
Fragmenting mycelium	0	25	0	0	0	82	0	100	100	100	100	100
Resistance to rifampin (25 µg/ml)	100	100	100	100	100	0	100	86	90	100	100	97
Tween hydrolysis (7 days)	0	0	0	0	0	0	83	0	0	0	0	0
Tween hydrolysis (14 days)	0	0	0	0	0	0	100	43	20	0	75	64
α-Esterase	0	0	100	0	0	0	0	0	100	75	0	5
β-Esterase	100	100	100	100	100	0	100	29	100	100	62	79
Acid phosphatase	100	0	0	0	0	0	0	43	0	75	75	5
Catalase (semiquantitative)	100	100	100	0	50	73	17	6	40	75	75	28
β-Galactosidase	0	0	0	0	0	0	100	100	100	100	100	100
Nitrate reduction	100	100	100	100	100	91	0	14	70	0	0	3
Acetamidase	100	50	0	0	100	100	100	0	30	25	0	100
Benzamidase	0	0	0	0	0	0	17	0	0	0	0	69
Urease	100	25	100	100	100	18	100	100	70	100	100	100
Nicotinamidase	100	0	100	20	0	82	100	57	80	50	100	95
Pyrazinamidase	100	0	100	20	0	82	100	43	60	25	88	95
Allantoinase	0	0	100	100	0	18	100	57	20	75	88	10
Growth at 45°C	0	0	0	0	0	0	0	0	0	0	0	82
Sucrose as C source	100	100	100	100	100	45	100	0	0	0	13	26
Propylene glycol as C source	100	100	100	100	100	100	100	0	0	0	0	64
Galactose as C source	0	0	0	0	0	0	100	0	0	100	0	3
Rhamnose as C source	0	0	100	0	0	0	0	0	0	0	0	85
Trehalose as C source	100	0	100	100	100	0	100	0	0	100	25	26
Inositol as C source	100	0	0	0	0	0	100	0	0	100	75	3
Mannitol as C source	0	0	100	100	100	100	100	0	0	75	100	3
Sorbitol as C source	0	0	100	100	100	91	100	100	0	0	0	0
Citrate as C source	20	50	50	100	100	100	100	86	0	100	13	5
Benzoate as C source	0	75	0	100	50	100	33	0	0	0	0	0
Serine as N and C source	0	0	0	0	0	9	100	0	0	0	100	8
Glucosamine as N and C source	20	100	50	100	100	82	100	57	0	75	100	95
Acetamide as N and C source	100	0	0	0	100	0	100	0	0	0	0	95
Monoethanolamine as N and C source	0	0	75	100	0	91	100	0	0	0	88	100
Benzamide as N source	0	0	75	40	0	0	0	0	70	0	0	69

^a Tests for the utilization of sole carbon sources included ammoniacal nitrogen in the medium.

mycobacteria and rapidly growing mycobacteria be placed in taxa comparable to *Nocardia* or *Gordona* (now *Rhodococcus*). This suggestion was supported by the results obtained from the present study.

Cluster D was divided into approximately two subclusters, one composed of the strains of *Rho-*

dococcus and another consisting of the strains of *Nocardia*. *R. aurantiacus* and *J. canicruria* (according to Goodfellow and Alderson [3], *J. canicruria* is *R. erythropolis*) were located in cluster D but were located on the outside of these subclusters. The single strain of *C. equi* studied was incorporated into cluster D. This

FIG. 7. General view of dendrogram. Cluster A, Subclusters 1 to 11; cluster B, subclusters 12 and 13; cluster C, subclusters 14 to 22; cluster D, subclusters 23 to 33.

appears to be reasonable, because *C. equi* was recently transferred by Goodfellow and Alderson (3) to *Rhodococcus* as *R. equi*.

N. asteroides was divided into two subclusters, 27 and 28. Previously, *N. asteroides* was divided by Tsukamura (15) into two species, *N. asteroides* in the strict sense and *N. farcinica*. The former was recently further divided into two subgroups (23). The same findings were obtained in the present study. The strains received as *N. asteroides* were divided into three subgroups, *N. asteroides* A, *N. asteroides* B, and *N. farcinica*. After the study of Tsukamura (15), Goodfellow (2), Kurup and Schmidt (7), and Mordarski et al. (9) divided *N. asteroides* into five, four, and three subgroups, respectively. One of the subgroups of Mordarski et al. (9) is probably identical to *N. farcinica* of Tsukamura (15). The determination of the relationships of the subgroups of these authors remains a problem for future study.

As shown previously by Tsukamura (14, 17, 21) and in the present study, slowly growing and rapidly growing mycobacteria seem to be valid taxa of generic (or subgeneric) rank. Strains of a few species of rapidly growing mycobacteria remained as outsiders, namely, those of *M. agri*, *M. smegmatis*, and *M. vaccae*, as did strains of scotochromogenic, rapidly growing mycobacteria isolated from a bovine thelitis lesion by Shimizu et al. (11).

The strains received as members of *Gordona* and those designated "*M.*" *rhodochrous* were regarded as belonging to a single genus, *Rhodococcus*, in numerical taxonomy studies carried out by Tsukamura (20) and by Goodfellow and Alderson (3), although some doubts about this conclusion have been raised (1, 6, 8, 10). The results of the present study support the previously proposed concept that the taxa heretofore designated as *Gordona*, "*M.*" *rhodochrous* complex, and *Nocardia rubra* belong to one and the same taxon. It was also shown that the genera *Rhodococcus* and *Nocardia* are more closely related to each other than they are to the mycobacteria.

In a previous numerical taxonomy study by Tsukamura (22), three species, *M. avium*, *M. intracellulare*, and *M. scrofulaceum* were combined into one, the correct name of which is *M. avium*. This finding was supported in the present study.

Strains of *M. thermoresistible* and those of *M. flavescens* formed a unique cluster, B, which is distinct from the clusters of both the slowly growing and the rapidly growing mycobacteria. In a previous study, in which some linked characters were used, *M. thermoresistible* was placed in the cluster of slowly growing mycobacteria

(14), and in another study, in which hypothetical median organisms were used, *M. flavescens* and *M. thermoresistible* were placed in the cluster of rapidly growing mycobacteria (21). The results obtained in the present study, in which a large number of strains were used and linked characters were omitted as far as possible, are considered to be more reliable than those of the two studies just mentioned.

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REPRINT REQUESTS

Address reprint requests to: Dr. M. Tsukamura, National Chubu Hospital, Obu, Aichi 474, Japan.

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