Numerical Analysis of Rapidly Growing, Nonphotochromogenic Mycobacteria, Including *Mycobacterium agri* (Tsukamura 1972) Tsukamura sp. nov., nom. rev.

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Numerical analyses of 165 strains of rapidly growing, nonphotochromogenic mycobacteria were carried out with 104 characters in one case and 84 in another. The results showed that the following taxa are distinct: *Mycobacterium fortuitum*; *M. chelonae* subsp. *chelonae*; *M. chelonae* subsp. *abscessus*; *M. smegmatis*; *M. chitae*; and "*M. agri*" (names in quotation marks are not on the Approved Lists of Bacterial Names, 1980). The distinctness of "*M. agri*" was confirmed by using 29 test strains. Strains of *M. smegmatis* were divided into two subgroups, "*M. smegmatis* subsp. *smegmatis*" and "*M. smegmatis* subsp. *lacticola*" on the basis of amidase patterns. Clusters of *M. fortuitum*, *M. chelonae* subsp. *chelonae*, and *M. chelonae* subsp. *abscessus* were distinguished at a level of 89% similarity by a numerical taxonomy method in which 84 characters were used, and these three clusters were connected to each other to form a large cluster at a level of 88%. Therefore, these three taxa were regarded as belonging to a complex. Within the cluster *M. fortuitum*, four subclusters were observed, but about one-half of the *M. fortuitum* strains studied did not belong to any of the four subclusters. The 78 strains of *M. fortuitum* studied were regarded as homogeneous, even though several biovars could be differentiated. Neither subcluster nor biovar of the *M. fortuitum* strains was shown to be significantly associated with disease in humans. Because *Mycobacterium agri* was not included on the Approved Lists of Bacterial Names, it is herein proposed as a revived name. The type strain of *M. agri* is 90012 (= ATCC 27406).

The number of species of rapidly growing, nonphotochromogenic mycobacteria is small. At present, the following species are generally accepted (13, 17-19): *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann 1899 (14); *M. fortuitum* Da Costa Cruz 1938 (7); *M. chelonae* Bergey et al. 1923 (1); *M. chitae* Tsukamura 1967 (27); *M. farcinogenes* Chamoiseau 1979 (6); and *Mycobacterium senegalense* (Chamoiseau) Chamoiseau 1979 (6). In 1972, Kubica et al. (13) created two subspecies of *M. chelonae*—*M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *abscessus*—having reduced "*Mycobacterium abscessus* Moore and Frerichs 1953" (15) (all names in quotation marks are not on the Approved Lists of Bacterial Names [19]) to subspecies rank. Runyon et al. (17) recognized only three species of these mycobacteria: *M. smegmatis*, *M. chelonae*, and *M. fortuitum*. Subsequently, Saito et al. (18), reporting as a study by the International Working Group for Mycobacterial Taxonomy, considered *M. chitae* as an acceptable species. In addition, in 1972, Tsuka-

mura (30) proposed "*Mycobacterium agri*" as a new species. However, this name was not included on the Approved Lists of Bacterial Names (19), and thus one of the objectives of this paper is to effect its valid publication.

To date, all known rapidly growing, nonphotochromogenic mycobacteria have been found in the environment (soil, dust, etc.) (36). Of these, the most commonly occurring is *M. fortuitum*, and only *M. fortuitum* and *M. chelonae* are regarded as opportunistic pathogens (35). The fact that the most commonly occurring organism is an opportunistic pathogen prompts the question of whether some subgroups of this organism are likely to be pathogenic. Bönicke (4) reported three biovars, and Tsukamura et al. (40) differentiated two subspecies, "*M. fortuitum* subsp. *fortuitum*" and "*M. fortuitum* subsp. *thermo-philum*," and stated that there are several biovars within the type subspecies. Recently, Grange and Stanford (10, 22) and Pattyn et al. (16) reported that there are several subtypes of *M. fortuitum*. Grange and Stanford (10) stated

that, of seven serovars, serovar I was most frequently associated with disease and was associated with biovar A of Bönicke, lipid type F, agglutination type F, and sensitin type 3 (10, 16). Thus, the second purpose of the present study was to determine whether there are some distinct subgroups, especially within the species *M. fortuitum*.

MATERIALS AND METHODS

Test strains. One hundred and sixty-five strains of rapidly growing, nonphotochromogenic mycobacteria were studied (Table 1).

Characters tested. One hundred and four characters (Table 2) were determined for each of the test strains. The methods of determining the characters were reported previously (25, 26, 31, 33).

Numerical classification. The matching coefficient (*M* value) between any two strains was calculated by the equation $M \text{ value (\%)} = (n_s \times 100\%) / (n_s + n_d)$, where n_s is the number of characters that show similar code symbols (++) or (--) and n_d is the number of characters which show different code symbols (+-). Clustering was done by the single-linkage method (20). 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 data for the original "strain x characters" matrix have been deposited with the World Data Center for Microorganisms, Brisbane, Australia.

Two methods of numerical classification were carried out, one using all 104 characters and another using 84 of the characters; omitted were 9 characters (no. 1 through 4, 9, and 64 through 67) ineffective in differentiating the strains; 4 characters (no. 30, 31, 35, and 36) which showed positive matches in almost all (but not all) strains; 3 characters (no. 26, 34, and 46) related to others; and 4 characters (no. 8, 98, 100, 101) in which the interpretation of results was not clear-cut.

Association with disease. Thirteen of the strains of *M. fortuitum* were considered to be associated with disease. Of these, two were Cruz's classical strains that were isolated from cold abscesses of humans (ATCC 6841 and ATCC 6842), two were isolated from lymphadenitis of swine, and nine were isolated from sputum specimens from patients who were considered to have disease due to *M. fortuitum*. Within 3 months, the nine patients each showed at least three isolations, one of which was used for this study, and, concomitantly with these isolations, the appearance of cavitory lesions in their lungs; these isolates were collected through the Mycobacteriosis Research Group of the Japanese National Chest Hospitals.

RESULTS

Almost similar results were obtained in the classification of the study strains by means of two numerical taxonomy analyses, one involving 104 characters and the other involving 84. The differentiation between strains received as *M. fortuitum* and those received as *M. chelonae* was not so clear-cut in the former analysis. Hence,

the results obtained in the latter analysis are shown in Fig. 1 to 3, wherein the differentiation between the two species is clear-cut.

Except for strains 104, 106, 107, and 108, the strains received as *M. fortuitum*, *M. chelonae* subsp. *chelonae*, or *M. chelonae* subsp. *abscessus* formed a cluster at a similarity level of 89%. Strains received as *M. smegmatis*, *M. chitae*, or "*M. agri*" formed clusters at levels of 95, 96, and 92%, respectively. Except for strains 125 and 132, the strains of *M. smegmatis* were divided into two subclusters at a level of 96%. One contained strain ATCC 14468, which was suggested as the neotype strain for *M. smegmatis* in *Bergey's Manual*, 8th edition (17), and another contained strain ATCC 607.

Within the *M. fortuitum* cluster, four small subclusters were observed, FOR1A, FOR1B, FOR2, and FOR3; about one-half of the strains did not form any subcluster. The first two subclusters belonged to the same subcluster according to the analysis in which 104 characters were used, but they could be differentiated in the analysis involving 84 characters.

The results obtained in this study show that the strains labeled "*M. agri*" form a distinct cluster, and these are here regarded as belonging to a distinct species. Because the name "*M. agri*" was not included on the Approved Lists (19), it is proposed here as a revived name for the same organism for which it was previously used (30, 32). A description of this organism is presented below.

Characters useful in distinguishing between the species of rapidly growing, nonphotochromogenic mycobacteria. As mentioned above, cluster formation was more clear-cut in the taxonomic analysis in which 84 characters were used. Therefore, the characters of the clusters (species) obtained in this analysis are shown in Table 2. Table 3 shows distinguishing characters of the subclusters of *M. fortuitum*.

Relationship between the association with disease and the biovar in the *M. fortuitum* strains. Subclusters FOR1A, FOR1B, FOR2, and FOR3 of *M. fortuitum* contained 11, 9, 4, and 5 strains, respectively. The number of strains associated with disease contained in these subclusters were three, one, one, and two, respectively. Therefore, there was no significant difference among the percentages of the disease-associated strains (χ^2 test; $P > 0.05$). Furthermore, correlations of disease with the amidase patterns, the patterns of utilization of nitrogen compounds as simultaneous nitrogen and carbon sources, and the patterns of utilization of carbohydrates as the carbon source in the presence of ammoniacal nitrogen were tested with these

TABLE 1. *Strains used*

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
1	E8363	<i>Mycobacterium fortuitum</i>		1	Sputum, humans
2	E8366	<i>M. fortuitum</i>		1	Sputum, humans
3	E8296	<i>M. fortuitum</i>		1	Sputum, humans
4	E8359	<i>M. fortuitum</i>		1	Sputum, humans
5	E8292	<i>M. fortuitum</i>		1	Sputum, humans
6	E8361	<i>M. fortuitum</i>		1	Sputum, humans
7	18109	<i>M. fortuitum</i>	ATCC 6842	2	Humans, cold Abscess ^b
8	18112	<i>M. fortuitum</i>	ATCC 6841 (type)	2	Humans, cold Abscess ^b
9	18224	<i>M. fortuitum</i>	E7921	1	Sputum, humans
10	42001	" <i>M. minetti</i> "	ATCC 19542 (type)	2	Cow
11	E9101	<i>M. fortuitum</i>	Patient 1	1	Sputum, humans ^b
12	18077	<i>M. fortuitum</i>	R48	3	?
13	18080	<i>M. fortuitum</i>	R449	3	?
14	18083	<i>M. fortuitum</i>	R520	3	?
15	18096	<i>M. fortuitum</i>	R945	3	?
16	18001	<i>M. fortuitum</i>	ATCC 19709	2	Soil
17	18158	<i>M. fortuitum</i>	E5792	1	Swine, lymph nodes ^b
18	18005	<i>M. fortuitum</i>	E302	1	Soil
19	18010	<i>M. fortuitum</i>	R389	3	?
20	18082	<i>M. fortuitum</i>	R490	3	?
21	18002	<i>M. fortuitum</i>	E334	1	Soil
22	18004	<i>M. fortuitum</i>	E313	1	Soil
23	18054	<i>M. fortuitum</i>	E1294	1	Soil
24	18102	<i>M. fortuitum</i>	R1028	3	?
25	18071	<i>M. fortuitum</i>	NCTC 1542	3	?
26	E8276	<i>M. fortuitum</i>		1	Sputum, humans
27	E8864	<i>M. fortuitum</i>	Patient 2	1	Sputum, humans ^b
28	E9099	<i>M. fortuitum</i>		1	Sputum, humans
29	E9100	<i>M. fortuitum</i>		1	Sputum, humans
30	E8298	<i>M. fortuitum</i>		1	Sputum, humans
31	18212	<i>M. fortuitum</i>	E7615 (Kamada)	1	Sputum, humans ^b
32	E8299	<i>M. fortuitum</i>		1	Sputum, humans
33	18231	<i>M. fortuitum</i>	E8000	1	Sputum, humans
34	18097	<i>M. fortuitum</i>	R962	3	?
35	18221	<i>M. fortuitum</i>	E7865	1	Sputum, humans
36	E6029	<i>M. fortuitum</i>		1	Sputum, humans
37	E6023	<i>M. fortuitum</i>		1	Sputum, humans
38	E6081	<i>M. fortuitum</i>	Patient 3	1	Sputum, humans ^b
39	18003	<i>M. fortuitum</i>	E330	1	Soil
40	18209	<i>M. fortuitum</i>	E7252	1	Sputum, humans
41	18233	<i>M. fortuitum</i>	E8112	1	Sputum, humans
42	18085	<i>M. fortuitum</i>	R640	3	?
43	18034	<i>M. fortuitum</i>	E1274	1	Soil
44	18035	<i>M. fortuitum</i>	E1275	1	Soil
45	18073	" <i>M. giae</i> "	ATCC 11440 (type)	2	Anuran organ
46	18074	" <i>M. peregrinum</i> "	ATCC 14467 (type)	2	Humans
47	18229	<i>M. fortuitum</i>	E7976 (Matsui)	1	Sputum, humans ^b
48	18007	<i>M. fortuitum</i>	E308	1	Soil
49	18089	<i>M. fortuitum</i>	R681	3	?
50	18099	<i>M. fortuitum</i>	R965	3	?
51	18105	<i>M. fortuitum</i>	R1208	3	?
52	18223	<i>M. fortuitum</i>	E7919 (Matsumoto)	1	Sputum, humans ^b
53	18230	<i>M. fortuitum</i>	E7994	1	Sputum, humans
54	E9082	<i>M. fortuitum</i>		1	Sputum, humans
55	E2671	<i>M. fortuitum</i>		1	Sputum, humans
56	18009	<i>M. fortuitum</i>	NCTC 8697	4	?
57	18087	<i>M. fortuitum</i>	R645	3	?
58	E8291	<i>M. fortuitum</i>		1	Sputum, humans
59	18090	<i>M. fortuitum</i>	R720	1	?
60	18098	<i>M. fortuitum</i>	R963	3	?
61	18180	" <i>M. fortuitum</i> subsp. <i>thermophilum</i> "	ATCC 27408	2	Soil
62	18205	<i>M. fortuitum</i>	E7064	1	Sputum, humans
63	18225	<i>M. fortuitum</i>	E7922	1	Sputum, humans
64	E5963	<i>M. fortuitum</i>	Patient 4	1	Sputum, humans ^b
65	E5766	<i>M. fortuitum</i>	Nishikido	1	Sputum, humans ^b

TABLE 1.—*Continued*

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
66	E8378	<i>M. fortuitum</i>		1	Sputum, humans
67	18207	<i>M. fortuitum</i>	E7119	1	Sputum, humans
68	E8379	<i>M. fortuitum</i>		1	Sputum, humans
69	E9020	<i>M. fortuitum</i>	Hayashida	1	Sputum, humans ^b
70	E8323	<i>M. fortuitum</i>		1	Sputum, humans
71	E8376	<i>M. fortuitum</i>		1	Sputum, humans
72	E9061	<i>M. fortuitum</i>		1	Sputum, humans
73	E8364	<i>M. fortuitum</i>		1	Sputum, humans
74	18103	<i>M. fortuitum</i>	R1202	3	?
75	18220	<i>M. fortuitum</i>	E7826	1	Sputum, humans
76	18222	<i>M. fortuitum</i>	E7916	1	Sputum, humans
77	18226	<i>M. fortuitum</i>	E7923	1	Sputum, humans
78	44002	<i>M. fortuitum</i> ?	E5782	1	Swine, lymph nodes ^b
79	22001	" <i>M. runyonii</i> "	380s	4	Humans
80	22011	" <i>M. runyonii</i> "	ATCC 14472 (type)	2	Humans
81	22012	" <i>M. abscessus</i> "	ATCC 19977 (type)	2	Human abscesses ^b
82	22014	" <i>M. abscessus</i> "	ATCC 23007	2	?
83	22019	" <i>M. abscessus</i> "	ATCC 23045	2	?
84	22021	" <i>M. abscessus</i> "	E2846	1	Sputum, humans
85	22003	" <i>M. runyonii</i> "	518r	4	Humans
86	22015	" <i>M. abscessus</i> "	ATCC 23016	2	?
87	22023	" <i>M. abscessus</i> "	E5359	1	Sputum, humans
88	22002	" <i>M. runyonii</i> ?"	481r	4	Humans
89	22004	" <i>M. abscessus</i> "	RJ-1	1	Sputum, humans ^b
90	19035	<i>M. chelonae</i> subsp. <i>chelonae</i>	E5870	1	Sputum, humans
91	19040	<i>M. chelonae</i> subsp. <i>chelonae</i>	ATCC 23000	2	?
92	19041	<i>M. chelonae</i>	ATCC 23013	2	?
93	19036	<i>M. chelonae</i> subsp. <i>chelonae</i>	E5880	1	Sputum, humans
94	19044	" <i>M. borstelense</i> "	ATCC 19235 (type)	2	Soil or water
95	19045	" <i>M. borstelense</i> "	ATCC 19237	2	Soil or water
96	19001	" <i>M. borstelense</i> "	SN281	5	Soil or water
97	19054	<i>M. chelonae</i>	NCTC 946 (type)	6	Cold blood animal
98	22008	" <i>M. abscessus</i> "	RJ-2	1	Sputum, humans ^b
99	E7918	<i>M. chelonae</i> subsp. <i>chelonae</i>	Patient 5	1	Sputum, humans ^b
100	22031	<i>M. chelonae</i> ?		1	Sputum, humans ^b
101	19037	<i>M. chelonae</i>	E6089	1	Sputum, humans
102	22029	" <i>M. abscessus</i> "	E7633	1	Sputum, humans
103	18232	<i>M. fortuitum</i> ?	E8061	1	Sputum, humans
104	19032	<i>M. chelonae</i>	E5301	1	Sputum, humans
105	19033	<i>M. chelonae</i>	E5377	1	Sputum, humans
106	19038	<i>M. chelonae</i> subsp. <i>chelonae</i>	E6535 (Kato)	1	Resected lung
107	22028	<i>M. chelonae</i> subsp. <i>abscessus</i>	E7556	1	Lesions, humans
108	22030	<i>M. chelonae</i> ?	E7674	1	Sputum, humans
109	41501	" <i>M. farcinogenes</i> var. <i>tchadense</i> "	NCTC 10955	7	Sputum, humans
110	18256	" <i>M. farcinogenes</i> var. <i>senegalense</i> "	NCTC 10956	7	?
111	17005	<i>M. smegmatis</i>	SN5	5	?
112	17014	<i>M. smegmatis</i>	Wa237	8	?
113	17017	<i>M. smegmatis</i>	R1103	8	?
114	17002	<i>M. smegmatis</i>	SN2	5	?
115	17007	<i>M. smegmatis</i>	SN7	5	?
116	17010	<i>M. smegmatis</i>	SN10	5	?
117	17016	<i>M. smegmatis</i>	R1102	8	?
118	17008	<i>M. smegmatis</i>	SN8	5	?
119	17011	<i>M. smegmatis</i>	Wa63	8	?
120	17012	<i>M. smegmatis</i>	Wa290	8	?
121	17015	<i>M. smegmatis</i>	Takeo	1	Chickens
122	17018	<i>M. smegmatis</i>	CDC	8	?
123	17022	<i>M. smegmatis</i>	Denken	1	Chickens
124	17023	<i>M. smegmatis</i>	Jucho	1	Chickens
125	17027	<i>Mycobacterium</i> sp. <i>M. smegmatis</i>	ATCC 14468 (suggested neotype)	2	?
126	17032	<i>M. smegmatis</i>	ATCC 23019	2	?
127	45001	<i>M. smegmatis</i>	ATCC 607	2	?
128	17035	<i>M. smegmatis</i>	ATCC 23037	2	?
129	17036	<i>M. smegmatis</i>	ATCC 23038	2	?
130	17034	<i>M. smegmatis</i>	ATCC 23032	2	?
131	17031	<i>M. smegmatis</i>	ATCC 23011	2	?

TABLE 1.—Continued

Serial no.	Laboratory no.	Received as:		Source ^a	Habitat
		Name	Strain		
132	17033	<i>M. smegmatis</i>	ATCC 23028	2	?
133	31003	<i>M. chitae</i>	ATCC 19628	2	Chicken organs
134	31004	<i>M. chitae</i>	ATCC 19629	2	Chicken organs
135	31005	<i>M. chitae</i>	ATCC 19630	2	Chicken organs
136	31002	<i>M. chitae</i>	ATCC 19627 (type)	2	Chicken organs
137	90021	" <i>M. agri</i> "	E6224	1	Soil
138	90023	" <i>M. agri</i> "	E6226	1	Soil
139	90017	" <i>M. agri</i> "	E6219	1	Soil
140	90004	" <i>M. agri</i> "	E6206	1	Soil
141	90011	" <i>M. agri</i> "	E6213	1	Soil
142	90012	" <i>M. agri</i> "	ATCC 27406 (type)	1	Soil
143	90013	" <i>M. agri</i> "	E6215	2	Soil
144	90015	" <i>M. agri</i> "	E6217	1	Soil
145	90016	" <i>M. agri</i> "	E6218	1	Soil
146	90009	" <i>M. agri</i> "	E6221	1	Soil
147	90010	" <i>M. agri</i> "	E6212	1	Soil
148	90008	" <i>M. agri</i> "	E6210	1	Soil
149	90003	" <i>M. agri</i> "	E6205	1	Soil
150	90019	" <i>M. agri</i> "	E6222	1	Soil
151	90024	" <i>M. agri</i> "	E6227	1	Soil
152	90025	" <i>M. agri</i> "	E6228	1	Soil
153	90006	" <i>M. agri</i> "	E6208	1	Soil
154	90014	" <i>M. agri</i> "	E6216	1	Soil
155	90026	" <i>M. agri</i> "	E6230	1	Soil
156	90001	" <i>M. agri</i> "	E6203	1	Soil
157	90005	" <i>M. agri</i> "	E6207	1	Soil
158	90031	" <i>M. agri</i> "	E6238	1	Soil
159	90028	" <i>M. agri</i> "	E6232	1	Soil
160	90029	" <i>M. agri</i> "	E6233	1	Soil
161	90007	" <i>M. agri</i> "	E6209	1	Soil
162	90018	" <i>M. agri</i> "	ATCC 27407	2	Soil
163	90027	" <i>M. agri</i> "	E6231	1	Soil
164	90030	" <i>M. agri</i> "	E6234	1	Soil
165	90002	" <i>M. agri</i> "	E6204	1	Soil

^a 1, M. Tsukamura, National Chubu Hospital, Obu, Aichi, Japan; 2, American Type Culture Collection, Rockville, Md.; 3, R. E. Gordon, Rutgers University, New Brunswick, N.J.; 4, K. Takeya, Kyushu University, Fukuoka, Japan; 5, R. Bönicke, Forschungsinstitut Borstel, Borstel, West Germany; 6, J. L. Stanford, Middlesex Hospital Medical School, London, England; 7, M. P. Lechevalier, Rutgers University; 8, H. Saito, Shimane Medical College, Izumo, Shimane, Japan.

^b These are the strains considered to have caused infection in humans (or, in two cases, in swine).

strains. There was no correlation of disease with any of these characters (Table 4).

DISCUSSION

The results presented here show that the strains of "*M. agri*" tested form a distinct cluster when analyzed by numerical taxonomy methods, and thus "*M. agri*" is a valid species of rapidly growing, nonphotochromogenic mycobacteria. The same finding was shown previously but with only several strains (39). *M. agri* is therefore the seventh species of the rapidly growing, nonphotochromogenic mycobacteria after the following six species: *M. smegmatis*, *M. fortuitum*, *M. chelonae*, *M. chitae*, *M. farcinogenes*, and *M. senegalense* (19).

Two strains, 109 and 110, received as *M. farcinogenes* appeared to be distinct from each other. In 1979, Chamoiseau (6) divided this species into two species, *M. farcinogenes* and *M. senegalense*. Strains 109 and 110 are the type

strains of the above-mentioned species, respectively (6). The results of the present study agreed with the finding of Chamoiseau. Both strains showed filamentous forms and, like the strains of *M. chelonae* subsp. *chelonae* and unlike the strains of the other species, were not tolerant to picric acid; strain 110 degraded *p*-aminosalicylate and formed a black formazan.

As mentioned above, the second method of analysis, in which 84 characters were used, resulted in a more clear-cut clustering. Therefore, we should like to discuss the results obtained by the second method.

M. fortuitum, *M. chelonae* subsp. *chelonae*, and *M. chelonae* subsp. *abscessus* formed clusters at the level of 89%, and these were connected with each other and formed one cluster at a level of 88%. These three taxa appeared to be closely related to each other and to be distinct from the other named taxa studied. Therefore, they may be regarded as a complex. In fact the strains of

TABLE 2. *Characters of rapidly growing, nonphotochromogenic mycobacteria*^a

Character	% of strains showing positive reaction						
	<i>M. fortuitum</i> (n = 78)	<i>M. chelonae</i> subsp. <i>abscessus</i> (n = 14)	<i>M. chelonae</i> subsp. <i>chelonae</i> (n = 12)	<i>M. smegmatis</i> subsp. <i>smegmatis</i> (n = 15)	<i>M. smegmatis</i> subsp. <i>lactica</i> (n = 5)	<i>M. chitae</i> (n = 4)	<i>M. agri</i> (n = 29)
1. Strong acid-fastness	95	100	92	100	100	100	100
2. Weak or partial acid-fastness	100	100	100	100	100	100	100
3. Permanent mycelium	0	0	0	0	0	0	0
4. Fragmenting mycelium	0	0	0	0	0	0	0
5. Long rods (more than 7 µm in length)	8	0	0	80	100	0	86
6. Intermediate rods (3-6 µm in length)	87	100	83	100	100	0	100
7. Short rods (less than 2 µm in length)	94	100	100	100	100	0	45
8. Cross-barring	0	0	0	0	0	0	0
9. Cord formation	0	0	0	0	0	0	0
10. Rough colonies	71	64	42	27	100	0	97
11. Colony pigmentation in dark	0	0	0	0	0	0	0
12. Photochromogenicity	0	0	0	0	0	0	0
13. Growth at 3 days (rapid growth)	100	100	100	100	100	100	100
14. Growth at 28°C	100	100	100	100	100	100	100
15. Growth at 37°C	100	100	100	100	100	100	100
16. Growth at 45°C	9	0	0	100	100	0	100
17. Growth at 52°C	0	0	0	0	60	0	7
18. Resistance to 0.2% <i>p</i> -aminosalicylate	100	100	100	100	100	100	100
19. Degradation of <i>p</i> -aminosalicylate	65	100	100	0	0	0	0
20. Resistance to NH ₂ OH·HCl (125 µg/ml)	100	100	100	100	100	100	69
21. Resistance to NH ₂ OH·HCl (250 µg/ml)	100	100	100	60	100	100	0
22. Resistance to NH ₂ OH·HCl (500 µg/ml)	100	100	100	0	0	0	0
23. Growth on Sauton agar	100	100	100	100	100	100	100
24. Tolerance to 0.1% sodium salicylate (Sauton agar)	100	100	25	100	100	0	7
25. Degradation of salicylate	67	93	58	0	0	0	0
26. Tolerance to 0.1% picric acid (Sauton agar)	100	93	25	100	100	100	100
27. Tolerance to 0.2% picric acid (Sauton agar)	99	93	8	100	100	100	100
28. Arylsulfatase (3 days)	94	93	100	87	20	0	24
29. Arylsulfatase (2 weeks)	100	100	100	100	100	100	100
30. Tolerance to thiophene-2-carboxylic acid hydrazide (1 µg/ml)	100	100	100	100	100	100	100
31. Resistance to 0.05% salicylate	100	100	100	100	100	100	100
32. Resistance to ethambutol (5 µg/ml)	99	100	100	93	100	100	0
33. Tolerance to 0.1% NaNO ₂ (Sauton agar)	100	100	92	100	100	100	100
34. Tolerance to 0.2% NaNO ₂ (Sauton agar)	100	100	83	100	100	100	100
35. Growth on 1% Tween-Sauton agar	100	100	92	100	100	100	100
36. Resistance to <i>p</i> -nitrobenzoic acid (0.5 mg/ml)	100	100	100	100	100	100	100
37. Resistance to rifampin (25 µg/ml)	100	100	100	100	100	100	100
38. Niacin production	0	0	8	0	0	0	0
39. Tween hydrolysis (7 days)	1	0	0	100	100	100	0
40. Tween hydrolysis (14 days)	8	7	0	100	100	100	100
41. Catalase (semiquantitative)	37	79	58	7	0	100	100
42. α-Esterase	29	21	58	7	0	100	100
43. β-Esterase	63	100	100	7	0	100	100
44. β-Galactosidase	17	86	100	100	100	0	72

TABLE 2.—Continued

Character	% of strains showing positive reaction						
	<i>M. fortui-</i> <i>tum</i> (n = 78)	<i>M. chelonae</i> subsp. <i>ab-</i> <i>scissus</i> (n = 14)	<i>M. chelonae</i> subsp. <i>che-</i> <i>lonae</i> (n = 12)	<i>M. smeg-</i> <i>mat</i> subsp. <i>smeg-</i> <i>mat</i> (n = 15)	<i>M. smeg-</i> <i>mat</i> subsp. <i>lac-</i> <i>ticola</i> (n = 5)	<i>M. chitae</i> (n = 4)	<i>M. agri</i> (n = 29)
45. Acid phosphatase	35	7	33	0	0	100	3
46. Nitrate reduction (6 h)	95	7	0	100	100	100	93
47. Nitrate reduction (24 h)	100	14	0	100	100	100	100
48. Acetamidase	87	14	8	93	0	100	66
49. Benzamidase	1	0	8	93	0	0	0
50. Urease	100	100	83	100	100	100	100
51. Isonicotinamidase	0	0	0	93	0	0	0
52. Nicotinamidase	64	50	50	93	0	100	100
53. Pyrazinamidase	67	57	33	100	0	100	100
54. Salicylamidase	0	0	0	13	0	0	0
55. Allantoinase	99	0	0	13	100	0	100
56. Succinamidase	0	0	0	93	20	0	0
57. Glutamate as N and C sources	100	100	100	100	100	100	100
58. L-Serine as N and C sources	56	100	75	100	100	0	0
59. Glucosamine as N and C sources	99	29	25	100	100	0	100
60. Acetamide as N and C sources	56	7	0	100	100	100	0
61. Benzamide as N and C sources	0	0	0	100	100	0	0
62. Monoethanolamine as N and C sources	99	100	100	100	100	0	0
63. Trimethylene diamine as N and C sources	100	86	0	100	100	0	0
64. Glucose as C source (glutamate-N)	100	100	100	100	100	100	100
65. Acetate as C source (glutamate-N)	100	100	100	100	100	100	100
66. Succinate as C source (glutamate-N)	100	100	100	100	100	100	100
67. Pyruvate as C source (glutamate-N)	100	100	100	100	100	100	100
68. Acetate as C source	100	100	100	100	100	100	100
69. Citrate as C source	18	7	100	100	100	0	24
70. Succinate as C source	100	86	42	100	100	75	100
71. Malate as C source	100	86	33	100	100	50	100
72. Pyruvate as C source	100	100	83	100	100	100	100
73. Benzoate as C source	3	0	0	100	100	0	0
74. Malonate as C source	4	0	0	100	100	0	21
75. Fumarate as C source	100	57	25	100	100	0	100
76. Fructose as C source	100	71	58	100	100	75	100
77. Sucrose as C source	40	7	0	100	40	25	0
78. Ethanol as C source	91	0	0	100	100	0	69
79. <i>n</i> -Propanol as C source	100	14	0	100	100	50	93
80. Propylene glycol as C source	78	0	0	100	100	0	21
81. 1,3-Butylene glycol was C source	71	0	0	100	100	0	0
82. 1,4-Butylene glycol was C source	14	0	0	40	60	0	0
83. 2,3-Butylene glycol was C source	60	0	0	100	100	0	0
84. <i>n</i> -Butanol as C source	100	79	0	100	100	100	0
85. Isobutanol as C source	100	29	0	100	100	100	0
86. Acid from glucose	100	93	100	100	100	0	100
87. Acid from mannose	100	93	100	100	100	0	100
88. Glucose as C source	100	100	100	100	100	100	100
89. Mannose as C source	100	100	100	100	100	0	100
90. Galactose as C source	0	0	0	100	100	0	0
91. Arabinose as C source	0	0	0	100	100	0	0
92. Xylose as C source	0	0	0	100	100	0	100
93. Rhamnose as C source	0	0	0	100	100	0	0
94. Trehalose as C source	41	36	50	100	100	0	0
95. Inositol as C source	13	0	0	100	100	0	100
96. Mannitol as C source	53	7	0	100	100	0	0
97. Sorbitol as C source	0	0	0	100	100	0	0

TABLE 2.—Continued

Character	% of strains showing positive reaction						
	<i>M. fortuitum</i> (n = 78)	<i>M. chelonaei</i> subsp. <i>abscessus</i> (n = 14)	<i>M. chelonaei</i> subsp. <i>chelonaei</i> (n = 12)	<i>M. smegmatis</i> subsp. <i>smegmatis</i> (n = 15)	<i>M. smegmatis</i> subsp. <i>lacticola</i> (n = 5)	<i>M. chitae</i> (n = 4)	<i>M. agri</i> (n = 29)
98. Acetamide as N source	90	100	50	100	100	100	86
99. Benzamide as N source	5	0	0	100	100	0	0
100. Urea as N source	95	100	42	100	100	100	69
101. Pyrazinamide as N source	91	100	33	100	100	100	100
102. Nicotinamide as N source	91	93	25	100	100	100	10
103. Nitrate as N source	92	100	33	100	100	100	3
104. Nitrite as N source	73	93	0	100	100	0	0

^a Unless otherwise noted, resistance to any agent was tested in Ogawa egg medium. Unless otherwise noted, utilization of carbohydrates as carbon source was tested in the presence of ammoniacal nitrogen.

FIG. 1. Dendrogram prepared from results of numerical classification of rapidly growing, nonphotochromogenic mycobacteria, using 84 characters. FOR1a, FOR1b, FOR3, and FOR2 are subclusters observed within the cluster of *M. fortuitum* strains.

these species had previously been considered as belonging to one species, *M. fortuitum*, by Gordon and Smith (9), and *M. chelonaei* subsp. *abscessus* had been regarded as a subspecies of *M.*

FIG. 2. Dendrogram prepared from results of numerical classification of rapidly growing, nonphotochromogenic mycobacteria, using 84 characters. CHE and ABS are clusters of *M. chelonaei* subsp. *chelonaei* and *M. chelonaei* subsp. *abscessus* strains, respectively.

FIG. 3. Dendrogram prepared from results of numerical classification of rapidly growing, nonphotochromogenic mycobacteria, using 84 characters. SME, LAC, CHI, and AGR are clusters of *M. smegmatis* subsp. *smegmatis*, "*M. smegmatis* subsp. *lacticola*," *M. chitae*, and "*M. agri*" strains, respectively.

fortuitum by Tsukamura et al. (40). Bojalil et al. (3) differentiated between *M. fortuitum* and "*M. runyonii*" (*M. chelonae* subsp. *abscessus*), and Takeya et al. (24) differentiated between these two taxa by the skin reaction to tuberculin. Later, Tsukamura et al. (38) stated that "*M. runyonii*" and "*M. abscessus*" were the same organism and, according to the rule of priority, "*M. runyonii*" was a later synonym of "*M. abscessus*" Moore and Frerichs (15). Bönicke and Stottmeier (5) proposed a new species, "*Mycobacterium borstelense*," in 1965. In 1970, Tsukamura (28) regarded *M. fortuitum*, "*M. abscessus*," and "*M. borstelense*" as distinct taxa on the basis of a numerical analysis of phenotypic characters. On the other hand, Stanford and Beck (21) stated that "*M. abscessus*," "*M. borstelense*," and the type strain of *M. chelonae*, which was maintained in the National Collection of Type Cultures, London, could not be differentiated by immunotaxonomic analysis and should be reduced to one taxon, *M. chelonae*. Stanford et al. (23) confirmed this finding. In contrast, Weiszfeiler et al. (42) observed a difference in virulence to mice between "*M. abscessus*" and "*M. borstelense*," which are now regarded as *M. chelonae* subsp. *abscessus* and *M. chelonae* subsp. *chelonae*, respectively. On the other hand, Jenkins et al. (12) could not differentiate between these two taxa on the basis of lipid analysis and seroagglutination reactions. Finally, an international cooperative study by Kubica et al. (13) revealed that these two taxa could be differentiated by a phenetic analysis but not by lipid and immunological analyses. Thus, they proposed that the taxa be two subspecies, *M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *abscessus*. In the phenetic analysis of the present study, the taxa *M. fortuitum*, *M. chelonae* subsp. *chelonae*, and *M. chelonae* subsp.

TABLE 3. Distinguishing characters among subgroups of *M. fortuitum*

Character	% of strains showing positive reaction			
	FOR1a (n = 11)	FOR1b (n = 9)	FOR2 (n = 4)	FOR3 (n = 5)
Degradation of <i>p</i> -aminosalicylate	0	100	50	100
Degradation of salicylate	0	100	25	100
Catalase (semiquantitative)	45	0	75	100
β -Galactosidase	0	0	100	0
Nicotinamidase	73	0	100	100
Pyrazinamidase	73	0	100	100
Serine as N and C sources	100	22	0	80
Acetamide as N and C sources	100	44	100	0
1,3-Butylene glycol as C source	91	67	75	0
Trehalose as C source	9	89	0	40
Mannitol as C source	0	44	0	100
No. of strains associated with disease	3 (27) ^a	1 (11)	1 (25)	1 (20)

^a Percentage of strains associated with disease in each group.

TABLE 4. Amidase patterns, patterns of utilization of nitrogen compounds as simultaneous nitrogen and carbon sources, and patterns of utilization of 10 carbohydrates as carbon sources of the 78 test strains of *M. fortuitum*, and their disease association

Character	Pattern ^a						No. of strains	No. of strains associated with disease (%)
Amidase ^b	1	3	5	6	8		48	6 (13)
	1	3			8		18	5 (28)
		3			8		11	2 (18)
	3	5	6	8			1	0 (0)
Utilization of nitrogen compounds as simultaneous N and C sources ^c	G	S	Gc	A	M	T	26	4 (15)
	G		Gc		M	T	15	1 (7)
			Gc	A	M	T	17	3 (18)
	G	S	Gc		M	T	17	5 (29)
	G			A	M		1	0 (0)
	G	S			M	T	1	0 (0)
	G				M	T	1	0 (0)
Utilization of 10 carbohydrates as sole C source ^d	G	M					27	5 (19)
	G	M	T				11	1 (9)
	G	M		I	Mt		4	1 (25)
	G	M	T	I	Mt		6	2 (33)
	G	M	T		Mt		15	1 (7)
	G	M			Mt		15	3 (20)

^a Shown by a set of characters which have shown positive reaction. Characters in the footnotes which do not appear in the table showed a negative reaction.

^b 1, Acetamidase; 2, benzamidase; 3, urease; 4, isonicotinamidase; 5, nicotinamidase; 6, pyrazinamidase; 7, salicylamidase; 8, allantoinase; 9, succinamidase.

^c G, Glutamate; S, serine; Gc, glucosamine; A, acetamide; B, benzamide; M, monoethanolamine; T, trimethylenediamine.

^d G, Glucose; M, mannose; Ga, galactose; A, arabinose; X, xylose; R, rhamnose; T, trehalose; I, inositol; Mt, mannitol; S, sorbitol.

abscessus appeared to be different taxa of the same rank, and the data suggest that they should be regarded as different species.

Grange and Stanford (10, 22) and Pattyn et al. (16) suggested that *M. fortuitum* be divided into several subgroups; one of the subgroups, which belonged to serovar I and was considered to be identical to biovar (biotype) A of Bönicke (4), was frequently associated with disease. Biovar A strains produce acid from glucose, mannose, and trehalose. In contrast, Haas et al. (11) stated that *M. fortuitum* strains formed a homogeneous group when viewed from the standpoint of cell proteins, and Vanden Berghe and Pattyn (41) showed that *M. fortuitum* strains were a homogeneous group with respect to the electrophoretic patterns of their proteins. Binko and Bartholomew (2) observed that strains of *M. fortuitum* produced isozymes that were immunologically identical. The results of the present study show that several subgroups are present within the species *M. fortuitum*, but that the subgroups can accommodate only one-half of the test strains. It was shown that each subgroup (Table 3) contained a few strains associated with disease and that none of the subgroups was statistically more significantly asso-

ciated with disease than any other. We could also differentiate a number of biovars (Table 4), but none of these appeared to be significantly more associated with disease than any other.

M. smegmatis was considered to be a homogeneous taxon by Gordon and Smith (8). Tsukamura et al. (29, 37, 38) showed the presence of two subgroups in this species, "*M. smegmatis* subsp. *smegmatis*" and "*M. smegmatis* subsp. *lacticola*." These taxa differ in their amidase patterns. The existence of the subgroups was confirmed in the present study.

Characters useful in differentiating the species included in the present study are shown in Table 2. Of these colony morphology is used frequently as a character helpful in differentiating between species. However, only the presence or absence of rough colonies in fresh isolates should be used as a distinguishing character, for, as shown previously with *M. smegmatis* (34), strains maintained in laboratories tend to show rough colonies, whereas freshly isolated strains usually produce smooth colonies.

M. agri (Tsukamura 1972) Tsukamura sp. nov., nom. rev. (ag'ri. Gr. n. *agrus* field; M. L. gen. n. *agri* of a field). The original isolate of this species was obtained from alkali-treated soil

inoculated on egg media incubated at 42°C. As shown in the present study, this taxon can readily be differentiated from all previously described species of rapidly growing, nonphotochromogenic mycobacteria. Furthermore, it is clearly different from all other mycobacteria, including the rapidly growing, scotochromogenic mycobacteria (39). Consequently, the name is herein revived for the same organism with which it was originally associated (30). The characters of this species are listed in Table 2.

The characters of the type strain 90012 (= ATCC 27406) are as follows. Acid-fast rods which often grow into long threads. Rough, non-pigmented colonies are produced in the dark and after exposure to light. Growth occurs after 3 days on egg media and at 28, 37, and 45°C; there is no growth at 52°C. Resistant to each of the following in egg media (Ogawa egg medium or Löwenstein-Jensen medium): 5% NaCl, *p*-nitrobenzoic acid (0.5 mg/ml); thiophene-2-carboxylic acid hydrazide (1 µg/ml). Niacin is not produced. Catalase positive (semiquantitative method). Tween not hydrolyzed after 10 days (slightly hydrolyzed after 14 days). β -Galactosidase is usually produced. Nitrate is reduced to nitrite. Susceptible to $\text{NH}_2\text{OH} \cdot \text{HCl}$ (0.25 mg/ml) in egg media; tolerant to 0.2% picric acid in Sauton agar; tolerant to 0.1% sodium nitrite in Sauton agar. Arylsulfatase negative after 3 days but positive after 14 days. Produces α - and β -esterases but not acid phosphatase.

Amidase pattern: acetamidase, urease, nicotinamidase, pyrazinamidase, and allantoinase are produced; benzamidase, isonicotinamidase, salicylamidase, and succinamidase are not produced. Glutamate and glucosamine are utilized as simultaneous nitrogen and carbon sources; serine, acetamide, benzamide, monoethanolamine, and trimethylene diamine are not so utilized. Glucose, mannose, xylose, inositol, fructose, acetate, succinate, malate, pyruvate, fumarate, ethanol, *n*-propanol, and propylene glycol are utilized as carbon sources in the presence of ammoniacal nitrogen; galactose, arabinose, rhamnose, trehalose, mannitol, sorbitol, sucrose, citrate, benzoate, malonate, *n*-butanol, isobutanol, 1,3-, 1,4-, and 2,3-butylene glycols are not thus utilized. Nitrite is not utilized as a sole source of nitrogen in the presence of glycerol as a carbon source.

Source: Soil.

Type strain: 90012; a culture of this strain has been deposited in the American Type Culture Collection, Rockville, Md., as ATCC 27406.

REPRINT REQUESTS

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