Reevaluation of *Mycobacterium fortuitum* (Synonym: *Mycobacterium ranae*)

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One hundred strains of *Mycobacterium fortuitum* (*M. ranae*) were characterized. Strains now recognized as belonging to the species *M. chelonae* were excluded. Seven serotypes of *M. fortuitum* showed some correlation with cell wall lipids, colony types, temperature tolerance, Bönicke's biotypes, and other properties. Serotype I was frequently associated with disease, whereas serotype II, although common in the environment, never was. *M. fortuitum* subsp. *fortuitum* and *M. fortuitum* subsp. *giae* belong to serotypes I and V, respectively; the other serotypes were not considered worthy of special subspecific designation. A list of the constant and variable characteristics of *M. fortuitum* is given.

In 1904 Rupprecht (28) described the occurrence of tuberculosis in a frog, and in the following year Küster (19) cultured the causative organism from such cases. These "frog tubercle bacilli" were named *Mycobacterium ranae* in the first edition of *Bergey's Manual of Determinative Bacteriology* in 1923, and this designation was employed in a number of good taxonomic studies by Aronson (1), Furth (8), Furth and Aronson (9), Gordon (10), Griffith (15), Mudd (23), and Wilson (42). The name *Mycobacterium fortuitum* was introduced in 1938 by da Costa Cruz (6) for a supposedly hitherto undescribed species. The synonymy of *M. fortuitum* and *M. ranae* was established in 1969 by Stanford and Gunthorpe (31), who strongly recommended the reinstatement of *M. ranae* as the legitimate name of the species. However, acting on the request by Runyon (27), the Judicial Commission (Minutes, Judicial Commission of the ICSB, to be published) of the International Committee on Systematic Bacteriology of the International Association of Microbiological Societies has conserved the specific epithet *fortuitum* against *ranae* in the name of this organism.

The name *M. fortuitum* has not always been used correctly in the literature, and it is likely that many strains identified as belonging to *M. fortuitum* were in fact strains of *M. chelonae* Bergey et al., as suggested by Weiszfeiler et al. (40). Furthermore, there has been a tendency to use the name *M. fortuitum* as a convenient label for any rapidly growing, nonchromogenic mycobacterium isolated from clinical material.

Thus Marks (21) had advocated the use of the term "fortuitum group" for the so-called species *M. fortuitum*, *M. peregrinum*, *M. abscessus*, and *M. borstelense*.

In the taxonomic studies of Tsukamura and his colleagues (34-37), the species *M. fortuitum* is defined as including a group known as *M. fortuitum* subsp. *runyonii*. Strains of this group have been shown by Stanford et al. (32) to belong to the species *M. chelonae*. On the other hand, *M. fortuitum* was regarded as a species distinct from *M. chelonae* (synonym: *M. abscessus* and *M. borstelense*) and from *M. peregrinum* on the basis of agglutination serology and lipid chromatography patterns by Jenkins et al. (16). Recent studies (18, 24) have shown that *M. fortuitum* includes the type strains of *M. ranae*, *M. minetti*, *M. giae*, and *M. peregrinum*.

There is, therefore, a need for a redefinition of *M. fortuitum* to include all currently recognized variants of this species as well as to differentiate it from other species of rapidly growing mycobacteria. It is the purpose of this paper to reevaluate *M. fortuitum* by delineating its boundaries and ascertaining the extent and nature of variation occurring within these boundaries. For this purpose, a serological, cultural, and biochemical analysis of 100 strains of diverse origins was undertaken.

MATERIALS AND METHODS

Bacterial strains. One hundred strains of *M. fortuitum* were studied; 66 were from national and private

culture collections and 34 were recent isolates from soil and vegetation in Uganda (34).

Cotype strains of *M. fortuitum* (ATCC 6841 [3]) and *M. ranae* (NCTC 2891 [2]), type strains of *M. giae* (ATCC 11440 [7]) and *M. peregrinum* (NCTC 10264 [3]), and a strain known as *M. minetti* NCTC 3631 (25) were included. The other strains in the study were isolated over the period 1923 to 1972 and were obtained from Belgium, Brazil, Denmark, Germany, Japan, Malta, Mexico, Nigeria, Uganda, the United Kingdom, the U.S.A., and Zaire. The original designations and origins of the 100 strains are listed in Table 1.

Cultural properties. The rate of growth and pigmentation on Lowenstein-Jensen medium at 32 and 42 C and growth on MacConkey medium at 32 C were observed.

The morphology of colonies on 1% nutrient agar was examined by means of a plate microscope (12).

Biochemical investigations. The strains were tested for their ability to hydrolyze Tween 80 within 10 days (39), to liberate sulfate ions from phenolphthalein disulfate within 3 and 10 days (41), and to reduce nitrate to nitrite ions within 4 and 24 h (4, 38). Strains grown on Lowenstein-Jensen medium were tested for nicotinic acid (niacin) production (17).

Utilization of carbohydrates was demonstrated by the method of Gordon and Smith (11), amidase activity by the technique of Bönicke (4), and hydrolysis of the pyrimidines cytosine, thymine, and uracil by the method of Röhrscheidt et al. (26).

Resistance to aminoglycoside antibiotics. One loopful of bacteria was suspended in 2 ml of nutrient broth, and one loopful of this suspension was spread onto dextrose sensitivity test agar (Oxoid) containing lysed blood. After drying, disks containing streptomycin (10 µg), neomycin (10 µg), kanamycin (30 µg), and gentamicin (10 µg) were placed on the agar. Zones of inhibition were read after 48 h of incubation at 32 C.

Lipid chromatography. The lipid composition of the strains was studied by the technique of Marks and Szulga (22). Thin-layer plates were prepared from silica gel (BDH Laboratory reagents), and the solvent used for the chromatographic run was a mixture of 75 parts *n*-propanol, 6 parts 0.880 ammonia, and 22 parts distilled water.

Serological studies. The application of immunodiffusion analysis to the taxonomic study of mycobacteria has been described previously (20, 29, 30). Antisera to serotypes I, II, and V of *M. fortuitum* were available from previous studies (13, 18, 24, 31), and additional antisera were prepared against a serotype III (a clinical isolate from Zaire), a serotype IV (NCTC 2006), a serotype VI (collection no. 91), and a serotype VII strain (collection no. R49).

RESULTS

Cultural properties. All strains grew within 7 days on Lowenstein-Jensen medium at 32 C, and 41 strains grew within the same period at

42 C. None of the strains were chromogenic. All strains grew on MacConkey medium at 32 C with binding of the indicator dye. On nutrient agar, three colony types were observed, one smooth type (S) and two rough types (R1 and R2) as previously described (12). The S colonies were glossy, mucoid, and dome shaped with an entire edge. R1 colonies were flat, opaque, brittle, and rhizoid, and R2 colonies were of a waxy appearance and consistency with an undulate edge and a central papilla. Forty-three strains contained S colonies with varying numbers of R1 and R2 colonies. Twelve strains were purely of the R1 type, 41 were purely of the R2 type, and 4 were a mixture of R1 and R2 colonies.

Biochemical properties. All strains hydrolyzed Tween 80 within 10 days. Arylsulfatase activity was detectable in 93 strains within 3 days and in all strains within 10 days. Ninety-nine strains reduced nitrite ions within 4 h, and one strain gave a weak reaction only after 24 h. Niacin was not produced by any of the strains.

On the basis of carbohydrate utilization, the strains were divisible into the three biotypes of Bönicke (5). Thus, 38 strains utilizing glucose, fructose, mannose, and trehalose were of biotype A, 17 strains also utilizing mannitol and sorbitol were of biotype B, and 45 strains additionally utilizing inositol were of biotype C. Arabinose, dulcitol, erythritol, galactose, lactose, raffinose, rhamnose, and xylose were not utilized by any strain.

Ninety-eight strains hydrolyzed urea, 98 hydrolyzed allantoin, and 84 hydrolyzed acetamide. Other amides hydrolyzed were malonamide (eight strains), succinamide (three strains), and benzamine (one strain). The hydrolysis of isonicotinamide and pyrazinamide was extremely variable quantitatively and not readily reproducible, but almost all strains hydrolyzed these compounds to some degree. None of the strains hydrolyzed nicotinamide or salicylamide.

Cytosine was hydrolyzed by all strains but, as in the case of isonicotinamide and pyrazinamide, there was marked quantitative variation. Ten strains possessed strong thymine and uracil hydrolase activity, whereas the other strains showed no activity in this respect.

Aminoglycoside resistance. All strains were sensitive to gentamicin (10 µg/disk), with zone sizes of 12 to 27 mm. Ninety-six strains were resistant to the 10 µg of streptomycin per disk, and four were sensitive, with zone sizes of 24 ± 1 mm. Ninety-two strains were sensitive to neomycin (10 µg/disk), with zone sizes of 12 to

TABLE 1. *Origin, designation, and variable properties of the 100 strains of Mycobacterium fortuitum*

Collection no.	Original designation ^a	Source	Year	Immunodiffusion serotype	Lipid type ^b	Colonial morphology	Growth at 42 C	Resistance to		Biotype	Hydrolysis of thymine and uracil	Hydrolysis of amides ^c							Arylsulfatase (3 day)	
								Streptomycin	Kanamycin and neomycin			1	2	3	5	6	8	9		10
4	NCTC 8573	Abscess	1954	I	F	R2	+	R	S	A	-	++							++	+
5	NCTC 2006	Terrapln	1925	IV	P	S	+	S	R	C	+	++							++	+
6	PGH H.	Sputum	1964	I	F	R2	+	R	S	A	-	++							++	+
7	PGH soil	Soil	1964	I	F	R2	+	R	S	A	-	++							++	+
19	PGH K.	Sputum	1964	IV	P	S	-	S	R	C	+	++							++	+
33	PGH C.U.	Abscess	1955	IV	P	S	+	R	R	C	+	++							++	+
45	NCTC 10395	Abscess	1938	I	F	R2	+	R	S	A	-	++							++	+
49	NCTC 10264	Bronchial aspirate	1962	V	P	R1, R2	-	R	S	B	-	++							++	-
79	NCTC 1542	Fish	1923	I	F	R2	+	R	S	A	-	++							++	+
80	NCTC 2291	Fish (halibut)	1927	I	F	R2	+	R	S	A	-	++							++	+
81	NCTC 2891	Frog	1905 (?)	I	F	R2	+	R	S	A	-	++							++	+
82	NCTC 3631	Cow	1932	I	F	R2	+	R	S	A	-	++							++	+
83	NCTC 8697	Abscess	1952	I	F	R2	+	R	S	A	-	++							++	+
90	PGH J.C.	Abscess	1957	I	F	R2	+	R	S	A	-	++							++	+
91	PGH R.W.	Abscess	1959	VI	P	S	-	S	S	B	+	++							++	+
92	PGH E.K.	Sputum	1965	I	F	R2	+	R	S	A	-	++							++	+
108	16575 PC	Sputum	1965-67	II	P	S	-	R	S	C	-	++							++	+
109	18519 PC	Sputum	1965-67	II	P	S	-	R	S	C	-	++							++	+
110	20459 PC	Sputum	1965-67	II	P	S	-	R	S	C	-	++							++	+
111	35998 PC	Sputum	1965-67	II	P	S	-	R	S	C	-	++							++	+
112	37030 PC	Sputum	1965-67	II	P	S	-	R	S	C	-	++							++	+
113	37379 PC	Sputum	1965-67	IV	P	S	-	R	S	C	-	++							++	+
404	480	Abscess	Recent	I	F	R2	+	R	R	S	A	++							++	+
431	ATCC 6841	Abscess	1938	I	F	R2	+	R	S	A	-	++							++	+
443	STH H.F.	Sputum	Recent	I	F	R2	+	R	S	A	-	++							++	+
487	750	Abscess	1969	III	P	S	+	R	R	S	C	++							++	+
490	1342	Abscess	1970	I	F	R	+	R	S	A	-	++							++	+
491	Mx491/70	Urine	1970	I	F	R	+	R	S	A	-	++							++	+
576	IMT 2076	Contaminated media	Recent	I	F	R	-	R	S	A	-	++							++	+
577	IMT 2042	Contaminated media	Recent	II	P	R1, R2	-	R	S	B	-	++							++	+

599	ATCC 19542	Cow	?	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
621	ATCC 23012	?	?	I	F	R1	+	R	S	A	-	++	++	+	+	+	+
629	ATCC 23020	Human	?	V	P	R1	-	R	S	A	-	++	++	+	+	+	+
630	ATCC 23021	Human	?	V	P	R1	-	R	S	B	-	++	++	+	+	+	+
632	ATCC 23023		?	V	P	R1	-	R	S	B	-	++	++	+	+	+	+
635	ATCC 23026	?	?	V	P	R2	-	R	S	B	-	++	++	+	+	+	+
638	ATCC 23029	?	?	I	F	R2	-	R	S	A	-	++	++	+	+	+	+
640	ATCC 23031	Human	?	I	N	R1	-	R	S	A	-	++	++	+	+	+	+
645	ATCC 23036	Human	?	V	N	R2	-	R	S	B	-	++	++	+	+	+	+
650	ATCC 23041	Abscess	?	IV	P	S	-	R	R	C	+	++	++	+	+	+	+
652	ATCC 23043	Soil	?	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
656	ATCC 23047	Human	?	V	N	R2	-	R	S	B	-	++	++	+	+	+	+
657	ATCC 23048	?	?	I	N	R2	-	R	S	A	-	++	++	+	+	+	+
675	IMT 800	Abscess	Recent	V	P	R1, R2	-	R	S	B	-	++	++	+	+	+	+
676	IMT 1811	Abscess	Recent	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
677	IMT 1812	Abscess	Recent	III	P	S	+	R	R	C	+	++	++	+	+	+	+
678	IMT 1828	Zoo aquarium	Recent	V	P	R1	-	R	S	B	-	++	++	+	+	+	+
679	IMT 1900	Abscess	Recent	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
680	IMT 1954	Contaminated media	Recent	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
681	IMT 2040	Contaminated media	Recent	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
799	SS 19	Sputum	Recent	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
800	SS 20	Sputum	Recent	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
802	SS 31	Gastric washings	Recent	V	N	R1	-	R	S	B	-	++	++	+	+	+	+
806	SS 928	Frog	?	V	N	R1	-	R	S	B	-	++	++	+	+	+	+
814	Mx 814	Myocardial abscess	1972	I	F	R2	-	R	S	A	-	++	++	+	+	+	+
817	30650 PL	Sputum	1972-73	V	P	R1	-	R	S	B	-	++	++	+	+	+	+
820	32343 PL	Abscess, chest wall	1972-73	I	F	R2	-	R	S	A	-	++	++	+	+	+	+
824	32211 PL	Sputum	1972-73	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
830	32687 PL	Urine	1972-73	V	P	R2	-	R	S	B	-	++	++	+	+	+	+
843	SS 516	Cow	?	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
844	SS 927		Same as no. 431	I	F	R2	+	R	S	A	-	++	++	+	+	+	+
862	C.U.4			IV	P	S	+	R	R	C	+	++	++	+	+	+	+

TABLE 1—continued

Collection no.	Original designation ^a	Source	Year	Immunodiffusion serotype	Lipid type ^b	Colonial morphology	Growth at 42 C	Resist- ance to		Hydrolysis of thymine and uracil	Hydrolysis of amides ^c							Arylsulfitase (3 day)
								Streptomycin	Kanamycin and neomycin		1	2	3	5	6	8	9	
868	<i>M. minetti</i>		Same as no. 82	I	F	R2	+	R	S	—			++			++		+
870	T 120	Soil	Recent	I	F	R2	+	S	S	—	++		++			++		+
R35		Grass, Lake Victoria		II	P	S	—	R	S	—	++		++					+
R46		Mud, Lango		II	P	S	—	R	S	—	++		++					+
R47		Mud, Lango		II	P	S	—	R	S	—	++		++					+
R49		Mud, Lango		VII	F'	S	—	R	S	—	++		++					—
R50		Mud, Bunyoro		II	P	S	—	R	S	—	++		++	+				+
R70		Mud, Bunyoro		II	P	S	—	R	S	—	++		++					+
R71		Mud, E. Mengo		II	P	S	—	R	S	—	++		++					+
R80		Mud, Lango		VII	F''	R2	—	R	S	—	++		++					+
R86		Mud, Bunyoro		II	P	S	—	R	S	—	++		++	+				+
R105		Mud, Bunyoro		II	P	S	—	R	S	—	++	++	++	+				+
R106		Mud, Ankole		II	P	S	—	R	S	—	++		++	+				+
		Injection abscess, Kampala		I	F	R2	+	R	S	—	++		++					+
R111		Injection abscess, Kampala		I	F	R2	+	R	S	—	++		++					+
R160		Mud, Lango		II	P'	S	—	R	S	—	++		++					—
R166		Mud, Bunyoro		II	P	S	—	R	S	—	++		++	+				+
R173		Mud, Lango		II	P	S	—	R	S	—	++		++	+				+
R243		Mud, Lango		II	P	S	—	R	S	—	++		++	+				+
R248		Mud, Lango		II	P	S	—	R	S	—	++		++	+				+
R261		Grass, Bunyoro		II	P	R1	—	R	S	—	++		++	+				+
R269		Mud, Bunyoro		II	P	S	—	R	S	—	++		++	+				+
R275		Grass, Bunyoro		II	P	S	—	R	S	—	++		++	+				+
R301		Mud, Bunyoro		II	P	S	—	R	S	—	++		++	+				+
		Grass, Near Kasese		II	P	S	—	R	S	—	++		++	+				+
R303		Grass, Near Kasese		II	P	S	—	R	S	—	++		++	+				+

R458	Mud, Bunyoro	II	P	S	—	R	S	C	—	++	+	++		+	+
R471	Mud, Bunyoro	II	P	S	—	R	S	C	—	++	+	++		+	+
R583	Grass, E. Mengo	II	P	S	—	R	S	C	—	++	+	++		+	+
R818	Mud, Mosaka	II	P	S	—	R	S	C	—	++	+	++		+	+
R820	Mud, Mosaka	II	P	S	—	R	S	C	—	++	+	++		+	+
R826	Mud, Madi	II	P	S	—	R	S	C	—	++	+	++		+	+
R839	Mud, Lango	II	P	S	—	R	S	C	—	++	+	++		+	+
R840	Mud, Bunyoro	II	P	R1, R2	—	R	S	C	—	++	+	++		+	+
R844	Mud, Bunyoro	II	N	R1	—	R	S	C	—	++	+	++		+	+
R850	Mud, Lango	II	N	R1	—	R	S	C	—	++	+	++		+	+
R852	Mud, Bunyoro	II	P	S	—	R	S	C	—	++	+	++		+	+
R885	Mud, Madi	II	P	S	—	R	R	C	—	++	+	++		+	+
R886	Mud, Lango	IV	P	S	—	R	R	C	+	++	+	++		+	+
R887	Mud, Lango	II	P	S	—	R	S	C	—	++	+	++		+	+

^a ATCC, American Type Culture Collection; Rockville, Md; NCTC, National Collection of Type Cultures, London, England; PGH, Paddington General Hospital; IMT, Institut de Medecine Tropicale, Antwerp; SS, Statens Serum-institut, Copenhagen.

b P, Peregrinum type; F, fortuitum type; N, nonspecific.

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 84

32 mm, and to kanamycin (30 µg/disk), with zone sizes of 15 to 34 mm; the other eight strains were resistant to both antibiotics.

Lipid chromatography. Two main lipid patterns were observed corresponding to the F (Fortuitum) and P (Peregrinum) patterns described by Jenkins et al. (16). Fifty-four strains were of the P type, 38 were of the F type, and 8 showed no specific lipid pattern. Three of the F types showed minor variations: two had an extra orange spot (F'), and one had an extra sky-blue spot (F'').

Immunodiffusion serology. As described in previous studies (29, 31), the precipitation lines were divisible into three groups: those shared with all other mycobacteria (group i antigens), those shared with other rapid growers (group iii), and species-specific antigens (group iv). In this study, nine group iv antigens were observed, but no strain possessed more than six. Three of these antigens were present in each of the 100 strains studied. On the basis of the variable possession of the other six antigens, seven serotypes were determined, five of which have been described previously (12, 18, 24). The use of antisera to serotypes III and IV has confirmed the antigenic structure of serotype IV but has shown that the serotype III strains possess an extra antigen peculiar to themselves. Two additional types, VI and VII, were determined; these contained one and two strains, respectively. Their antigenic structure has been confirmed by the use of type-specific antisera. The antigenic structures of the seven serotypes are shown in Fig. 1.

Thirty-eight strains were of serotype I, 35 were of serotype II, 2 were of serotype III, 7 were of serotype IV, 13 were of serotype V, 1 was of serotype VI, and 2 were of serotype VII.

The cotype strains of *M. fortuitum* and *M. ranae*, and a strain referred to as *M. minetti*, were of serotype I, and the type strains of *M.*

SEROTYPE

1

11

111

IV

V

VI

VII

FIG. 1. *Species-specific antigens of Mycobacterium fortuitum.*

giae and *M. peregrinum* were of serotype V.

The variable characteristics of the 100 strains of *M. fortuitum* are shown in Table 1. The correlation of these variable characteristics is shown in Table 2. A summary of the constant and variable characteristics is provided in Table 3.

DISCUSSION

The results of this and previous studies have shown that there is considerable cultural, biochemical, and antigenic variation within the serologically defined species *M. fortuitum*. Table 2 shows the variation found within each serotype. A close correlation exists between the various properties listed. Thus, all serotype I strains failed to utilize the alcoholic sugars inositol, mannitol, and sorbitol; all strains of serotypes V, VI, and VII utilized mannitol and sorbitol; and, with a single exception, the strains of serotype II, III, and IV utilized inositol. The lipid patterns are also related to serotype, the Fortuitum pattern (16) occurring in serotypes I and VII and the Peregrinum pattern in the other serotypes. Naturally occurring resistance to kanamycin and neomycin, which is due to enzymatic degradation of the drugs (14), is restricted to serotypes III and IV. Such resistance correlates closely with thymine and uracil hydrolase activity: the ten strains that hydrolyze these pyrimidines include the eight strains that are resistant to kanamycin and neomycin. The other two strains possess strong succinamidase activity, the significance of which is unknown. Colonial morphology is partly related to serotype: thus, S forms occur only in serotypes II, III, IV, VI, and VII, and, of

the 38 serotype I strains, 36 are of the R2 type.

Studies on single, smooth colony isolates have shown that irreversible smooth-to-rough variation occurs (12). However, in the majority of cases such variation is not accompanied by

TABLE 3. *Characteristics of Mycobacterium fortuitum encountered in diagnostic bacteriology*

Characteristic	Percent of strains positive
Growth within 7 days on Lowenstein-Jensen medium at 32 C	100
Growth on MacConkey medium with binding of dye	100
Nitratase activity within 24 h	100
Tween 80 hydrolysis within 10 days	100
Arylsulfatase activity within 10 days	100
Utilization of glucose, mannose, trehalose, and fructose	100
Hydrolysis of cytosine	100
Pigment production in light and dark	0
Production of niacin	0
Hydrolysis of nicotinamide and salicylamide	0
Utilization of arabinose, dulcitol, erythritol, lactose, galactose, raffinose, rhamnose, and xylose	0
Growth at 42 C	41
Resistance to kanamycin and neomycin	8
Arylsulfatase activity within 3 days	93
Hydrolysis of thymine and uracil	10
Hydrolysis of urea	98
Hydrolysis of allantoin	98
Hydrolysis of acetamide	84
Hydrolysis of malonamide	8
Hydrolysis of succinamide	3
Hydrolysis of benzamide	1
Utilization of mannitol and sorbitol	62
Utilization of inositol	45

TABLE 2. *Occurrence of the variable properties of Mycobacterium fortuitum in the seven immunodiffusion serotypes*

Sero-type	No. of strains	Lipid type ^a			Colony type				Resistance to kanamycin and neomycin	Growth at 42 C	Utilization of carbohydrates			Hydrolysis of	
		P	F	N	S	R1	R2	R1/R2			Ino-sitol	Man-nitol	Sor-bitol	Thy-mine	Acet-amide
I	38		36 ^b	2		2	36			35					37
II	37	35		2	32	3		2			36	37	37		36
III	2	2			2				2	2	2	2	2	2	2
IV	7	7			7				6	3	7	7	7	7	7
V	13	9		4		7	4	2				13	13		1
VI	1	1			1					1		1	1	1	1
VII	2		2 ^c		1		1					2	2		
Total	100	54	38	8	43	12	41	4	8	41	45	62	62	10	84

^a P, Peregrinum type; F, Fortuitum type; N, nonspecific.

^b Includes one strain of lipid type F'.

^c Two strains of lipid types F' and F''.

change in immunodiffusion serotype or biotype and is thus analogous to the simple smooth-to-rough variation that occurs in many other bacterial genera.

The major patterns of intraspecific variation are shown in Table 4 according to serotype. Strains of serotypes III and IV cannot be distinguished from each other culturally or biochemically but differ from all other types in giving the most positive reactions. In contrast, strains of serotypes I and V in particular differ from the biotype C strains (serotypes II, III, and IV) by the lack of several characteristics. In a previous study (12), it was shown that strains of serotypes I and V could be derived at low frequency from a genetically more complete serotype II strain. As this change was irreversible, it was concluded that a major mutation, probably deletional, had occurred. It was postulated that such mutation could play an important role in the origin of intraspecific variants in this and other mycobacterial species. Such a concept would also explain the occurrence of forms intermediate between the major variants which could arise by small deletions or point mutations. For example, one serotype IV strain was sensitive to kanamycin and neomycin, a serotype II strain failed to utilize inositol, and another failed to hydrolyze acetamide.

Although a large number of strains of diverse geographical origin have been examined, it is probable that additional variants exist, some of which may be of limited geographical distribution. The strains of serotypes I, II, IV, and V studied were from widely distributed areas throughout the world, but the two serotype III strains were isolated near Kinshasa, Zaire, and the two type VII strains came from mud in the Lango district of Uganda. Some of the minor variations within the species may be associated with lysogeny. Despite the extent of variation

within *M. fortuitum*, there are, of course, many properties constant for this species so that the strains form a distinct cluster on numerical analysis (18). Although there are some points of similarity between serotype I strains of *M. fortuitum* and strains of *M. chelonae*, there is no reason for confusion to occur. Both groups of strains utilize glucose, mannose, and trehalose at 28 C, but strains of *M. chelonae* are unable to do so at 40 C; in addition, the latter are unable to reduce nitrate or hydrolyze allantoin, do not grow at 42 C, differ in colonial morphology, and possess a different lipid pattern. On immunodiffusion analysis, they bear no closer relationship to *M. fortuitum* than to any other rapidly growing mycobacterium (32).

The strains analyzed in this study were isolated from humans and other animals and from environmental sources. Those isolated from human sources were divisible into two groups: those clearly associated with disease, i.e., the only pathogen isolated from abscesses, and those isolated from sputum or urine with no proven association with disease. Table 5 shows the distribution of strains according to these groups and to the serotypes. Strains of serotype I are most commonly associated with disease, whereas no definite cases of disease are attributed to strains of serotype II. It is of some relevance that the great majority of strains isolated from the Ugandan environment were of serotype II, whereas two strains isolated from injection abscesses from the same geographical area were both of serotype I. It is also noteworthy that the type strains are all of either serotype I or V, probably because until recently the great majority of mycobacteria in culture collections were isolated from clinical material rather than from the environment.

We do not consider that all the variants described merit subspecific status. However, it

TABLE 4. Summarized correlation of the variable characteristics of *Mycobacterium fortuitum*

Serotype	Biotype ^a	Colony morphology	Lipid type ^b	Growth at 42 C	Resistance to neomycin and kanamycin	Hydrolysis of	
						Thymine	Acetamide
I	A	R2	F	+	—	—	+
II	C	S	P	—	—	—	+
III	C	S	P	+	+	+	+
IV	C	S	P	+	+	+	+
V	B	R1, R2	P	—	—	—	—
VI	B	S	P	+	—	+	+
VII	B	S, R2	F ^c	—	—	—	—

^a Biotypes of Bönicke (1966).

^b P, Peregrinum type; F, Fortuitum type.

^c F' and F'' strains.

TABLE 5. *Origin of the strains of Mycobacterium fortuitum according to immunodiffusion serotype*

Serotype	Disease		Clinical material	Environment	Contaminated media	Unknown
	Human	Animal				
I	14	6	9	3	3	3
II			5	31	1	
III	2					
IV	3	1	2	1		
V	2	1	8	1		1
VI	1					
VII				2		

has been suggested by Pattyn et al. (24) that the names *M. fortuitum* subsp. *fortuitum* and *M. fortuitum* subsp. *giae* (*M. peregrinum*) be applied to strains of serotypes I and V, respectively. In view of the close correlation between the biotypes of Bönicke and other variable properties, this typing system may be used to advantage as an alternative, with division of the biotype C strains into those sensitive and those resistant to neomycin and kanamycin. Additional methods for the subdivision of *M. fortuitum* are available. The use of agglutination serology and delayed hypersensitivity reactions in guinea pigs have been described previously (24). The species may also be subdivided by means of bacteriophage typing (13).

Now that *M. fortuitum* is the correct name for a well-defined mycobacterial species, it is hoped that in the future it will be used as such and not as a broad term for any rapidly growing, nonchromogenic mycobacterium isolated from clinical material, which has frequently been the case in the past.

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REPRINT REQUESTS

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