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Proposal of *Mycobacterium peregrinum* sp. nov., nom. rev., and Elevation of *Mycobacterium chelonae* subsp. *abscessus* (Kubica et al.) to Species Status: *Mycobacterium abscessus* comb. nov.

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We studied the taxonomic positions of the rapidly growing organism *Mycobacterium fortuitum* and phenotypically related organisms. We confirmed that "*Mycobacterium peregrinum*" ATCC 14467^T (T = type strain) is genetically independent of *M. fortuitum* ATCC 6841^T by using various DNA hybridization conditions. Strains that were genetically identified as "*M. peregrinum*" were phenotypically differentiated from *M. fortuitum* ATCC 6841^T. Thus, we propose that "*M. peregrinum*" should be revived as an independent species, *Mycobacterium peregrinum* sp. nov., nom. rev. The type strain is strain ATCC 14467. *M. fortuitum* subsp. *acetamidolyticum* ATCC 35931^T exhibited a high level of DNA relatedness to *M. fortuitum* ATCC 6841^T. The hybridized DNAs maintained stable heteroduplexity at high stringency; thus, we confirmed that *M. fortuitum* subsp. *acetamidolyticum* is identical to *M. fortuitum* ATCC 6841^T. We found that *M. chelonae* subsp. *abscessus* ATCC 19977^T is genetically different from *M. chelonae* subsp. *chelonae* NCTC 946^T on the basis of the results of quantitative hybridization even under optimal conditions. There was no reason to maintain this organism as a subspecies of *M. chelonae*. Thus, we propose that *M. chelonae* subsp. *abscessus* should be elevated to species status as *Mycobacterium abscessus* (Kubica et al.) comb. nov. The type strain is strain ATCC 19977.

"*Mycobacterium peregrinum*" was first proposed as a rapidly growing *Mycobacterium* species distinct from *Mycobacterium fortuitum* (2). Later, Stanford and Gunthorpe (18) reported that serologically these two taxa belong to a single species. However, in an international cooperative study Kubica et al. (10) could not confirm the previously described differences between "*M. peregrinum*" and *M. fortuitum* and concluded that "*M. peregrinum*" is a subjective synonym of *M. fortuitum*. Thus, the name "*M. peregrinum*" was omitted from the Approved Lists of Bacterial Names (17).

The synonomy of these taxa was also supported by the results of Minnikin et al. (14). These workers analyzed the mycolic acid patterns of mycobacteria and reported that they found no data which contradicted the classification of "*M. peregrinum*" as a subjective synonym of *M. fortuitum*.

Pattyn et al. (15) recommended that "*M. peregrinum*" should be listed as a subspecies of *M. fortuitum* on the basis of the results of lipid chromatography and seroagglutination studies, but no proposal was officially submitted. In contrast, the authors of several papers have supported the listing of "*M. peregrinum*" as a distinct species. Data obtained from DNA studies by Baess (1) and Lévy-Frébault et al. (12), from a β-lactamase study by Wallace et al. (24), and from an antigenic study by Tsang et al. (19) indicated that "*M. peregrinum*" should be classified as an independent species.

The DNA-DNA complementarity value between "*M. peregrinum*" and *M. fortuitum* was reported to be 49 to 57% by Baess (1), and these data were supported by the data of Lévy-Frébault et al. (12), although the strains and experimental conditions used in the two studies were different.

Mycobacterium chelonae is divided into two subspecies, *M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *abscessus* (10). The levels of genetic relatedness for these two subspecies reported in two different studies (1, 12) are conflicting.

In this study we investigated the genetic relatedness of members of the *M. fortuitum* complex under three different conditions by changing the stringency of DNA hybridization experiments and characterized the type strain and clinical strains of the *M. fortuitum* complex by using differential characteristics for phenotypic identification.

MATERIALS AND METHODS

Organisms. We used *M. fortuitum* ATCC 6841^T (T = type strain), *M. chelonae* subsp. *chelonae* NCTC 946^T, *M. chelonae* subsp. *abscessus* ATCC 19977^T, *M. fortuitum* subsp. *acetamidolyticum* ATCC 35931^T, and "*M. peregrinum*" reference strain ATCC 14467^T, as well as 25 clinical isolates identified as *M. fortuitum* by conventional biochemical tests, including tests for growth rate, colony morphology, colonial pigmentation, pigment reaction to light, tolerance to 0.2% picrate, degradation of *p*-aminosalicylate, and arylsulfatase, nitrate reductase, Tween 80 hydrolase, and urease activities. These tests were performed by using a commercial mycobacterial identification kit (Kobayashi Pharmaceutical Co., Ltd., Osaka, Japan) designed in accordance with the recommendations of the *Mycobacterium* Taxonomy Committee of the Japanese Society for Tuberculosis Disease (6, 16).

Characterization of strains. Growth temperature tests were performed by using Ogawa egg medium at 28, 37, and 43°C for 2 weeks. Organisms were grown on MacConkey agar (8) as follows: a 10-μl portion of a bacterial suspension (McFarland 1) was inoculated onto plates containing medium, and the preparations were incubated at 28 and 37°C for 1 week. Acid production from carbohydrates and the use of organic

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compounds as sole carbon sources were investigated by using the methods of Tsukamura (21, 22). The growth test on Ogawa egg medium containing 250 μg of hydroxylamine per ml was done by using the method of Tsukamura (20). The iron uptake test was performed by using the method of Wayne and Doubek (26). Tolerance to 5% sodium chloride (9) was tested by growing organisms in Ogawa egg medium containing 5% (wt/vol) NaCl at 28 and 37°C for 2 weeks. Amidase activities were tested by using the method of Bönicke (3). Susceptibility to cefoxitin and susceptibility to cefmetazol were tested as follows: the drug concentration in Middlebrook 7H10 agar ranged from 3.13 to 100 μg/ml, and 20 ml of prepared medium was solidified in each plate. The test cultures, which were grown for 7 days at 37°C on Ogawa egg medium, were suspended in distilled water to a turbidity equal to a McFarland no. 1 standard; 10 μl of bacterial suspension was inoculated onto each plate, and the plates were incubated for 7 days under an atmosphere containing 5% CO_2.

Colorimetric microplate hybridization. The colorimetric microplate hybridization test was performed as described previously (11). Briefly, chromosomal DNA extracted from a test organism was labeled with photoreactive biotin. The denatured, labeled DNA was mixed with hybridization solution and hybridized with reference DNAs that were immobilized in microdilution wells at 60°C for 2 h.

Hybridized DNA was detected by measuring the A_{630}, using streptavidin horseradish peroxidase (Zymed), 3,3', 5,5'-tetramethylbenzidine, and H_2O_2. In this method, as described below, the homologous DNA of a test strain is not immobilized in a well; therefore, the absolute homology value is not available. The relative color intensity for each well was calculated as the maximum absorbance value minus the control absorbance value in a test, which was defined as 100%. When the relative color intensity of the well which exhibited the second strongest color intensity was less than 70%, the labeled strain was considered identified.

In this study, we used a microdilution plate in which 18 reference mycobacterial DNAs were immobilized in individual wells in addition to DNA from *Escherichia coli* ATCC 25922 (as a control); the reference DNAs which we used were DNAs from *Mycobacterium bovis* ATCC 19210^T, *Mycobacterium kansasii* ATCC 12478^T, *Mycobacterium marinum* ATCC 927^T, *Mycobacterium simiae* ATCC 25275^T, *Mycobacterium scrofulaceum* ATCC 19981^T, *Mycobacterium gordonae* ATCC 14470^T, *Mycobacterium szulgai* ATCC 10831^T, *Mycobacterium avium* ATCC 25291^T, *Mycobacterium intracellulare* ATCC 13950^T, *Mycobacterium gastri* ATCC 15754^T, *Mycobacterium xenopi* NCTC 10042^T, *Mycobacterium nonchromogenicum* ATCC 19530^T, *Mycobacterium terrae* ATCC 15755^T, *Mycobacterium triviale* ATCC 23292^T, *M. fortuitum* ATCC 6841^T, *M. chelonae* subsp. *chelonae* NCTC 946^T, *M. chelonae* subsp. *abscessus* ATCC 19977^T, and "*M. peregrinum*" ATCC 14467^T.

Genetic relatedness among type strains. Purified DNA was prepared as described previously (11). The level of genetic relatedness among type strains was investigated by using the method of Ezaki et al. (4). Hybridization was performed at 40, 50, or 60°C for 8 h in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 50% formamide.

RESULTS

The results of tests performed to identify 25 clinical isolates by using colorimetric microplate hybridization are shown in Table 1. Although the relative color intensities were calculated for 18 immobilized reference DNAs, only four sets of data related to the *M. fortuitum* complex are shown in Table 1.

TABLE 1. Identification of 25 clinical isolates by using the colorimetric microplate hybridization method

Test strain	Reactions with the following organism in microdilution wells[a]:			
	M. fortuitum ATCC 6841^T	*M. peregrinum* ATCC 14467^T	*M. chelonae* subsp. *chelonae* NCTC 946^T	*M. chelonae* subsp. *abscessus* ATCC 19977^T
KPM 4002	100[b]	58	<0	<0
KPM 4006	100	8	1	3
KPM 4007	100	15	2	1
KPM 4008	100	47	<0	4
KPM 4011	100	6	<0	<0
KPM 4012	100	28	<0	<0
KPM KY042	100	3	<0	<0
KPM KY044	100	67	24	44
KPM KY045	100	49	<0	<0
KPM KY069	100	13	2	10
KPM KY156	100	23	<0	<0
KPM KY555	100	47	13	23
KPM KY733	100	50	17	17
KPM KY349	100	15	<0	15
KPM 4004	27	100[c]	4	6
KPM 4005	45	100	5	9
KPM 4013	49	100	23	28
KPM 4014	24	100	4	9
KPM KY063	20	100	14	26
KPM KY177	49	100	31	18
KPM KY487	17	100	9	11
KPM KY546	48	100	2	6
KPM KY688	46	100	3	8
KPM KY469	68	100	6	15
KPM KY813	100[d]	80	<0	<0

[a] The values are relative color intensities calculated from the maximum A_{630} value minus the control absorbance value in a test which was defined as 100%. We used a microplate containing 18 immobilized reference DNAs from *Mycobacterium* species. Only data for organisms belonging to the *M. fortuitum* complex are shown.
[b] Identified as *M. fortuitum*.
[c] Identified as "*M. peregrinum*."
[d] Unidentified because criteria were not satisfactory.

A total of 14 and 10 strains gave the highest color intensities with the DNAs from *M. fortuitum* ATCC 6841^T and "*M. peregrinum*" ATCC 14467^T, respectively. These strains were identified as members of these two species. One strain (strain KPM KY813) reacted similarly with the DNAs from *M. fortuitum* and "*M. peregrinum*." Although this strain reacted most strongly with *M. fortuitum* DNA, the second relative color intensity was more than 70%. Thus, the results did not satisfy our identification criteria (11). Quantitative hybridizations in which we used strain KPM KY813, *M. fortuitum* ATCC 6841^T, and "*M. peregrinum*" ATCC 14467^T DNAs revealed that strain KPM KY813 does not belong to an established species.

The results of 41 phenotypic tests are shown in Table 2. The strains of "*M. peregrinum*" that were identified by colorimetric microplate hybridization were distinguished clearly from *M. fortuitum* by their failure to grow on MacConkey agar at 37°C and on Ogawa egg medium at 43°C and by acid production from mannitol and trehalose. More than 60% of the strains of *M. fortuitum* did not degrade *p*-aminosalicylate, in contrast to all of the strains of "*M. peregrinum*." The strain that was not identified by the

TABLE 2. Phenotypic properties of strains related to *M. fortuitum* and 25 clinical isolates

Characteristic	Type strain					Strains identified by microplate hybridization as:		
	M. fortuitum ATCC 6841^T	*M. peregrinum* ATCC 14467^T	*M. fortuitum* subsp. *acetamidolyticum* ATCC 35931^T	*M. chelonae* subsp. *chelonae* NCTC 946^T	*M. chelonae* subsp. *abscessus* ATCC 19977^T	*M. fortuitum* (14 strains)	*M. peregrinum* (10 strains)	Unidentified (1 strain)
Growth at 37°C in less than 7 days	+	+	+	+	+	100[a]	100[a]	+
Colony form	S[b]	RS	S	RS	RS	S (50%) or RS (50%)	S (80%) or RS (20%)	RS
Growth on Ogawa egg medium at:								
28°C	+	+	+	+	+	100	100	+
37°C	+	+	+	+	+	100	100	+
43°C	+	−	+	−	−	92.9	0	−
Growth on MacConkey agar at:								
28°C	+	+	−	+	+	100	100	+
37°C	+	−	−	−	+	92.9	0	−
Growth in the presence of 5% NaCl at:								
28°C	+	+	−	+	+	100	100	+
37°C	+	+	−	−	+	78.6	50	−
Growth in the presence of:								
Picrate (0.2%)	+	+	−	−	+	100	100	+
Hydroxylamine (500 μg/ml)	+	+	+	+	+	100	90	+
Pigmentation in dark or in light	−	−	−	−	−	0	0	−
Iron uptake 37°C	+	+	+	−	−	92.9	100	+
Degradation of *p*-aminosalicylate	−	+	−	+	+	35.7	100	+
Enzymatic activity								
Arylsulfatase (3 days)	+	+	+	+	+	100	100	+
Tween 80 hydrolase	−	+	−	−	−	28.6	10	−
Nitrate reductase	+	+	+	−	−	100	100	+
Amidase reactions								
Benzamide	−	−	−	−	−	7.1	20	−
Urea	+	+	+	+	+	100	100	+
Isonicotinamide	−	−	−	−	−	0	10	−
Nicotinamide	+	−	+	+	+	50	60	−
Pyrazinamide	+	+	+	+	+	57.1	60	+
Alantoin	+	+	+	−	+	100	100	+
Succinamide	−	−	−	−	−	7.1	0	−
Acetamide	+	+	+	−	−	100	90	+
Acid produced from:								
Mannitol	−	+	−	−	−	21.4	100	+
Inositol	−	−	−	−	−	7.1	10	−
Dulcitol	−	−	−	−	−	0	0	−
Arabinose	−	−	−	−	−	0	0	−
Xylose	−	+	−	−	−	0	0	−
Mannose	+	+	−	−	−	85.7	100	+
Galactose	−	−	−	−	−	0	0	−
Rhamnose	−	−	−	−	−	0	0	−
Trehalose	−	+	−	−	−	21.4	90	+
Sorbitol	−	−	−	−	−	7.1	0	−
Glucose	+	+	−	−	−	92.9	100	+
Saccharose	−	−	−	−	−	0	0	−
Sole carbon sources								
Fructose	+	+	+	−	−	100	100	+
Glucose	+	+	−	−	−	100	100	+
Oxalate	−	−	−	−	−	0	0	−
Citrate	−	−	−	+	−	0	0	−

[a] The values in this column are the percentages of positive strains.
[b] S, smooth type; RS, intermediate smooth and rough types.

colorimetric hybridization method had properties just like those of "*M. peregrinum*."

If the inoculum was heavy, some strains of "*M. peregrinum*" exhibited slight growth on MacConkey agar even at 37°C, so this test had to be performed by using dilute bacterial suspensions as the inocula.

Tests for amidase activity or other enzymatic activities were not effective in differentiating these species.

The MICs of cefmetazol and cefoxitin for 28 strains, including the type strains of "*M. peregrinum*," *M. fortuitum*, and *M. fortuitum* subsp. *acetamidolyticum*, are shown in Fig. 1. Figure 1 shows that "*M. peregrinum*" tended to be

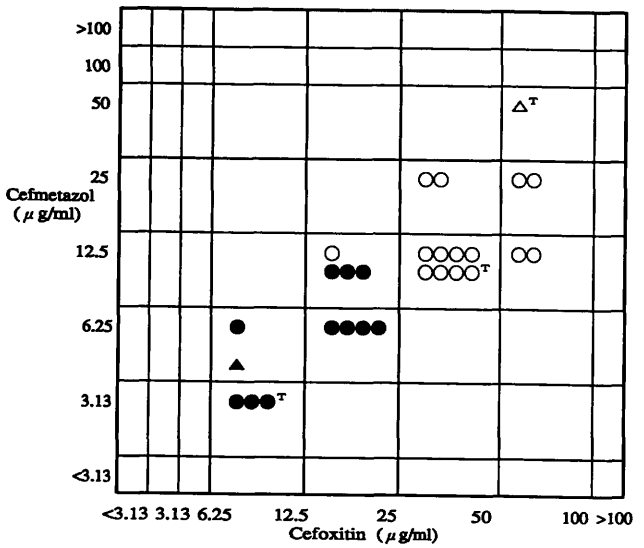

FIG. 1. MICs of cefoxitin and cefmetazol for 28 strains. Symbols: ○, *M. fortuitum*; ●, *M. peregrinum*; ▲, strain not identified by colorimetric microplate hybridization; △, *M. fortuitum* subsp. *acetamidolyticum*. T = type strain.

susceptible to low concentrations of each drug. With cefoxitin, the MICs for 14 of the 15 *M. fortuitum* strains tested (93%) were more than 50 μg/ml, while the MICs for all of the "*M. peregrinum*" strains were less than 25 μg/ml. With cefmetazol, the MICs for all strains of *M. fortuitum* were more than 12.5 μg/ml, and the MICs for only 3 of the 11 "*M. peregrinum*" strains tested (27%) were 12.5 μg/ml.

The MICs of both cefoxitin and cefmetazol for *M. fortuitum* subsp. *acetamidolyticum* ATCC 35931^T were high. The strain that was not identified by colorimetric hybridization was in the low-MIC group.

The results of quantitative DNA-DNA hybridization experiments are shown in Table 3. It is known that the DNAs of mycobacteria have relatively high G+C contents (5). The G+C contents of most of these organisms range from 61 to 67 mol%; the one exception is *Mycobacterium leprae*, which has a G+C content of 56 mol% as determined by a spectrophotometric assay. The DNAs of the type strains of *M. chelonae* subsp. *chelonae*, *M. chelonae* subsp. *abscessus*, and *M. fortuitum* have G+C contents of 62, 64, and 63 mol%, respectively, according to Imaeda et al. (5). We obtained values of 66 and 67 mol% for *M. fortuitum* and "*M. peregrinum*," respectively, by using the high-performance liquid chromatography method. The following formula derives G+C content from the melting temperature (T_m) (12): G+C content = 2.44 (T_m − 69.3). This formula is usually available for expediently determining the T_m from the G+C content except in cases of extremely high or low G+C contents. When this expedient method was used, the T_m values for the strains related to *M. fortuitum* were 95 to 97°C. Thus, the optimal hybridization temperature (T_m − 25°C) in 2× SSC was 70 to 72°C. However, we performed hybridization in 2× SSC containing 50% formamide; under these conditions the optimal hybridization temperature was 40 to 42°C because 1% formamide lowered the hybridization temperature by 0.6°C.

The levels of genomic relatedness between "*M. peregrinum*" ATCC 14467^T and *M. fortuitum* ATCC 6841^T were 42.2 to 60.4% under optimal conditions (40°C).

TABLE 3. Levels of DNA relatedness among the type strains related to *M. fortuitum* and strain KPM KY813

Immobilized strain	% Homology with the following labeled strains:																	
	M. fortuitum ATCC 6841^T			*M. peregrinum* ATCC 14467^T			*M. fortuitum* subsp. *acetamidolyticum* ATCC 35931^T			*M. chelonae* subsp. *chelonae* NCTC 946^T			*M. chelonae* subsp. *abscessus* ATCC 19977^T			KPM KY813		
	40°C[a]	50°C	60°C	40°C	50°C	60°C	40°C	50°C	60°C	40°C	50°C	60°C	40°C	50°C	60°C	40°C	50°C	60°C
M. fortuitum ATCC 6841^T	100[b]	100	100	42.2	39.7	24.3	76.5	80.6	67.9	10.4	11.7	2.9	12.3	0.9	1.8	36.6	32.4	8.4
M. peregrinum ATCC 14467^T	60.4	44.5	17.7	100	100	100	53.3	45.3	17.5	12.3	7.4	2.9	15.0	6.8	3.1	43.7	38.3	13.0
M. fortuitum subsp. *acetamidolyticum* ATCC 35931^T	95.6	88.7	81.5	42.4	41.2	33.3	100	100	100	5.9	8.5	4.7	9.8	<0	<0	36.2	32.2	9.4
M. chelonae subsp. *chelonae* NCTC 946^T	8.0	7.8	8.0	8.6	9.6	18.0	10.1	4.6	1.9	100	100	100	34.1	21.0	3.6	14.0	3.2	2.1
M. chelonae subsp. *abscessus* ATCC 19977^T	11.6	2.4	<0	8.5	2.6	5.8	9.0	1.0	1.1	35.1	31.1	13.6	100	100	100	15.9	4.6	1.3
KPM KY813	81.2	50.4	13.3	62.5	50.1	38.7	66.9	51.6	22.6	16.9	10.4	5.0	17.6	1.3	<0	100	100	100

[a] Hybridization temperature.
[b] The values are averages of four replicates.

TABLE 4. Differential characteristics for *M. fortuitum* and *M. peregrinum*

Characteristic	Reactions of type strains		Reactions of clinical isolates	
	M. fortuitum ATCC 6841	*M. peregrinum* ATCC 14467	*M. fortuitum* (14 strains)	*M. peregrinum* (10 strains)
Growth at 43°C on Ogawa egg medium	+	−	92.9[a]	0
Growth on MacConkey agar at 37°C	+	−	92.9	0
Degradation of *p*-aminosalicylate	−	+	35.7	100
Acid produced from:				
Mannitol	−	+	21.4	100
Trehalose	−	+	21.4	90

[a] The values are the percentages of positive strains.

Strain KPM KY813 was more closely related to *M. fortuitum* ATCC 6841^T than to "*M. peregrinum*" ATCC 14467^T, but we found that the hybridized DNA was not maintained under stringent conditions.

M. fortuitum subsp. *acetamidolyticum* ATCC 35931^T and *M. fortuitum* ATCC 6841^T exhibited 80.6 to 88.7% relatedness even under stringent conditions. When *M. fortuitum* subsp. *acetamidolyticum* ATCC 35931^T was proposed as a subspecies of *M. fortuitum* (23), the authors found 94% genomic relatedness between the two type strains. Our data confirmed that *M. fortuitum* subsp. *acetamidolyticum* ATCC 35931^T and *M. fortuitum* ATCC 6841^T belong in a single species.

On the other hand, *M. chelonae* subsp. *chelonae* NCTC 946^T and *M. chelonae* subsp. *abscessus* ATCC 19977^T exhibited only 35% DNA relatedness under optimal conditions.

DISCUSSION

According to current bacterial taxonomy standards, species comprise strains that exhibit more than 70% DNA-DNA complementarity (7, 25). Many bacterial classifications have been reevaluated by using this criterion. We reevaluated genetic relatedness among established mycobacterial species in a previous report (11). We also noted that there are still many problems to be solved in the classification of genus *Mycobacterium*.

Proposal of *M. peregrinum* sp. nov., nom. rev. "*M. peregrinum*" ATCC 14467^T was first described as an organism that is genetically independent from *M. fortuitum* ATCC 6841^T (1); however, the results of international cooperative work have shown that strains identified as "*M. peregrinum*" are virtually the same as *M. fortuitum*, and the name "*M. peregrinum*" was not included on the Approved Lists of Bacterial Names (17).

Later, Lévy-Frébault et al. (12) confirmed that strains identified as "*M. peregrinum*" are genetically different from the type strain of *M. fortuitum*, strain ATCC 6841; our data derived from three different experimental conditions supported this observation.

Strains identified as "*M. peregrinum*" by DNA-DNA hybridization were phenotypically distinguishable from *M. fortuitum*. Thus, we propose that "*M. peregrinum*" should be revived as a distinct species.

Description of the type strain of *Mycobacterium peregrinum* sp. nov., nom. rev. *Mycobacterium peregrinum* (L. adj. *peregrinus*, strange, foreign) (2). The characteristics of the type strain are as follows: acid-fast rods (1.5 to 4 μm long by 0.5 μm wide); colonies on egg medium are intermediate between smooth and rough and white to slightly yellowish but nonphotochromogenic. Growth occurs after 7 days on egg medium at 28 and 37°C but not at 43°C. On MacConkey agar, growth occurs at 28°C but not at 37°C. This organism tolerates 5% NaCl and 500 μg of hydroxylamine per ml in Ogawa egg medium and 0.2% picrate in Sauton agar medium. Tests for iron uptake and degradation of *p*-aminosalicylate are positive. This bacterium produces arylsulfatase (3 days), nitrate reductase, and Tween 80 hydrolase. Urease, pyrazinamidase, alantoinase, and acetamidase are present, while benzamidase, isonicotinamidase, nicotinamidase, and succinamidase are absent. Acid is produced from mannitol, xylose, mannose, trehalose, and dextrose, but not from inositol, dulcitol, arabinose, galactose, rhamnose, sorbitol, and saccharose.

M. peregrinum utilizes fructose and glucose as sole carbon sources but does not utilize oxalate or citrate. The type strain is strain ATCC 14467.

Differential characterestics of *M. peregrinum*. Characteristics that differentiate *M. peregrinum* from *M. fortuitum* are shown in Table 4. *M. peregrinum* is a rapid grower, growing to naked eye visibility on egg medium within 7 days at 37°C. It produces arylsulfatase (3 days) and degrades *p*-aminosalicyclic acid. These characteristics differentiate this species from nonpathogenic rapid growers. Reduction of nitrate to nitrite is the characteristic that differentiates it from *M. chelonae* and *Mycobacterium abscessus*.

M. peregrinum can be distinguished from *M. fortuitum* by its failure to grow at 43°C on egg medium and at 37°C on MacConkey agar and by acid production from mannitol and trehalose. Its relatively low tolerance to cefmetazol and cefoxitin is helpful for differentiation (Fig. 1).

Proposal to elevate *M. chelonae* subsp. *abscessus* (Kubica et al.) to species status as *Mycobacterium abscessus* (Kubica et al.) comb. nov. The two subspecies of *M. chelonae*, *M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *abscessus*, were proposed on the basis of the results of a numerical cluster analysis (10). After this proposal, the level of genetic relatedness between the two subspecies was reported in two different studies (1, 12). Baess reported that *M. chelonae* subsp. *chelonae* ATCC 19235 and *M. chelonae* subsp. *abscessus* ATCC 19977^T were genetically closely related because the two strains exhibited 99% DNA relatedness as determined by spectrophotometric hybridization. However, strain ATCC 19235 is not the type strain, and thus the data were not conclusive for deciding whether the two subspecies are identical.

Conflicting results came from a different laboratory. Lévy-Frébault et al. (12) used six strains of *M. chelonae* subsp. *chelonae* and six strains of *M. chelonae* subsp. *abscessus*, including the type strain of each subspecies. The data of these authors clearly indicated that *M. chelonae* subsp.

chelonae and *M. chelonae* subsp. *abscessus* are independent species because the DNA reannealing values supported recognition as independent species.

We think that there is no reason for keeping *M. chelonae* subsp. *abscessus* as a subspecies of *M. chelonae*. *M. chelonae* subsp. *abscessus* can be differentiated from *M. chelonae* subsp. *chelonae* by ordinary biochemical tests, as shown in Table 1. Thus, we propose that *M. chelonae* subsp. *abscessus* should be elevated to species status as *M. abscessus* (Kubica et al.) comb. nov..

Description of the type strain of *Mycobacterium abscessus* comb. nov. The characteristics of the type strain are as follows: acid-fast rods (1.0 to 2.5 μm long by 0.5 μm wide); colonies on egg medium are intermediate between smooth and rough, white to grayish, and nonphotochromogenic. Growth occurs after 7 days on egg medium at 28 and 37°C but not at 43°C. On MacConkey agar, growth occurs at 28°C and even at 37°C. This organism tolerates 5% NaCl and 500 μg of hydroxylamine per ml in Ogawa egg medium and 0.2% picrate in Sauton agar medium. The iron uptake test is negative, but the test for degradation of *p*-aminosalicylate is positive. *M. abscessus* produces arylsulfatase (3 days), but not nitrate reductase and Tween 80 hydrolase. Amidase, urease, nicotinamidase, pyrazinamidase, and alantoinase are present, while benzamidase, isonicotinamidase, succinamidase, and acetamidase are absent. Does not produce acid from the following carbohydrates: mannitol, inositol, dulcitol, arabinose, xylose, mannose, galactose, rhamnose, trehalose, sorbitol, glucose, and saccharose. Does not utilize fructose, glucose, oxalate, and citrate as sole carbon sources. The type strain is strain ATCC 19977.

Differential characterestics of *M. abscessus*. Among the pathogenic rapidly growing mycobacteria, *M. chelonae* and *M. abscessus* can be distinguished from *M. fortuitum* or *M. peregrinum* by their failure to reduce nitrate and take up iron, as described in *Bergey's Manual of Systematic Bacteriology* (27). Tolerance to 5% NaCl in egg medium and tolerance to 0.2% picrate in Sauton agar medium are characterestics that distinguish *M. abscessus* from *M. chelonae*. Furthermore, *M. abscessus* does not utilize citrate as a sole carbon source, whereas *M. chelonae* does.

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