Mycobacterium intermedium sp. nov.

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Strains of a new type of slowly growing mycobacterium were repeatedly isolated from sputum from a patient with pulmonary disease. This photochromogenic organism grew at 22, 31, 37, and 41°C, possessed catalase, acid phosphatase, esterase, β -galactosidase, and arylsulfatase activities, and hydrolyzed Tween. It did not produce nicotinic acid or have nitrate reductase, acetamidase, benzamidase, isonicotinamidase, nicotinamidase, pyrazinamidase, succinamidase, and acid phosphatase activities. Urease activity was variable. The organism is susceptible to ethambutol and resistant to isoniazid and streptomycin. A mycolic acid analysis revealed the presence of α -mycolates, α' -mycolates, and keto-mycolates. The results of comparative 16S rRNA sequencing placed this organism at an intermediate position between the rapidly and slowly growing mycobacteria. On the basis of the pattern of enzymatic activities and metabolic properties, the results of fatty acid analyses, and the unique 16S rRNA sequence, we propose that this organism represents a new species, for which we propose the name *Mycobacterium intermedium*. The type strain is strain 1669/91; a culture of this strain has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSM 44049.

Mycobacteria are aerobic, nonmobile bacteria that are characteristically acid fast. The property of acid fastness, which is due to waxy materials in the cell wall, is particularly important for recognizing mycobacteria. Members of the genus *Mycobacterium* are widespread in nature and range from soil-dwelling saprophytes to pathogens of humans and animals (26, 32). Isolates belonging to a previously unrecognized *Mycobacterium* species were isolated repeatedly from sputum from a patient with pulmonary disease. In this paper we describe the results of a taxonomic study of this organism and propose that it should be named *Mycobacterium intermedium* sp. nov.

MATERIALS AND METHODS

Bacterial strains. Three isolates, strains 8537/90, 1669/91^T (T = type strain), and 1924/91, were recovered from sputum specimens obtained from a patient with pulmonary disease. The isolation method used was the standard method used for isolation of mycobacteria from clinical specimens (26). Colony morphology and the ability to grow at various temperatures (22, 31, 37, 41, and 45°C) were determined after 4 weeks of incubation on Löwenstein-Jensen slants. The other strains used in this study were obtained from the strain collection of the National Reference Laboratory for Mycobacteria, Forschungsinstitut Borstel, Borstel, Germany.

Identification. The following properties were determined as described previously (14): production of nicotinic acid (24); acetamidase, benzamidase, urease, isonicotinamidase, nicotinamidase, pyrazinamidase, and succinamidase activities (3); nitrate reduction (4); Tween hydrolysis (31); acid phosphatase activity (9); production of esterases (10) and arylsulfatases (18); catalase activity (15); β -galactosidase activity (12); and resistance to isoniazid (0.25 μ g/ml), streptomycin (4.0 μ g/ml), ethambutol (5.0 μ g/ml), and rifampin (2.0 μ g/ml) (25).

Lipid analyses. Lipid analyses were carried out by using two different, complementary methods. Mycolic acids were analyzed by thin-layer chromatography of whole-organism methanolysates (20). Freeze-dried bacteria (50 mg) were degraded by treatment at 75°C with 3 ml of methanol-toluene-sulfuric acid (30:15:1, vol/vol/vol) for 16 h. Long-chain components were extracted with hexane, and traces of acid were removed by passage through a short column containing ammonium hydrogen carbonate. The hexane extracts were examined by thin-layer chromatography on plates (20 by 20 cm) coated with a 0.25-mm layer of silica, using a solution containing petroleum ether (boiling point, 60 to 80°C) and diethyl ether (85:15, vol/vol) as the developing phase. For two-dimensional chromatography on plates (10 by 10 cm), triple development with a mixture containing petroleum ether and acetone (95:5, vol/vol) in the first direction was followed by single development with toluene-acetone (97:3, vol/vol) in the second direction.

Fatty acids were analyzed by using fatty acid methyl esters obtained from wet biomass (approximately 40 mg) by saponification, methylation, and extraction as described previously (8, 16, 19, 21). The fatty acid methyl ester mixtures were separated by gas-liquid chromatography, using the Microbial Identification System (Microbial ID, Newark, Del.), which consisted of a Hewlett-Packard model 5980 gas chromatograph fitted with a 5% phenylmethyl silicone capillary column (0.2 mm by 25 m), a flame ionization detector, a Hewlett-Packard model 3392 integrator, a Hewlett-Packard model 7673A automatic sampler, and a Hewlett-Packard model 216 computer (Hewlett-Packard, Palo Alto, Calif.). Peaks were automatically integrated, and fatty acid identities and percentages were calculated by using the Microbial Identification System Library Generation Software (Microbial ID). The gas chromatographic parameters were as follows: the carrier gas was ultrahigh-purity hydrogen; the column head pressure was 60 kPa; the injection volume was 2 μ l; the column split ratio was 100:1; the septum purge rate was 5 ml/min; the column temperature

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TABLE 1. Distinguishing characteristics of selected *Mycobacterium* strains and species^a

Characteristic	Strain 8537/90	Strain 1669/91 ^T	Strain 1924/91	<i>M. kansasii</i> ^b	<i>M. simiae</i> ^b	<i>M. asiaticum</i> ^b	<i>M. malmoeense</i> ^b
Enzymatic activities							
Production of Nicotinic acid	0	0	0	0	+/-	0	0
Acetamidase	0	0	0	0	0	0	0
Benzamidase	0	0	0	0	0	0	0
Urease	+/-	+	+	+	+	0	+/-
Isonicotinamidase	0	0	0	0	0	0	0
Nicotinamidase	0	0	0	+	+/-	0	+
Pyrazinamidase	0	0	0	0	+/-	0	+
Succinamidase	0	0	0	0	0	0	0
Tween hydrolysis (10 days)	+	+	+	+	0	+	+
Nitrate reductase	0	0	0	0	0	0	0
Arylsulfatase (10 days)	+	+	+	+	0	+	0
Acid phosphatase	+	+	+	+	0	+	0
α -Esterase	+	+	+	0	+	+	(+)
β -Esterase	+/-	+/-	+/-	0	+	+	(+)
Catalase at 22°C	+	+	+	+	+	+	+
Catalase at 68°C	+	+	+	+	+	+	0
β -Galactosidase	+	+	+	0	0	0	0
Photochromogenic	+	+	+	+	+	+	0
Growth at:							
22°C	+	+	+	+	+	0	+/-
31°C	+	+	+	+	+	+	+
37°C	+	+	+	+	+	+	+/-
41°C	+	+	+	+	+	+	0
45°C	0	0	0	0	0	0	0
Susceptibility to:							
Isoniazid	R	R	R	R	R	R	R
Streptomycin	R	R	R	R	R	R	R
Ethambutol	S	S	S	S	R	R/S	S
Rifampin	S	S	S/R	S	S/R	R	S/R

^a 0, negative; +/-, variable; (+), weakly positive; +, positive; S, susceptible; R, resistant.

^b Obtained from the strain collection of the National Reference Laboratory for Mycobacteria, Forschungsinstitut Borstel, Borstel, Germany.

was increased from 170 to 270°C at a rate of 5°C/min; the injection port temperature was 250°C; and the detector temperature was 300°C.

rRNA sequencing and phylogenetic analysis. The 16S rRNA gene was amplified by using the polymerase chain reaction technique, and the sequence was determined as described previously (5, 7). With the sequencing strategy which we used we determined the nucleotides of isolate 1669/91^T at 1,441 contiguous positions. The sequence obtained was aligned with selected 16S rRNA sequences (23, 29, 30) as described previously (23). For the phylogenetic analysis, regions of alignment uncertainty were omitted (positions 38 to 49, 172 to 175, and 777 to 789 of the sequence, corresponding to *Escherichia coli* positions 83 to 95, 212 to 217, and 841 to 851; in addition, positions 1271 to 1441 of the sequence were omitted because the previously published sequence of *Mycobacterium asiaticum* [29] lacks this part of the molecule); this reduced the number of positions used from 1441 to 1242. Pairwise distances were calculated by weighting nucleotide differences and insertions-deletions equally (Hamming distances). A phylogenetic tree was constructed by using the neighborliness method (27) as described previously (23).

Nucleotide sequence accession number. The EMBL nucleotide sequence accession number for strain 1669/91^T is X67847.

RESULTS AND DISCUSSION

Cells of strains 8537/90, 1669/91^T, and 1924/91 grown on Löwenstein-Jensen agar were coccobacilli (2.0 by 2.6 μ m)

and acid-alcohol fast. No spores, capsules, or aerial hyphae were observed.

On Löwenstein-Jensen slants the isolates grew as eugonic, smooth, photochromogenic colonies within 14 to 21 days when they were incubated at temperatures ranging from 22 to 41°C. The properties of strains 8537/90, 1669/91^T, and 1924/91 are compared with the properties of other slowly growing species of mycobacteria in Table 1. These species were selected on the basis of the similarity of the properties investigated. The enzymatic activities and metabolic properties demonstrate that our strains differ from previously described species (32). Our strains possess catalase, acid phosphatase, esterase, β -galactosidase, and arylsulfatase activities and hydrolyze Tween. Urease activity is variable. The strains are susceptible to ethambutol and resistant to isoniazid and streptomycin.

One-dimensional thin-layer chromatography of whole-organism acid methanolysates of strains 8537/90, 1669/91^T, and 1924/91 produced a simple pattern which could not be separated from the patterns of the other species tested (*Mycobacterium malmoeense*, *Mycobacterium simiae*, *Mycobacterium kansasii*, and *Mycobacterium asiaticum*). When two-dimensional thin-layer chromatography was used, more precise separation of all of the components was obtained. Strains 8537/90, 1669/91^T, and 1924/91 contain α -mycolates, α' -mycolates, and keto-mycolates. This mycolic acid pattern is found in *M. simiae* and *M. malmoeense* but not in most other slowly growing mycobacteria, which are characterized by the presence of α -, keto-, and methoxy-mycolates, e.g., *M. kansasii*, *M. asiaticum*, *Mycobacterium goodii*, *My-*

cobacterium haemophilum, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium gastri*, *Mycobacterium marinum*, *Mycobacterium microti*, *Mycobacterium szulgai*, and *Mycobacterium ulcerans* (6, 20; data not shown). Better separation of strains 8537/90, 1669/91^T, and 1924/91 from other mycobacteria was obtained by comparing the results of the short-chain fatty acid analyses (17). The gas-liquid chromatography patterns in Table 2 revealed consistent differences between isolates 1669/91^T, 1924/91, and 8537/90 on the one hand and *M. simiae*, *M. kansasii*, *M. malmoense*, and *M. asiaticum* on the other hand. Isolates 1669/91^T, 1924/91, and 8537/90 appeared to have the same fatty acid composition as determined by the gas-liquid chromatography analysis. *M. kansasii* differed from these isolates by the presence of dimethyl myristic acid (2,4-DiMe 14:0), a peak characteristic of *M. kansasii*. In addition, *M. kansasii* and *M. simiae* contained *cis*-11-hexadecenoic acid (16:1 *cis*-11) and lacked *cis*-10-hexadecenoic acid (16:1 *cis*-10). *M. asiaticum* differed from our isolates quantitatively with respect to the myristic acid (14:0) content and qualitatively by the absence of tuberculostearic acid (10-Me 18:0 TBSA). In contrast to *M. malmoense*, our isolates contained only moderate concentrations of tuberculostearic acid. In addition, *M. malmoense* differed by the absence of dodecanoic acid (12:0).

An analysis of the hypervariable region in the 16S rRNA sequence corresponding to *E. coli* positions 129 to 270, which is known to be specific for mycobacteria at the species level (22), revealed that isolates 8537/90, 1669/91^T, and 1924/91 had identical sequences. The sequence which we determined differed from the homologous regions of all previously described species of slowly growing mycobacteria (2, 11, 13, 22, 23, 29, 30; unpublished data) (Fig. 1). Strain 1669/91^T was chosen to determine an almost complete 16S rRNA sequence (Fig. 2). The sequence obtained was different from more than 30 previously published mycobacterial sequences, including the sequences of *M. kansasii*, *M. asiaticum*, *M. simiae*, *M. terrae*, and *M. malmoense*. A phylogenetic tree that shows the natural relationships among the mycobacteria (Fig. 3) was constructed by using equally weighted (Hamming) distances (Table 3). Together with *M. simiae*, strain 1669/91^T occupies a phylogenetically intermediate position between slowly growing and rapidly growing mycobacteria. This intermediate position is characterized by a slow growth rate at the phenotypic level, yet at the molecular level *M. simiae* and strain 1669/91^T exhibit the molecular signature of rapidly growing mycobacteria (i.e., a short helix 18) (23, 28, 29). Interestingly, strain 1669/91^T appears to be the most recent species in the slowly growing mycobacterial lineage before the molecular signature of a long helix was acquired.

Strains 8537/90, 1669/91^T, and 1924/91 were isolated from sputum from a patient with pulmonary disease, presented clinically as chronic obstructive bronchitis. Sputum specimens from this patient were repeatedly positive for acid-fast bacteria on microscopic examination. A long-term chemotherapeutic regimen consisting of isoniazid, rifampin, ethambutol, and pyrazinamid resulted in significant improvement of the patient's condition. Isolation of a mycobacterium species other than *M. tuberculosis* raises the question of the pathogenic potential of the mycobacterium isolated. The American Thoracic Society (1) has specified that definite diagnosis depends on evidence of disease, such as a pulmonary infiltrate that is visible on a chest roentgenogram. Multiple colonies of the same *Mycobacterium* species should either be isolated repeatedly or be isolated from a closed

TABLE 2. Microbial Identification System whole-cell fatty acid analysis of *M. intermedium* isolates and related organisms

Strain or species	% of peak area																	Identification (similarity index) ^a	
	12:0	2-Me 12:0	14:0	2-Me 14:0	2,4-DiMe 14:0	15:0	16:1 B- <i>cis</i> -6	16:1 <i>cis</i> -9	16:1 <i>cis</i> -10	16:1 <i>cis</i> -11	16:0	17:1	17:0	18:2 <i>cis</i> -9,12	18:1 <i>cis</i> -9	18:1 <i>cis</i> -11	18:0		10-Me 18:0 TBSA
1669/91 ^T	1.52	0.34	2.59	1.56		0.69	0.99	3.49	7.33	0.61	41.01	0.75	0.68	0.49	30.64	0.96	2.01	4.34	Not in data base <i>M. kansasii</i> (0.730) <i>M. simiae</i> (0.863) <i>M. asiaticum</i> (0.730)
1924/91	1.89	0.38	5.71	1.76		0.31	0.89	5.78	5.92	0.61	43.19	0.38	0.27	0.33	23.34	0.82	2.90	5.12	
8537/90	2.08	0.4	5.84	1.72		0.35	1.04	5.92	6.85	0.58	42.30	0.39	0.26	0.4	24.26	0.79	2.24	4.33	
<i>M. malmoense</i> ^b			4.49			2.34	1.98	1.01	7.67		34.64		0.93	1.81	22.03		5.76	15.41	
<i>M. kansasii</i> ^b	0.29	0.7	3.40	0.36	4.12	0.29	0.90	2.13		5.25	38.88	0.43	0.60	0.55	30.22	0.85	4.32	5.63	
<i>M. simiae</i> ^b	0.34		7.50			0.36	1.28	1.68		4.55	39.68		0.57	0.34	28.96		6.63	7.21	
<i>M. asiaticum</i> ^b	2.92		19.53			0.35	0.47	3.83	4.22		38.78		0.56	0.61	19.84		7.74		

^a Identification based on Microbial Identification System data.

^b Strains were obtained from the strain collection of the Forschungsinstitut Borstel.

TGA TCT GCC CTG CAC TTC GGG ATA AGC CTG GGA AAC TGG GTC TAA TAC CGG ATA GG ACCA CGG GAT GCA TGCTCT-TGT GGT GGA AAG	<i>M.tb. complex</i>
.A. ACTTC .GCC..AG.A	<i>M.8537/90</i>
.A. ACTTC .GCC..AG.A	<i>M.1924/91</i>
.A. ACTTC .GCC..AG.A	<i>M.1669/91</i>
CA. TTA .GCTA	<i>M.avium, M.paratuberculosis, M.lepramurium</i>
CA. TTA .GCTA	<i>M.intracellulare</i>
CA. TTA .GCTA	<i>M.intracellulare serovar 7</i>
CA. TTA .GCTA	<i>M.intracellulare serovar 18</i>
.A. TT .AA .GCC..	<i>M.leprae</i>
CA. TT .GCC..	<i>M.scrofulaceum</i>
.A. C .A .GCC..	<i>M.malmoense</i>
CA. AC. TT .GCC..	<i>M.gastri, M.kansasii</i>
.A. C .A .GCC..	<i>M.szulgai</i>
... C. TTC TGCGG-G..	<i>M.asiaticum</i>
.A. A. A. A. C A. C..	<i>M.xenopi</i>
.A. A. A. A. C A. C..	<i>M.gordonae I</i>
.A. A. A. A. C A. C..	<i>M.gordonae II</i>
C. T. C..	<i>M.gordonae III</i>
.A. T .AA .GCC..	<i>M.marinum, M.ulcerans</i>
... T .A .G .AT .C. GTG-	<i>M.haemophilum</i>
... CTT. TC-	<i>M.nonchromogenicum</i>
.A. TT .GCC..	<i>M.terrae</i>
... T .T. AC. GTC-	<i>M.simiae</i>
	<i>M.cookii</i>

FIG. 1. Alignment of 16S rRNA sequences from slowly growing mycobacteria in the species-specific signature region. The first nucleotide corresponds to *E. coli* position 129. The *M. tuberculosis* (*M.tb.*) sequence was used as the reference sequence; nucleotides different from *M. tuberculosis* nucleotides are indicated. The dashes indicate deletions. Sequences were obtained from references 2, 11, 13, 22, 23, 29, and 30 and from our study.

lesion, and the results of careful laboratory studies should not suggest another cause for the disease process. Although we repeatedly isolated the mycobacterium described in this paper from the patient's sputum, the question of its pathogenic potential cannot be answered with certainty with the data available.

Characteristics which differentiate *M. intermedium* from other related mycobacteria. *M. intermedium* can be differentiated from *M. kansasii* by the results of tests for nicotin-

amidase, α -esterase, β -esterase, and β -galactosidase activities. *M. gordonae* is readily distinguished from *M. intermedium* by its scotochromogenic pigmentation, its lack of growth at 41°C, and its negative β -galactosidase test. *M. intermedium* can be confused with *M. asiaticum*. These two species are phenotypically very similar with respect to the properties investigated, but differ in the ability to grow at 22°C and in β -galactosidase activity. In contrast to *M. intermedium*, *M. asiaticum* exhibits dysgonic growth. In addition, *M. intermedium* is characterized by a unique signature sequence (Fig. 1), which differentiates it from *M. asiaticum* and other previously described *Mycobacterium* species. Direct sequence determinations of amplified 16S ribosomal DNAs have been used recently for differentiation of *Mycobacterium* species (22).

Taxonomic description of *Mycobacterium intermedium* sp. nov. *Mycobacterium intermedium* (in.ter.me' di.um. L.

	10	20	30	40	50
1	GGCGGCGTGTCTTAACACATGCAAGTCGAACGGAAAGGCCCTTCGGGGGT				
51	ACTCGAGTGGCGAAGCGGGTGAGTAACACGTGGGTAACTTCGCCCTGCACAC				
101	TGGGATAAGCCTGGGAACTGGGTCTAATACCGGATAGGACCTCTCGGCG				
151	CATCGCTAGGAGGTGGAAGCTTTTGGCGGTGTGGGATGGGCGCGGCGCT				
201	ATCAGCTTGTGGTGGGTGACGGCTACCAAGGCGACGACGGGTAGCCG				
251	GCCTGAGAGGGTGTCCGCCACACTGGGACTGAGATACGGCCAGATCC				
301	TACGGAGGCGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGC				
351	AGCGACGCCGCTGGGGATGACGGCCTTCGGGTGTGTAACCTCTTTCAG				
401	CAGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCACCGGCCAAT				
451	ACGTGCCAGCAGCGCGGTAACTACGTAGGCTGCGAGCGTTGTCCGGAAT				
501	ACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTTCGCTGTAATCT				
551	CACGGCTTAAGTGTGAGCGTGGGCGATACGGGCGAGTACAGTACTGC				
601	AGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGA				
651	GGAACACCGGTGGCGAAGGGCGGTCTCTGGGCGTAACTGACGCTGAGGA				
701	GCGAAAGCGTGGGAGCGGAACAGGATTAGATACCTGGTAGTCCACGCGG				
751	TAAACGCTGGGTACTAGGTGTGGGTTTCTTCTTGGGATCCGTGCCGTA				
801	GCTAACGCATTAAAGTACCCCGCTGGGAGTACGGCGCAAGGCTAAAAAC				
851	TCAAAGGAATTGACGGGGCGCGCACAAAGCGGCGGAGCATGTGGATTAAT				
901	TCGATGCAACGCGAAGAACCTTACCTGGTTTGCATGCAAGGACGCGT				
951	CTAGAGATAGGCGTTCCTTGTGGCCTGTGTGAGGTGGTGCATGGCTGT				
1001	CGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACAGCGCAAC				
1051	CCTGTCTCATGTTGCCAGCGGGTAAATGCCGGGACTCGTAGAGACTGC				
1101	CGGGGTCAACTCGGAGGAAGGTGGGATGACGTCAGTCATCATGCCCCCT				
1151	TATGTCCAGGCTTCACACATGCTACCAATGGCCGGTACAAAGGGCTGCGA				
1201	TGCCCGGAGGTAAAGCAATCCTTTAAAGCGGTCTCAGTTCGGATCGG				
1251	GGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCA				
1301	GCAACGCTGCCGTGAATACGTTCCCGGCGCTGTACACACCCCGCTCAC				
1351	GTCATGAAAGTCGTTAACCCCGAAGCCAGTGGCCCTAACCTTTGGGAGGG				
1401	AGCTGTCGAAGGTGGGATCGGCGATTGGGACGAAGTCGTAA				

FIG. 2. 16S rDNA sequence of strain 1669/91^T (total length, 1,441 nucleotides). The first and last nucleotides correspond to *E. coli* 16S rDNA positions 38 and 1500, respectively. The positions that were omitted for the phylogenetic analysis are underlined.

FIG. 3. Phylogenetic tree based on 16S rRNA sequences, showing the position of strain 1669/91^T. The tree was rooted by using *Nocardia asteroides* as an outgroup. Bar = 10 nucleotide differences.

TABLE 3. Hamming distances derived from 16S rRNA sequences^a

Taxon	Hamming distance																			
	<i>N. asteroides</i>	<i>M. fortuitum</i> biovar <i>fortuitum</i>	<i>M. fortuitum</i> biovar <i>peregrinum</i>	<i>M. smegmatis</i>	<i>M. flavescens</i>	<i>M. simiae</i>	<i>M. intermedium</i> 1669/91 ^T	<i>M. terrae</i>	<i>M. nonchromogenicum</i>	<i>M. xenopi</i>	<i>M. gordonae</i>	<i>M. bovis</i>	<i>M. leprae</i>	<i>M. marinum</i>	<i>M. scrofulaceum</i>	<i>M. gastri/M. kansasii</i>	<i>M. szulgai</i>	<i>M. malmoense</i>	<i>M. intracellulare</i>	<i>M. avium</i>
<i>Mycobacterium fortuitum</i> biovar <i>fortuitum</i>	74																			
<i>M. fortuitum</i> biovar <i>peregrinum</i>	71	15																		
<i>M. smegmatis</i>	87	30	37																	
<i>M. flavescens</i>	81	26	34	12																
<i>M. simiae</i>	78	45	48	44	43															
<i>M. intermedium</i> 1669/91 ^T	85	46	46	47	48	28														
<i>M. terrae</i>	101	47	56	51	52	56	60													
<i>M. nonchromogenicum</i>	107	51	54	57	56	63	67	23												
<i>M. xenopi</i>	115	71	77	72	75	69	67	48	56											
<i>M. gordonae</i>	100	64	65	62	67	48	51	36	48	57										
<i>M. bovis</i>	108	59	64	62	68	56	45	34	46	49	31									
<i>M. leprae</i>	113	74	79	73	77	61	52	53	58	59	41	25								
<i>M. marinum</i>	107	56	61	64	70	58	46	34	45	50	30	8	27							
<i>M. scrofulaceum</i>	107	64	65	64	70	43	35	52	55	59	34	28	35	26						
<i>M. gastri/M. kansasii</i>	109	68	69	68	74	47	33	45	54	55	27	17	28	19	17					
<i>M. szulgai</i>	107	66	66	67	71	46	35	44	50	52	26	16	22	18	19	10				
<i>M. malmoense</i>	109	66	66	67	71	46	37	46	50	54	26	18	22	20	19	12	2			
<i>M. intracellulare</i>	108	71	71	70	75	52	39	46	52	54	30	22	26	22	19	14	12	12		
<i>M. avium</i>	109	70	70	70	76	53	42	46	50	57	29	20	25	20	22	15	13	13	8	
<i>M. asiaticum</i>	105	62	65	62	66	45	51	32	47	55	18	21	36	22	34	25	20	22	28	26

^a Sequences were obtained from references 23, 29, and 30.

neut. adj. *intermedium*, intermediate, referring to the phylogenetic position of this organism between the rapidly and slowly growing mycobacteria). Cells are acid-alcohol-fast coccobacilli (2.0 by 2.6 μ m). Does not form spores, capsules, or aerial hyphae. Visible growth from dilute inocula requires 2 to 3 weeks; colonies on Löwenstein-Jensen agar are eugonic, smooth, photochromogenic, and 3 to 5 mm in diameter. Growth occurs at 22, 31, 37, and 41°C; optimum growth occurs at temperatures between 31 and 37°C. Susceptible in vitro to ethambutol. Biochemical characteristics are shown in Table 1. *M. intermedium* synthesizes α -mycolates, α' -mycolates, and keto-mycolates. A phylogenetic analysis based on an evaluation of 16S rRNA sequences places *M. intermedium* in an intermediate position between the rapidly and slowly growing mycobacteria (Fig. 3). Cultures were isolated from the sputum of a patient with pulmonary disease. Strain 1669/91 is the type strain; a culture of this strain has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSM 44049.

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