JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1995, p. 2805–2811 Vol. 33, No. 11
0095-1137/95/$04.00+0

Mycobacterium conspicuum sp. nov., a New Species Isolated from Patients with Disseminated Infections

BURKHARD SPRINGER,[1] ENRICO TORTOLI,[2] INGEBORG RICHTER,[1] REIMAR GRÜNEWALD,[3] SABINE RÜSCH-GERDES,[4] KARIN USCHMANN,[3] FREDY SUTER,[5] M. DAVID COLLINS,[6] REINER M. KROPPENSTEDT,[7] AND ERIK C. BÖTTGER[1]*

Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover, 30625 Hannover,[1] Landeskrankenhaus Lostau, 39291 Lostau,[3] Forschungsinstitut Borstel, 23845 Borstel,[4] and Deutsche Sammlung von Mikroorganismen und Zellkulturen, 38124 Braunschweig,[7] Germany; Servizio Malattie Infettive, 21052 Busto, Arsizio,[5] and Laboratorio di Microbiologia e Virologia, 50139 Florence,[2] Italy; and Institute of Food Research, Agriculture and Food Research Council, Reading RG6 2EF, United Kingdom[6]

Received 18 May 1995/Returned for modification 12 July 1995/Accepted 5 August 1995

A new type of slowly growing, nonphotochromogenic mycobacterium was recovered from two patients with disseminated disease. The growth characteristics, acid fastness, and mycolic acids were consistent with those for *Mycobacterium* species. The results of biochemical investigations, lipid analyses, and comparative 16S rRNA sequencing showed that these isolates represent a new slowly growing *Mycobacterium* species which is named *Mycobacterium conspicuum*.

Mycobacteria are aerobic, nonmobile bacteria that are characteristically acid fast (28). 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 (29). Identification of mycobacteria has traditionally relied upon biochemical tests and lipid analysis (10, 24). More recently, molecular methods, in particular, 16S rRNA sequencing, have been developed to improve conventional identification procedures (20). During routine application of 16S rRNA sequence determination for the identification of mycobacteria, it has become clear that the genus *Mycobacterium* is much more diverse than previously anticipated and harbors a significant number of yet to be described pathogens (8, 11, 12, 16). In this report we describe a new *Mycobacterium* species, *Mycobacterium conspicuum*, that was isolated repeatedly from two patients with disseminated mycobacterial infection.

CASE REPORTS

Case report 1. The patient was a 27-year-old male with an uncharacterized cellular immunodeficiency (CD4 count, 21/mm^3; CD4/CD8 ratio, 0.2) who presented with pneumonia. Serological tests for human immunodeficiency virus types 1 and 2 were negative. The former medical and family histories were unremarkable except for a brother with general pancytopenia who died of a disseminated infection with *Mycobacterium avium*. Sputum was cultured for mycobacteria, and a triple regimen that included isoniazid, rifampin, and pyrazinamide was started under the impression that the patient had pulmonary tuberculosis. Despite treatment, acid-fast bacilli remained present in the patient's sputum and the patient's condition worsened. Therapy was subsequently changed to isoniazid, prothionamide, and ethambutol, resulting in clinical improvement.

Three months later the patient was readmitted to the hospital because of multiple skin lesions. A biopsy specimen was obtained, and the specimen revealed the presence of acid-fast bacilli. Therapy with rifampin, streptomycin, prothionamide, thiacetazone, and ethambutol was introduced. Four months later the patient was readmitted because of signs of disseminated mycobacterial infection. Following intravenous application of a multiple-drug therapy including rifampin, streptomycin, prothionamide, and ethambutol the patient's condition improved. Subsequently, therapy was changed to an oral application that included thiacetazone, and this therapy was given for 12 months. Following the discontinuation of drug therapy the patient was readmitted 6 months later, again with signs of disseminated mycobacterial infection (acid-fast bacilli present in pleural fluid, sputum, skin lesions, and stool). Despite treatment with streptomycin, clarithromycin, and rifabutin, the patient died 2 months later.

Case report 2. The patient was a 24-year-old male, was an intravenous drug abuser, and was infected with human immunodeficiency virus. The previous medical history included *Pneumocystis carinii* pneumonia, *Candida* esophagitis, and wasting. Acid-fast bacilli were seen in multiple samples of sputum and feces, and mycobacteria were repeatedly recovered by culture from blood and sputum. The symptoms at the time of isolation of mycobacteria were weight loss, fever, and pancytopenia; the CD4 count was 40/mm^3. A triple drug regimen including rifabutin, ciprofloxacin, and ethambutol resulted in clinical improvement and disappearance of the fever. The patient was dismissed because of his mental status (AIDS dementia complex). Three months later the patient died with severe wasting.

MATERIALS AND METHODS

Bacterial strains. Several isolates (3895/92^T, 3226/93, 3227/93, 3228/93, 3229/93, and 3230/93) were recovered from blood, sputum, and skin biopsy specimens of patient 1. Of the mycobacteria recovered from cultures of blood from patient 2, isolate FI 19134 was chosen for further analysis.

The isolation methods used were the standard methods used for the isolation of mycobacteria from clinical specimens, i.e., radiometric culturing in combination with Löwenstein-Jensen slants (24). Löwenstein-Jensen slants were always incubated under ambient conditions. 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 the 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 previ-

* Corresponding author. Phone: 49-511-532 4348. Fax: 49-511-532 4366.

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

Characteristic	*M. conspicuum*	*M. asiaticum*[b]	*M. gordonae*[b]	*M. kansasii*[b]	*M. malmoense*[b]
Enzymatic activity					
Production of nicotinic acid	0	0	0	0	0
Acetamidase	0	0	0	0	0
Benzamidase	0	0	0	0	0
Urease	0	0	±	+	±
Isonicotinamidase	0	0		0	0
Nicotinamidase	0	0	±	+	+
Pyrazinamidase	0	0	±	0	+
Succinidamidase	0	0	0	0	0
Tween hydrolysis (10 days)	+	+	+	+	+
Nitrate reductase	0	0	0	+	0
Arylsulfatase (10 days)	+	+	(+)	+	0
Acid phosphatase	±	+		+	0
Tellurite reduction	0	0	0	0	0
Catalase at 22°C	<2 cm	+	+	+	+
Catalase at 68°C	<1 cm	+	+	+	0
β-Galactosidase	0	0	0	0	0
Photochromogenic	0	+	0	+	0
Growth at:					
22°C	+	0	+	+	±
31°C	+	+	+	+	+
37°C	0	+	+	+	±
41°C	0	+	0	+	0
Growth on:					
NaCl (5%)	0		0	0	
MacConkey agar	0				
TCH	+		+	+	
Hydroxylamine	+				

[a] 0, negative; ±, variable; (+), weakly positive; +, positive.
[b] Obtained from the strain collection of the National Reference Laboratory for Mycobacteria, Forschungsinstitut Borstel.

ously (10, 24, 26): production of nicotinic acid, acetamidase, benzamidase, urease, isonicotinamidase, nicotinamidase, pyrazinamidase, and succinamidase; nitrate reduction; Tween hydrolysis; acid phosphatase; arylsulfatase; catalase; β-galactosidase; tellurite reduction; and growth on NaCl (5%), MacConkey agar,

TABLE 2. Drug susceptibility testing

Drug	MIC (μg/ml)		
	3895/92^T	3227/93	FI 19134
Isoniazid	2	4	2
Streptomycin	4	4	4
Ethambutol	4	2	1
Rifampin	8	8	4
Ciprofloxacin	1	1	1
Amikacin	1	2	1
Rifabutin	1	1	0.25
Clofazimine	0.25	0.25	0.25
Clarithromycin	1	1	1
Sparfloxacin	0.5	0.5	1
Azithromycin	8	16	16
Ofloxacin	4	4	4
Kanamycin	2	4	2
Capreomycin	8	4	8
Pyrazinamide	>400	>400	>400
p-Aminosalicylic acid	2	8	4

TABLE 3. Microbial Identification System whole-cell fatty acid analysis of *M. conspicuum* 3895/92^T and related organisms

Strain or species[a]	% of peak area																Identification (similarity index)[b]
	12:0	2-Me 12:0	14:0	2-Me 14:0	2,4-DiMe 14:0	15:0	16:1 B-*cis*-6	16:1 *cis*-9	16:1 *cis*-10	16:1 *cis*-11	16:0	17:1 *cis*-9	18:0 alc[c]	17:0	18:2 *cis*-9,12	18:1 *cis*-9	18:1 *cis*-11	18:0	10-Me 18:0 TBSA[d]	20:0 alc[e]	
3895/92^T	1.54	1.1	6.71			0.94	0.44	0.78	2.81		18.98		7.41	1.38		13.60	1.23	6.60	6.70	29.78	(<0.3)
M. gordonae	0.61		8.61	3.69		0.28	0.63	5.01	6.42		33.67	0.37		1.86	0.75	34.70		3.40			*M. gordonae* (0.816)
M. kansasii	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	31.22	0.85	4.32	5.63		*M. kansasii* (0.730)
M. asiaticum	2.92		19.53			0.35	0.47	3.83	4.22		39.78			0.56	0.61	19.84		7.74			*M. asiaticum* (0.730)
M. malmoense			4.45			2.34	1.98	1.01	7.67		34.64			0.93	1.81	22.03		5.76	15.41		Not in database

[a] Strains were obtained from the strain collection of the Forschungsinstitut Borstel.
[b] Identification based on Microbial Identification System.
[c] 18:0 alc, octadecanol.
[d] TBSA, tuberculostearic acid (10-methyl-octadecenoic acid).
[e] 20:0 alc, eicosanol.

TABLE 4. Comparison of ECLs of the unknown C_{15} and C_{17} fatty acid methyl esters with ECLs of related fatty acids

ECL	Fatty acid
14.000	14:0
14.315	2-Methyl-14:0
14.393	10-Methyl-14:0
14.487	Unknown A
14.570	2,4-Dimethyl-14:0
14.629	15-Methyl-14:0 (iso-15:0)
14.722	14-Methyl-14:0 (aiso-15:0)
14.856	*cis*-9-15:1
15.000	15:0
16.000	16:0
16.315	2-Methyl-16:0
16.393	10-Methyl-16:0
16.488	Unknown B
16.570	2,4-Dimethyl-16:0 (18 C atoms)
16.629	15-Methyl-16:0 (iso-17:0)
16.722	14-Methyl-16:0 (anteiso-$C_{17:0}$)
16.73 to 16.86	$C_{17:1}$ (ECL depends on position of double bond)
17.000	$C_{17:0}$

TCH, and hydroxylamine. Drug susceptibility testing and determination of MICs were done by a radiometric method as described previously (22, 23).

Lipid analyses. Lipid analyses were carried out by thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and high-performance liquid chromatography (HPLC).

TLC was done with whole organism methanolysates. 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 TLC on plates (20 by 20 cm) coated with a 0.25-mm layer of silica by 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 (3, 6, 17). The fatty acid methyl ester mixtures were separated by gas-liquid chromatography by 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 ultra-high-purity hydrogen, the column head pressure was 60 kPa, the injection volume was 2 μl, the column split ratio was 100:1, the septum purge rate was 5 ml/min, the column temperature 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.

HPLC was performed on the mycolic acids extracted from whole cells as reported previously (2). Briefly, the fatty acids were saponified with a 25% KOH solution in 50% ethanol for 1 h in an autoclave at 121°C. After acidification with 18.5% HCl, mycolic acids were extracted with chloroform and were derivatized to their UV-adsorbing *p*-bromophenacyl esters (5); after a further acidification a final chloroform extraction was performed. A total of 100 μl of the extract was spiked with 5 μl of a high-molecular-weight standard (Ribi; ImmunoChem, Hamilton, Mont.), and 5 μl was injected into a reverse-phase C_{18} Ultrasphere-XL cartridge column of a HPLC System Gold model (Beckman, Palo Alto, Calif.) equipped with a 166 model detector set at 260 nm. The elution conditions changed linearly from 98% methanol–2% dichloromethane to an 80-20% mixture in 1 min and to a 35-65% mixture over the next 9 min (flow rate, 2.5 ml/min). At the end of the run the column was reequilibrated for 1 min to the initial conditions.

The representative pattern of the strains was visually compared with our library of *Mycobacterium* HPLC profiles and with those of all of the species whose profiles had been published; for a more accurate comparison, single peaks were identified and labelled on the basis of their relative retention times, calculated by dividing the retention time of each peak by the retention time of the internal standard (7).

rRNA sequencing and phylogenetic analysis. Nucleic acid extraction and signature sequencing were performed as described previously (12). Strain 3895/92^T was chosen for a more detailed analysis. The nearly complete 16S rRNA gene was amplified by PCR, and the sequence was determined as described previously (21). With the sequencing strategy which we used, we determined the nucleotides of isolate 3895/92^T at 1,433 contiguous positions. The sequence obtained was aligned with selected 16S rRNA sequences as described previously (21). For the phylogenetic analysis regions of uncertainty in the alignment were omitted (positions 38 to 49 and positions 1399 to 1433 of the sequence, corresponding to *Escherichia coli* positions 83 to 95 and positions 1449 to 1542, respectively); this reduced the number of positions used from 1,433 to 1,386. Pairwise distances were calculated by weighting nucleotide differences and insertions-deletions equally (Hamming distances). A phylogenetic tree was constructed by using the neighbor joining method as described previously (21, 25).

Nucleotide sequence accession number. The EMBL nucleotide sequence accession number for strain 3895/92^T is X88922.

RESULTS AND DISCUSSION

Cells of the various isolates grown on Löwenstein-Jensen agar were coccobacilli and acid-alcohol fast. No spores, capsules, or aerial hyphae were observed.

On Löwenstein-Jensen slants the isolates grew as dysgonic,

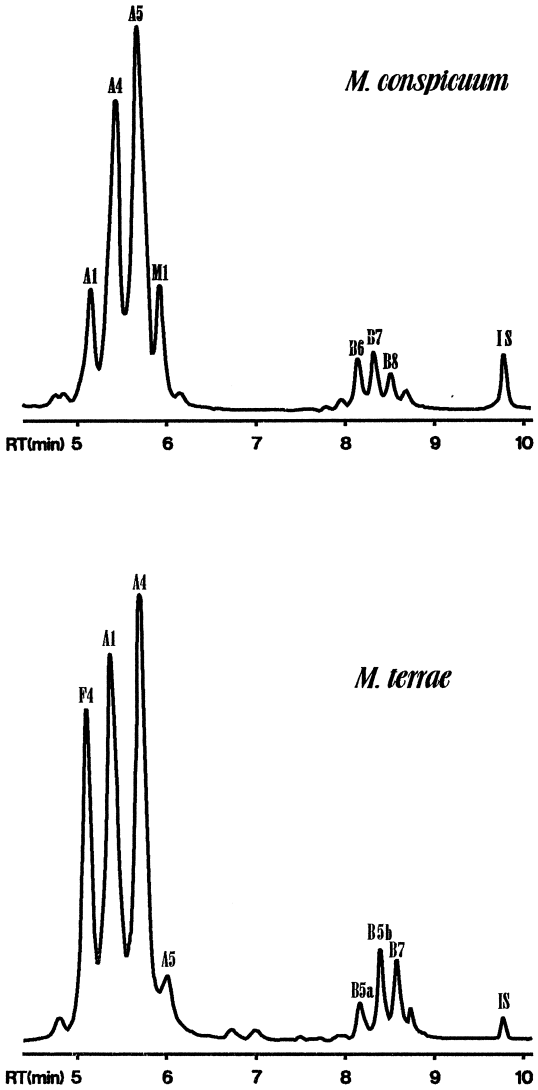

FIG. 1. Comparison of representative high-performance liquid chromatograms of the mycolic acid bromophenacyl esters of *M. conspicuum* and *M. terrae* (peaks are labelled as described previously [7]). IS, internal standard; RT, retention time.

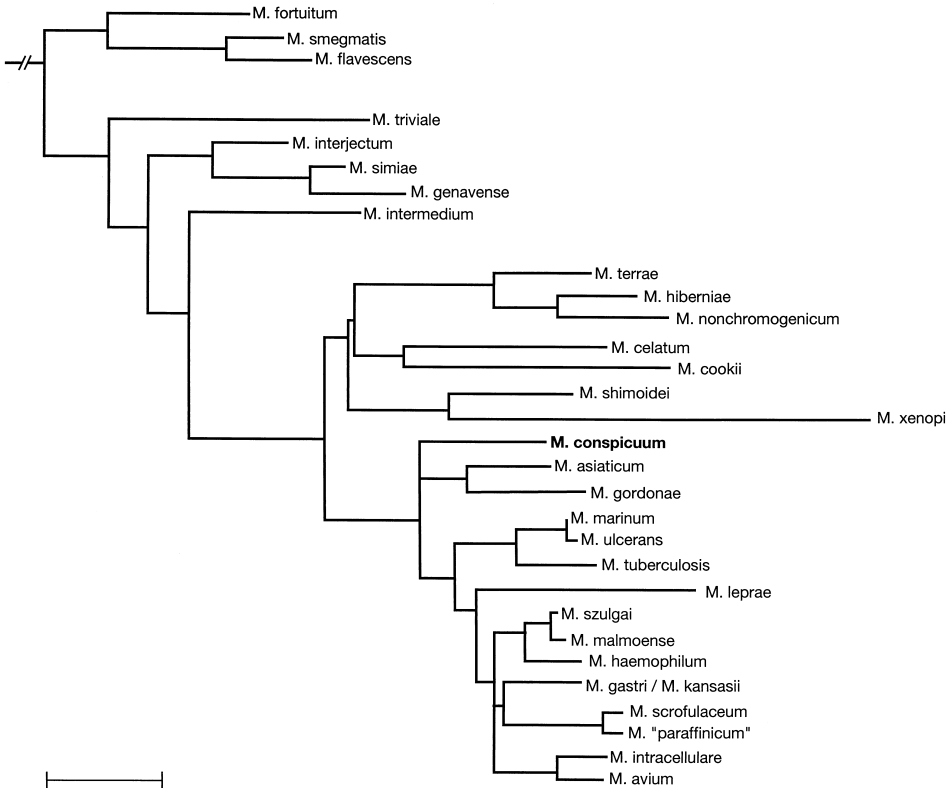

FIG. 2. Phylogenetic tree based on 16S rRNA sequences illustrating the position of *M. conspicuum*. The tree was rooted by using *Nocardia asteroides* as the outgroup. The bar indicates 10 nucleotide differences.

smooth, yellowish, nonphotochromogenic colonies within 14 to 21 days when they were incubated at temperatures ranging from 22 to 31°C. The properties of the various isolates are given in Table 1. The enzymatic activities and metabolic properties demonstrate that our strains differ from previously described species. Among all tests for enzyme activities, only tests for Tween hydrolysis and arylsulfatase activity were consistently positive; acid phosphatase activity was variable. The strains do not grow at 37°C except in liquid medium (BACTEC 12B); no growth was seen on MacConkey agar or in the presence of 5% NaCl. Detailed determinations of the MICs of a variety of drugs with antimycobacterial activity are given in Table 2.

Strain 3895/92^T was chosen for lipid analysis by TLC, GLC, and HPLC. The separation of the whole organism's acid methanolysates by TLC developed in two dimensions produced a multispot pattern composed of alpha-, keto-, and omega-dicarboxy-mycolates plus a spot of alcohols. This pattern is widely distributed among mycobacteria (14, 15, 18).

The separation of the fatty acid methyl esters by gas-liquid chromatography (Table 3) by using the Microbial Identification System revealed the expected fatty acid pattern containing unbranched saturated and unsaturated fatty acids as well as tuberculostearic acid (13). Small amounts (1.1%) of 2-methyl-branched dodecanoic acid (2-Me-12:0) could also be found. The hydrolysis products of the wax esters octadecanol and eicosanol, which are coeluted with the fatty acid methyl esters, were present in high amounts (7 and 30%, respectively). Two fatty acids with unknown structures showing an equivalent chain length (ECL) of 14,487 for peak A (2.0%) and 16,488 for peak B (2.5%) could also be detected. For peak A the electron impact mass spectrum displayed peaks at masses of 256 (M^+), 227 ($M\text{-}C_2H_5$)$^+$, 225 ($M\text{-}CH_3O$)$^+$, and 74 [$CH_3O\text{-}C(OH)=CH_2$]$^+$ and 87 ($CH_3O\text{-}CO\text{-}CH_2\text{-}CH_2$)$^+$; and for peak B the electron impact mass spectrum displayed masses of 284 (M^+), 255 ($M\text{-}CH_3O$)$^+$, 253 ($M\text{-}CH_3O$)$^+$, and 74 [$CH_3O\text{-}C(OH)=CH_2$]$^+$ and 87 ($CH_3O\text{-}CO\text{-}CH_2\text{-}CH_2$)$^+$; all of these are typical of fatty acid methyl esters. The mass peaks of peak A correspond to a saturated fatty acid methyl ester with an uneven number of carbon atoms (C_{15}), as do those of peak B (C_{17}). By comparing the ECLs of peaks A and B (14,489 and 16,488, respectively) with the ECLs of known fatty acids of the same chain length, it can be assumed that both are methyl-branched saturated fatty acids with a methyl branch between positions C-10 and C-15 (Table 4).

The occurrence of tuberculostearic acid together with undecanol and eicosanol in the fatty acid pattern classifies strain 3895/92^T into a group of mycobacteria, which includes *M. avium-M. intracellulare*, *M. scrofulaceum*, *M. terrae*, *M. nonchromogenicum* and *M. phlei* (19). Strain 3895/92^T has the same mycolic acid pattern as the mycobacteria listed above, which is composed of alpha-, keto-, and omega-dicarboxy-mycolates (4). In contrast to the five mycobacteria, 3895/92^T also synthesizes two branched fatty acids with 15 and 17 carbon atoms, respectively. The occurrence of these two unknown branched fatty acids (peaks A and B, respectively) differentiates 3895/92^T from all other mycobacteria.

Analysis of mycolic acids by HPLC for isolates 3895/92^T, 3227/93, and FI 19134 revealed an identical profile which is characterized by an early cluster of major peaks; this is followed by a minor, clearly separated second cluster (Fig. 1). Such a profile is different from any other published mycobacterial pattern; in our HPLC library, the new pattern most closely resembled that of *M. terrae*.

TABLE 5. Hamming distances derived from 16S rRNA sequences

Hamming distance																														Taxon
M. fortuitum	*M. smegmatis*	*M. flavescens*	*M. simiae*	*M. genavense*	*M. terrae*	*M. nonchromogenicum*	*M. xenopi*	*M. gordonae*	*M. tuberculosis*	*M. leprae*	*M. marinum*	*M. scrofulaceum*	*M. gastri/M. kansasii*	*M. szulgai*	*M. malmoense*	*M. intracellulare*	*M. avium*	*M. intermedium*	*M. interjectum*	*M. triviale*	*M. haemophilum*	*M. asiaticum*	*M. "paraffinicum"*	*M. conspicuum*	*M. shimoidei*	*M. celatum*	*M. cookii*	*M. hiberniae*	*M. ulcerans*	
78	91	85	84	83	107	113	131	105	116	119	112	112	114	112	114	113	114	90	86	91	110	110	111	111	112	110	111	112	113	*N. asteroides*
	30	26	47	47	49	53	84	65	63	76	57	65	69	67	67	72	71	47	44	46	71	62	64	67	64	60	66	53	58	*M. fortuitum*
		12	46	50	53	59	85	63	66	75	65	65	69	68	68	71	71	48	44	50	70	64	64	64	68	67	68	61	66	*M. smegmatis*
			45	48	54	58	88	68	72	79	71	71	75	72	72	76	77	49	43	47	74	68	70	68	72	70	69	62	72	*M. flavescens*
				11	60	67	84	49	60	63	59	44	48	47	47	53	54	29	16	44	48	45	43	42	62	57	63	66	60	*M. simiae*
					59	71	88	53	63	70	62	55	59	55	55	61	57	39	24	48	57	48	54	53	66	58	62	70	63	*M. genavense*
						23	60	39	40	57	37	55	48	47	49	49	49	63	57	60	52	36	56	43	44	41	44	20	38	*M. terrae*
							68	51	52	62	48	58	57	53	53	55	53	70	65	57	56	49	59	47	46	55	44	16	49	*M. nonchromogenicum*
								71	62	73	64	73	69	66	68	68	71	81	83	72	69	70	73	66	46	60	74	67	65	*M. xenopi*
									34	42	30	34	27	26	26	30	29	51	43	62	29	17	35	27	44	42	49	48	31	*M. gordonae*
										28	11	31	20	19	21	25	23	48	53	67	24	26	30	27	39	41	51	45	12	*M. tuberculosis*
											28	36	29	23	23	27	26	53	54	75	23	37	37	34	44	52	61	59	29	*M. leprae*
												26	19	18	20	22	20	46	52	64	21	21	27	25	36	40	48	39	1	*M. marinum*
													17	19	19	19	22	35	38	61	19	33	3	26	40	49	55	57	27	*M. scrofulaceum*
														10	12	14	15	33	44	65	14	24	16	19	36	48	56	50	20	*M. gastri/M. kansasii*
															2	12	13	35	38	63	8	19	20	17	33	43	51	48	19	*M. szulgai*
																12	13	37	38	65	8	21	20	17	35	45	53	50	21	*M. malmoense*
																	8	39	45	69	14	27	20	23	39	47	58	49	23	*M. intracellulare*
																		42	45	67	13	25	23	24	37	47	57	48	21	*M. avium*
																			22	51	36	50	34	47	56	65	70	63	47	*M. intermedium*
																				42	37	38	39	40	61	56	62	62	53	*M. interjectum*
																					65	61	60	59	57	59	57	60	65	*M. triviale*
																						24	20	19	35	47	56	53	22	*M. haemophilum*
																							34	20	44	38	44	44	22	*M. asiaticum*
																								27	40	49	56	58	28	*M. "paraffinicum"*
																									41	45	49	44	26	*M. conspicuum*
																										40	48	49	37	*M. shimoidei*
																											40	53	41	*M. celatum*
																												46	49	*M. cookii*
																													40	*M. hiberniae*

TABLE 6. Sequence homology of selected mycobacterial 16S rRNA sequences with *Mycobacterium* sp. strain 3895/92^T

Strain	% Homology[a]
M. fortuitum	95.2
M. xenopi	95.2
M. smegmatis	95.4
M. triviale	95.7
M. genavense	96.2
M. cookii	96.5
M. nonchromogenicum	96.6
M. intermedium	96.6
M. celatum	96.8
M. hiberniae	96.8
M. terrae	96.9
M. simiae	97.0
M. shimoidei	97.0
M. interjectum	97.1
M. leprae	97.5
M. "paraffinicum"	98.1
M. gordonae	98.1
M. tuberculosis	98.1
M. scrofulaceum	98.1
M. ulcerans	98.1
M. marinum	98.2
M. avium	98.3
M. intracellulare	98.3
M. asiaticum	98.6
M. gastri	98.6
M. kansasii	98.6
M. haemophilum	98.6
M. szulgai	98.8
M. malmoense	98.8

[a] Percent homology is based on 1,386 of the 1,433 determined 16S rRNA sequence positions after omission of the regions of alignment uncertainty.

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 (12, 20), revealed that isolates 3895/92^T, 3226/93, 3227/93, 3228/93, 3229/93, 3230/93, and FI 19134 had identical sequences. The sequence which we determined differed from those of the homologous regions of all previously described species of slowly growing mycobacteria. Strain 3895/92^T was chosen for use in determining an almost complete 16S rRNA sequence. A phylogenetic tree that shows the natural relationships among the mycobacteria (Fig. 2) was constructed by using equally weighted (Hamming) distances (Table 5). The short interknot branch lengths observed do not allow the position of 3895/92^T in this tree to be determined with certainty. On the basis of 16S rRNA sequence homology, strain 3895/92^T shares greater than 98.5% sequence homology with a number of mycobacterial species, including *M. asiaticum*, *M. gastri*, *M. kansasii*, *M. haemophilum*, *M. szulgai*, and *M. malmoense* (Table 6).

The strains described in this report were isolated from two immunocompromised patients presenting with disseminated mycobacterial infections. The vulnerability of immunocompromised patients, in particular patients with AIDS, to infections with nontuberculous mycobacteria, e.g., *M. avium*, *M. genavense*, and *M. celatum*, is well known (1, 9, 27). The two case reports described here suggest that *M. conspicuum* has a pathogenic potential similar to those of other nontuberculous pathogenic mycobacteria.

Characteristics which differentiate *M. conspicuum* from other mycobacteria. *M. conspicuum* shows a quite distinct metabolic reaction pattern which can rarely be confused with those of other mycobacterial species. *M. conspicuum* synthesizes alpha- and keto-mycolates and wax esters. The fatty acid pattern is composed of saturated and unsaturated unbranched fatty acids and tuberculostearic acid. The alcohols octadecanol and eicosanol are also found. Diagnostic are the occurrence of two branched fatty acids ($C_{15:0}$ and $C_{17:0}$), which could be detected in small amounts. *M. conspicuum* is characterized by a unique 16S rRNA sequence, as well as a distinct HPLC profile, which differentiates it from previously described *Mycobacterium* species.

Taxonomic description of *Mycobacterium conspicuum* sp. nov. *Mycobacterium conspicuum* (Lat. con.spi.cuus, for conspicuous, the unique biochemical profile of this species). Cells are acid-alcohol-fast coccobacilli. Does not form spores, capsules, or aerial hyphae. Visible growth from dilute inocula requires 2 to 3 weeks; colonies on Löwenstein-Jensen agar are dysgonic and nonphotochromogenic. Growth occurs at 22 and 31°C. Biochemical characteristics are given in Table 1; drug susceptibilities are given in Table 2. *M. conspicuum* synthesizes alpha- and keto-mycolates and wax esters; the short-chain fatty acid profile is given in Table 3. A phylogenetic tree based on an evaluation of 16S rRNA sequences places *M. conspicuum* within the group of slowly growing mycobacteria. The organism was isolated in cultures of samples from two patients with disseminated infection. Strain 3895/92^T is the type strain; a culture of this strain has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSM 44136.

ACKNOWLEDGMENTS

This study was supported in part by grants from the Bundesministerium für Forschung und Technologie (Verbund Mykobakterielle Infektionen) and the Commission of the European Communities (High Resolution Automated Microbial Identification). E.C.B. is supported by a Hermann-and-Lilly-Schilling professorship.

We thank Susanne Maibom for typing the manuscript.

REFERENCES

1. **Böttger, E. C., A. Teske, P. Kirschner, S. Bost, H. R. Chang, V. Beer, and B. Hirschel.** 1992. Disseminated infections with "*Mycobacterium genavense*" in patients with AIDS. Lancet **340:**76–80.
2. **Butler, W. R., L. Thibert, and J. O. Kilburn.** 1992. Identification of *Mycobacterium avium* complex strains and some similar species by high-performance liquid chromatography. J. Clin. Microbiol. **30:**2698–2704.
3. **Daffé, M., M. A. Lanéelle, C. Asselineau, V. Lévy-Frébault, and H. David.** 1983. Taxonomic value of mycobacterial fatty acids: proposal for a method of analysis. Ann. Inst. Pasteur Microbiol. **134B:**241–256.
4. **Dobson, G., D. E. Minnikin, S. M. Minnikin, J. H. Parlett, M. Goodfellow, M. Ridell, and M. Magnusson.** 1985. Systematic analysis of complex mycobacterial lipids, p. 237–265. *In* M. Goodfellow and D. E. Minnikin (ed.), Chemical methods in bacterial systematics. Academic Press, London.
5. **Durst, H. D., M. Milano, E. J. Kikta, S. A. Connelly, and E. Grushka.** 1975. Phenacyl esters of fatty acids via crown ether catalysts for enhanced ultraviolet detection in liquid chromatography. Anal. Chem. **47:**1797–1801.
6. **Eorolen, E., and O.-P. Lehtonen.** 1988. Optimal data processing procedure for automating bacterial identification by gas-liquid chromatography of cellular fatty acids. J. Clin. Microbiol. **26:**1745–1753.
7. **Glickman, S. E., J. O. Kilburn, W. R. Butler, and L. S. Ramos.** 1994. Rapid identification of mycolic acid patterns of mycobacteria by high-performance liquid chromatography using pattern recognition software and a *Mycobacterium* library. J. Clin. Microbiol. **32:**740–745.
8. **Haas, W., P. Kirschner, S. Ziesing, H. J. Bremer, and E. C. Böttger.** 1993. Cervical lymphadenitis in an immune competent child caused by a previously unknown mycobacterium. J. Infect. Dis. **167:**237–240.
9. **Horsburgh, C. R.** 1991. Mycobacterium avium complex infection in the acquired immunodeficiency syndrome. N. Engl. J. Med. **324:**1332–1338.
10. **Kent, P. T., and G. P. Kubica.** 1985. Public health laboratory. A guide for the level III laboratory. U.S. Department of Health and Human Services publication (CDC) 86-8230. Centers for Disease Control, Atlanta.
11. **Kirschner, P., A. Meier, and E. C. Böttger.** 1993. Genotypic identification and detection of mycobacteria: facing novel and uncultured pathogens, p. 173–190. *In* D. H. Persing, F. Tenover, T. J. White, and T. F. Smith (ed.),

Diagnostic molecular microbiology. American Society for Microbiology, Washington, D.C.
12. **Kirschner, P., B. Springer, U. Vogel, A. Meier, A. Wrede, M. Kiekenbeck, F. Bange, and E. C. Böttger.** 1993. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a two-year experience in a clinical laboratory. J. Clin. Microbiol. **31:**2882–2889.
13. **Kroppenstedt, R. M.** 1985. Fatty acid and menaquinone analysis of actinomycetes and related organisms, p. 173–199. *In* M. Goodfellow and D. E. Minnikin (ed.), Chemical methods in bacterial systematics. Academic Press, London.
14. **Levy-Frebault, V., and F. Portaels.** 1992. Proposed minimal standards for the genus *Mycobacterium* and for description of slowly growing *Mycobacterium* species. Int. J. Syst. Bacteriol. **43:**315–323.
15. **Luquin, M., V. Ausina, F. Lopez Calahorra, F. Belda, M. Garcia Barcelo, C. Celma, and G. Prats.** 1991. Evaluation of practical chromatographic procedures for identification of clinical isolates of mycobacteria. J. Clin. Microbiol. **29:**120–130.
16. **Meier, A., P. Kirschner, K. H. Schröder, J. Wolters, R. M. Kroppenstedt, and E. C. Böttger.** 1993. *Mycobacterium intermedium* sp. nov. Int. J. Syst. Bacteriol. **43:**204–209.
17. **Miller, L. T.** 1982. Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. J. Clin. Microbiol. **16:**584–586.
18. **Minnikin, D. E., S. M. Minnikin, J. H. Parlett, M. Goodfellow, and M. Magnusson.** 1984. Mycolic acid patterns of some species of mycobacterium. Arch. Microbiol. **139:**241–256.
19. **Parez, J. J., M. Fauville-Dufaux, J. L. Dossogne, E. de Hoffmann, and F. Pouthier.** 1994. Faster identification of mycobacteria using gas liquid and thin layer chromatography. Eur. J. Clin. Microbiol. Infect. Dis. **13:**717–725.
20. **Rogall, T., T. Flohr, and E. C. Böttger.** 1993. Differentiation of *Mycobacterium* species by direct sequencing of amplified DNA. J. Gen. Microbiol. **136:**1915–1920.
21. **Rogall, T., J. Wolters, T. Flohr, and E. C. Böttger.** 1990. Towards a phylogeny and definition of species at the molecular level within the genus *Mycobacterium*. Int. J. Syst. Bacteriol. **40:**323–330.
22. **Siddiqui, S. H.** 1992. Radiometric (Bactec) tests for slowly growing mycobacteria, p. 5.14.1–5.14.25. *In* H. D. Isenberg and J. H. Hindler (ed.), Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
23. **Siddiqui, S. H., L. B. Heifets, M. H. Cynamon, N. M. Hooper, A. Laszlo, J. P. Libonati, P. J. Lindholm-Levy, and N. Pearson.** 1993. Rapid broth macrodilution method for determination of MICs for *Mycobacterium avium* isolates. J. Clin. Microbiol. **31:**2332–2338.
24. **Sommers, H. M., and R. C. Good.** 1985. *Mycobacterium*, p. 216–248. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
25. **Sourdis, J., and M. Nei.** 1988. Relative efficiencies of the maximum parsimony and distance-matrix methods in obtaining the correct phylogenetic tree. Mol. Biol. Evol. **5:**298–311.
26. **Springer, B., P. Kirschner, G. Rost-Meyer, K.-H. Schröder, R. M. Kroppenstedt, and E. C. Böttger.** 1993. *Mycobacterium interjectum*, a new species isolated from a patient with chronic lymphadenitis. J. Clin. Microbiol. **31:** 3083–3089.
27. **Tortoli, E., C. Piersimoni, D. Bacosi, A. Bartoloni, F. Betti, L. Bono, C. Burrini, G. de Sio, C. Lacchini, A. Mantella, P. Orsi, V. Penati, M. Simonetti, and E. C. Böttger.** 1995. Isolation of the newly described species *Mycobacterium celatum* from AIDS patients. J. Clin. Microbiol. **33:**137–140.
28. **Wayne, L. G., and G. P. Kubica.** 1986. The mycobacteria, p. 1435–1457. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
29. **Wayne, L. G., and H. A. Sramek.** 1992. Agents of newly recognized or infrequently encountered mycobacterial diseases. Clin. Microbiol. Rev. **5:**1–25.