Mycobacterium alvei sp. nov.

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A new species of rapidly growing, nonphotochromogenic mycobacteria, *Mycobacterium alvei*, is described. The inclusion of this organism in the genus *Mycobacterium* is based on its acid fastness, its mycolate pattern, and its G+C content. A study of six strains showed that they form a homogeneous group with an internal phenotypic similarity value of $97 \pm 2.22\%$. DNA relatedness studies showed that the six *M. alvei* strains which we studied form a single DNA hybridization group which is less than 49% related to 14 other species of the genus *Mycobacterium*; the ΔT_m values determined for the strains which exhibited higher levels of DNA homology were all greater than 7.9°C. A lipid analysis showed that tuberculostearic acid was present. Docosanoic and tetracosanoic acid methyl esters were detected as mycolic acid cleavage products. All six isolates which we tested contained α -mycolic acids and relatively large amounts of a new kind of mycolic acid containing a methoxy group at ω -1 position, a characteristic that has not been described previously in mycobacteria. Strain CR-21 is the type strain; a culture of this strain has been deposited in the Collection Nationale de Cultures de Microorganismes de l'Institut Pasteur, Paris, France, as strain CIP 103464.

Mycolic acids are useful taxonomic markers for differentiation of mycobacteria at both the genus level and the species level. Mycolic acids are α -branched, β -hydroxy fatty acids which are characterized in mycobacteria by very long chains (up to 90 carbon atoms long) and by the presence of various functional groups in the longest (mero) chain (4, 11). Depending on the nature of these functional groups, different chromatographic behaviors are observed when preparations are subjected to thin-layer chromatography (TLC). Six types of mycolates have been recognized in this way; these mycolates are mycolate types I and II (long and short mycolates with no additional oxygenated functions), III (methoxymycolates), IV (ketomycolates), V (epoxymycolates), and VI (dicarboxymycolates). Mycolate TLC patterns for the different mycobacterial species have been published previously either in catalogs or in previous studies (4, 21, 30, 32, 33, 45).

While the results of TLC analyses reflect structural variations in the mero chain, gas chromatography analyses under pyrolysis conditions reveal variations in the α -alkyl branch. The pyrolysis esters are also useful markers for differentiation of mycobacteria at both the genus level and the species level. Mycobacteria release saturated pyrolysis esters with 22, 24, or 26 carbon atoms, unlike other mycolic acid-containing organisms, which have shorter saturated and sometimes unsaturated, α -alkyl branches (12, 43). Within the genus *Mycobacterium*, variation in the pyrolysis ester chain length is an additional differential characteristic that is especially useful for subdividing groups of species that produce the same TLC mycolate pattern.

Unlike nonhydroxylated fatty acids and glycolipid contents, which may vary from strain to strain (1, 4, 14), both TLC and gas chromatography data based on mycolate analyses are reliable characteristics that are common to all strains of a species and are used in the routine identification scheme used by reference laboratories (8, 25). Only some BCG strains produce a mycolate pattern that is devoid of

type III mycolates and thus is distinct from the authenticated profiles containing type I, III, and IV mycolates encountered in all *Mycobacterium bovis* strains and other tubercle bacilli (4, 34). Except for the patterns of these selected variants, a mycolate profile that is distinct from the profile of the type strain may indicate that a culture has been misidentified or is mixed (21, 23). Unusual mycolate characteristics as determined by TLC or gas chromatography strongly suggest the presence of new taxonomic groups, as previously shown in the description of *Mycobacterium fallax* or armadillo-derived mycobacteria (24, 38). Recently, Luquin et al. (28) described a new type of mycolate which has a chromatographic behavior that is distinct from the behaviors of type I to VI mycolates, as shown by TLC.

Between 1981 and 1989, six strains that belong to the genus *Mycobacterium* and are able to synthesize the new type of mycolate were isolated from environmental sources and clinical samples. The properties of these strains indicate that they belong to a new species. In this paper, we describe the characteristics of these apparently unusual strains and designate a new species, *Mycobacterium alvei*.

MATERIALS AND METHODS

Bacterial strains. A total of six strains were studied. Three of these strains (strains CR-21^T [T = type strain], CR-162, and CR-273) were isolated from water samples from the Llobregat River in Barcelona, Spain; one strain (strain CR-177) was isolated from soil; and the remaining two strains (strains CR-429 and CR-507) were isolated from human sputum samples. In addition to these six isolates, we studied the following strains of species which are on the Approved Lists of Bacterial Names (40) or in the Index of the Bacterial and Yeast Nomenclature Changes (36): *Mycobacterium agri* ATCC 27406^T; *Mycobacterium aichiense* ATCC 27280^T; *Mycobacterium aurum* ATCC 23366^T; *Mycobacterium austroafricanum* ATCC 33464^T; *Mycobacterium chelonae* subsp. *chelonae* NCTC 946^T and CIP 801159; *Mycobacterium chelonae* subsp. *abscessus* ATCC

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19977^T; *Mycobacterium chitae* ATCC 19627^T; *Mycobacterium chubuense* ATCC 27278^T; *Mycobacterium diernhoferi* ATCC 19340^T; *Mycobacterium duvalii* NCTC 358^T; *Mycobacterium fallax* CIP 8139^T; *Mycobacterium flavescens* ATCC 14474^T; *Mycobacterium fortuitum* ATCC 6841^T, ATCC 14467, CIPT 27, and CIPT 810539; *Mycobacterium gadium* ATCC 27726^T; *Mycobacterium gastri* ATCC 15754^T; *Mycobacterium gilvum* NCTC 10742^T; *Mycobacterium gordonae* ATCC 14470^T and CIPT 0210008; *Mycobacterium kansasii* ATCC 12478^T; *Mycobacterium komossense* ATCC 33013^T; *Mycobacterium marinum* ATCC 927^T; *Mycobacterium moriokaense* ATCC 43059^T; *Mycobacterium neoaurum* ATCC 25795^T; *Mycobacterium obuense* ATCC 27023^T; *Mycobacterium parafortuitum* ATCC 19686^T; *Mycobacterium phlei* ATCC 11758^T; *Mycobacterium porcinum* ATCC 33776^T; *Mycobacterium poriferae* ATCC 35087^T; *Mycobacterium pulveris* ATCC 35154^T; *Mycobacterium rhodesiae* ATCC 27024^T; *Mycobacterium senegalense* NCTC 10956^T; *Mycobacterium smegmatis* ATCC 19420^T; *Mycobacterium sphagni* ATCC 33027^T; *Mycobacterium terrae* ATCC 15755^T; *Mycobacterium thermoresistibile* ATCC 19527^T; *Mycobacterium tokaiense* ATCC 27282^T; *Mycobacterium triviale* ATCC 23292^T; *Mycobacterium tuberculosis* ATCC 27294^T and ATCC 25177; and *Mycobacterium vaccae* ATCC 15483^T. In addition, the frequency distribution of the test results and the results of a lipid analysis for mycobacterial isolates obtained from human host and environmental sources were included for comparative purposes; these isolates included 13 *M. chelonae* subsp. *chelonae* strains, 6 *M. chelonae* subsp. *abscessus* strains, 8 *M. fallax* strains, 7 *M. flavescens* strains, 12 *M. fortuitum* strains, 9 *M. gastri* strains, 28 *M. gordonae* strains, 21 *M. kansasii* strains, 4 *M. marinum* strains, 7 *M. smegmatis* strains, 13 *M. terrae* strains, 11 *M. triviale* strains, and 47 *M. tuberculosis* strains.

Characterization of strains. Colony morphology, the ability to grow at various temperatures (25, 30, 37, 45, and 52°C), pigment production, and photoreactivity were determined as previously described (15, 16, 46–48). The niacin test was performed by using test strips (Difco Laboratories, Detroit, Mich.). Strains were tested for iron uptake by using a modification of the procedure of Szabo and Vandra (42), as described by Silcox et al. (39). The catalase test was performed by using the method of Kubica and Pool (17). The nitrate reductase and arylsulfatase tests were performed as described by Vestal (46). The β -glucosidase, urease, penicillinase, and trehalase tests were performed as recommended by David and Jahan (7) and David et al. (9). Tests for resistance to hydroxylamine, sodium chloride, hydrolysis of Tween 80, and β -galactosidase were performed as described by Wayne et al. (47, 48). Tests to determine the use of glucose, inositol, mannitol, and sodium citrate as sole carbon sources in the presence of ammoniacal nitrogen were performed by using the procedure of Silcox et al. (39). Tests to determine degradation of salicylate, tolerance to picric acid, and acid formation from carbon sources were performed as described by Tsukamura (44). Growth in the presence of different antibiotics was determined by using the method of Canetti et al. (2).

Lipid analysis. To isolate lipid components, all of the strains were cultivated on plates containing Middlebrook 7H10 agar and incubated at 30 to 37°C in the presence of 5% CO₂. Saponification was performed by treating wet organisms with a 5% (wt/vol) potassium hydroxide solution in methanol-benzene (8:2, vol/vol) and heating the mixture overnight under reflux. After acidification with 20% sulfuric acid, the fatty acids were extracted in diethyl ether and

methyated with diazomethane. To check for the presence of epoxymycolates, an acidic methanolysis procedure (6, 10, 30, 31) was performed. This treatment destroyed epoxydes and transformed them into much more polar compounds. The potent carcinogens benzene and diazomethane were used with due regard for the necessary safety procedures. The patterns of mycolates were determined on high-performance thin-layer chromatographic plates (10 by 10 cm; HPTLC Alufolien Kieselgel 60 F₂₅₄; Merck) by using either double development with petroleum ether-diethyl ether (85:15, vol/vol) (solvent A) or dichloromethane (solvent B). The positions of the separated components were revealed by spraying the plates with a 10% solution of molybdophosphoric acid in ethanol, followed by charring. Purification and structural analysis of mycolates were performed as previously described (19, 28). The patterns of nonhydroxylated fatty acids were determined by capillary gas chromatography as described previously (25).

DNA-DNA hybridization. For DNA extraction and subsequent DNA-DNA hybridization, selected strains (see Table 4) were cultivated in 1 to 3 liters of nutrient broth (Difco) supplemented with a powder base containing 7H9 Middlebrook broth medium (Difco), 1% glycerol, and 0.05% Tween 80. Flasks were incubated at 30 or 37°C with gentle agitation for 10 to 21 days. DNA was extracted as described previously (22, 24). Briefly, the bacteria were converted to wall-deficient forms and then lysed by adding sodium dodecyl sulfate as previously described and proteinase K (Boehringer, Mannheim, Germany) instead of pronase. The DNA was then purified by using the usual methods. DNA from the type strain of the proposed new mycobacterial taxon, strain CIP 103464, was labeled in vitro by nick translation (nucleotides were obtained from the Radiochemical Centre, Amersham, England) as previously described (13), except that we modified the concentrations of some of the reagents, as follows: 3 μ l of DNA (800 μ g/ml), 40 μ l of S1 nuclease (1 U/ml; Sigma Chemical Co., St. Louis, Mo.), and 15 μ l of DNase I (10⁻⁸ g/ml). Unlabeled DNAs were sheared by sonication in order to get DNA fragments that were 500 to 800 bp long. The molecular weight of sonicated DNA was checked by gel electrophoresis, using phage lambda hydrolyzed by restriction enzyme *Hind*III. The S1 nuclease method (3) in which the S1 nuclease-trichloroacetic acid procedure (13) was used was modified as previously described (24). The temperature (T_m) at which 50% of the DNA became hydrolyzable by S1 nuclease was determined by using the procedure developed by Crosa et al. in 1973 (3) and modified as described previously (22).

Numerical taxonomy analysis. The data obtained from our study of 58 taxonomic properties were recorded as positive or negative, and the matching coefficient (M value) was calculated by using the following equation: M value = $(ns \times 100)/(ns + nd)$, where ns is the number of characters for which two strains give the same results (both positive or both negative) and nd is the number of characters for which the strains give different results (one positive and one negative) (41). The computer which we used was an IBM model 3083/XE/VM/SP-HPC computers. The program which we used is included in the Clustan 2 packet of programs (Computing Laboratory, University of St. Andrews, St. Andrews, Scotland).

RESULTS AND DISCUSSION

Cells of the six new strains grown on Löwenstein-Jensen agar and on Middlebrook 7H10 agar were short, gram-

FIG. 1. Mature *M. alvei* CIP 103464^T colonies on Middlebrook 7H10 medium. (A) Micrograph. Magnification, $\times 120$. (B) No magnification.

positive, acid-fast rods which often formed clumps, but not cords. Spores, capsules, and true branching were not observed. At 30°C, growth occurred within 4 to 5 days on Löwenstein-Jensen and Middlebrook 7H10 media and on nutrient agar. However, at 37°C, all of the strains grew more slowly (growth appeared within 10 to 15 days). Dilute inocula on Middlebrook agar yielded large, eugonic, buff-colored, rough colonies (Fig. 1). The phenotypic properties of the six *M. alvei* strains are shown in Table 1. A phenotypic similarity value of $97.0 \pm 2.2\%$ among the six strains showed the high degree of homogeneity of these organisms and permitted differentiation of these strains from other mycobacterial species as a distinct group. Comparisons of *M. alvei* CIP 103464^T with the type strains of 37 other mycobacterial species revealed degrees of similarity of 79.3% with *M. fallax*, 74.1% with *M. fortuitum* and *M. senegalense*, and 70.7% with *M. chelonae* (Table 2).

Lipid analysis. A gas chromatography analysis revealed the presence of hexadecanoate, octadecenoate, and tuberculostearate (10-methyloctadecanoate) as the major fatty acid methyl esters in lipid extracts of the six *M. alvei* strains. The mycolic acid cleavage products were docosanoate and tetracosanoate. Secondary alcohols and additional methyl-branched fatty acids were not detected. On thin-layer chromatograms all six strains produced an exceptional pattern of mycolates that was composed of nonoxygenated mycolates and one more polar oxygenated mycolate, which had an R_f value that was close to the R_f value of wax ester mycolate (solvent B) or between the R_f values of epoxymycolate and wax ester mycolate (solvent A) (Fig. 2 and Table 3). The formula of this new oxygenated mycolate shown in Fig. 3 was derived from data from a spectroscopic analysis and chemical degradation (28). The oxygenated mycolate of *M. alvei* was a novel mycolic acid which contained a methoxy group at the ω -1 position, instead of the ω -17-to- ω -18 position as in the previously described methoxymycolates (5, 28, 35). This is in accordance with the unusual chromatographic behavior of this compound, since a methoxy group on the

penultimate carbon of the mero chain should result in a significantly higher polarity of the molecule compared with a molecule with a methoxy group in the middle of an aliphatic chain. Another remarkable difference is that the methoxy mycolate had two double bonds in its long meroaldehyde chain instead of one as in previously described mycolates containing additional oxygenated groups (5, 28, 35).

DNA relatedness. The ratios of absorbance for DNA solutions ranged from 1.9 to 2.1 and from 1.7 to 1.8 for the A_{260}/A_{280} and A_{260}/A_{230} ratios, respectively. The results of DNA-DNA hybridization experiments are shown in Table 4. The type strains of species which produced mycolate patterns similar to the pattern of the new taxon were selected for DNA-DNA hybridization experiments, along with other rapidly growing mycobacterial species. Representatives of the type species of the genus and of the slowly growing species *M. gordonae* and *M. triviale* were also included. The use of the hybridization and S1 nuclease treatment conditions described above allowed levels of nonspecific hybridization (in control tubes containing herring and labeled DNAs) ranging from 8.1 to 9.2%. Five strains of *M. alvei* were 80 to 100% related to strain CIP 103464^T, with ΔT_m values less than 1°C. The levels of DNA relatedness between strain CIP 103464^T and other strains ranged from 5 to 49%; the ΔT_m values determined for the higher levels of DNA complementarity were all more than 7.9°C. The guanine-plus-cytosine (G+C) contents of DNAs were calculated from the melting temperatures by using the equation of Owen and Pitcher (37). The G+C content of strain CIP 103464^T was 68 mol%; this value was an underestimation of the G+C content as sonicated DNAs were used for to determine the melting temperatures.

Description of type strain CR-21 (= CIP 103464) of *Mycobacterium alvei* sp. nov. *Mycobacterium alvei* (al've.i. L.gen. n.alvei, bed of a river, referring to the place where this species was first isolated). The cells of *M. alvei* are short rods that are 1 to 3 μ m long and 0.5 to 0.7 μ m wide; they are mainly strongly acid fast, except for a small number (less

TABLE 1. Properties of the *M. alvei* type strain and five additional strains

Characteristic	Type strain ^a	Five other strains ^b
Morphology		
Rods (>1 µm long)	+	100
Coccobacillary (<1 µm long)	—	0
Cord formation	—	0
Smooth colonies	—	0
Rough colonies	+	100
Pigmentation in the dark	—	0
Pigmentation in the light	—	0
Growth at:		
25°C	+	100
30°C	+	100
30°C in <5 days	+	100
37°C	+	100
37°C in <5 days	—	0
45°C	—	0
52°C	—	0
Growth on nutrient agar at:		
30°C	+	100
37°C	+	100
Growth on MacConkey agar without crystal violet	—	0
Iron uptake	—	0
Niacin	—	0
Enzymatic activities		
Arylsulfatase (3 days)	+	100
Catalase (22°C)	+	100
Catalase (68°C)	+	100
β-Glucosidase (3 h)	+	100
β-Galactosidase (18 h)	+	100
Nitrate reductase (2 h)	+	100
Penicillinase (5 h)	+	100
Trehalase (5 h)	+	100
Urease (18 h)	+	100
Tween hydrolysis (10 days)	+	100
Salicylate degradation (7 days)	—	0
Acid produced from:		
Glucose	+	100
Dulcitol	—	0
Fructose	+	100
Inositol	—	0
Mannitol	—	0
Rhamnose	—	0
Xylose	—	0
Trehalose	+	100
Use of the following compounds as sole carbon sources:		
Glucose	+	100
Inositol	—	0
Mannitol	—	0
Fructose	+	100
Sodium citrate	—	0
Growth in the presence of:		
Picric acid (0.2%)	—	60 ^c
NaCl (5%)	—	0
NH ₂ OH (500 mg/liter)	+	40 ^d
D-Cycloserine (30 mg/liter)	+	100
Ethambutol (2 mg/liter)	—	0
Streptomycin (4 mg/liter)	+	100
Kanamycin (20 mg/liter)	—	0
Capreomycin (40 mg/liter)	—	0
Isoniazid (0.1 mg/liter)	+	100
Isoniazid (1 mg/liter)	+	100
Isoniazid (10 mg/liter)	—	60 ^c
Rifampin (40 mg/liter)	+	100
p-Aminosalicylic acid (0.5 mg/liter)	+	100
Thiacetazone (2 mg/liter)	+	100
Thiacetazone (10 mg/liter)	+	100

^a —, negative; +, positive.^b The values are the percentages of strains that are positive.^c Strains CR-62, CR-429, and CR-507 are positive.^d Strains CR-62, CR-429, and CR-507 are negative.TABLE 2. Levels of similarity between *M. alvei* CR-21^T (= CIP 103464^T) and type strains of 38 other mycobacterial species and subspecies

Species or subspecies	% Similarity to strain CIP 103464 ^T
<i>M. fallax</i>	79.3
<i>M. fortuitum</i>	74.1
<i>M. senegalense</i> ^a	74.1
<i>M. chelonae</i> subsp. <i>chelonae</i>	70.7
<i>M. chelonae</i> subsp. <i>abscessus</i>	70.7
<i>M. morioakaense</i>	69.8
<i>M. chitae</i>	69.0
<i>M. flavescens</i>	69.0
<i>M. agri</i>	69.0
<i>M. sphagni</i>	67.5
<i>M. rhodesiae</i>	67.5
<i>M. aichiense</i>	67.5
<i>M. smegmatis</i>	67.2
<i>M. austroafricanum</i>	65.7
<i>M. marinum</i>	65.5
<i>M. parafortuitum</i>	65.3
<i>M. tokaiense</i>	65.0
<i>M. chubuense</i>	65.0
<i>M. kansasii</i>	63.8
<i>M. triviale</i>	63.8
<i>M. porcinum</i>	63.8
<i>M. thermoresistibile</i>	63.3
<i>M. obuense</i>	62.5
<i>M. neoaurum</i>	62.5
<i>M. terrae</i>	62.1
<i>M. vaccae</i>	62.1
<i>M. diernhoferi</i>	62.0
<i>M. gilvum</i>	61.1
<i>M. duvalii</i>	61.1
<i>M. komossense</i>	60.9
<i>M. gadium</i>	60.0
<i>M. pulveris</i>	59.6
<i>M. phlei</i>	57.9
<i>M. aurum</i>	57.1
<i>M. poriferae</i>	55.8
<i>M. tuberculosis</i>	55.2
<i>M. gordonae</i>	53.4
<i>M. gastri</i>	51.7

^a Only 51 properties were compared.

than 10%) of cyanophil forms. The cells are gram-positive rods and frequently appear to be coccoid. Smears prepared from Youmans medium (without Tween 80) and Middlebrook 7H12 medium contain clumps of rods but not cords. Colonies on Löwenstein-Jensen medium and on Middlebrook 7H10 agar are eugonic, rough, and nonpigmented in the dark and also after exposure to light. Growth occurs within 5 days at 30°C, the optimum growth temperature. At 37°C, the organisms grow more slowly (growth appears within 10 to 15 days), and they do not grow at 45 or 52°C. The type strain produces thermostable catalase and is positive for arylsulfatase (3 days), nitrate reductase, urease, β-glucosidase, and β-galactosidase. No growth occurs in MacConkey agar without crystal violet. Salicylate is not degraded to catechol. Niacin is not produced. Iron uptake is negative. Growth is inhibited in the presence of sodium chloride (5%) and picric acid (0.2%). Inositol, mannitol, and sodium citrate are not utilized as sole carbon sources. Acid is produced from glucose, fructose, and trehalose but not from dulcitol, inositol, mannitol, rhamnose, and xylose. The type strain is resistant to D-cycloserine, streptomycin, isoniazid (0.1 and 1 mg/liter), rifampin, p-aminosalicylic acid,

FIG. 2. Thin-layer chromatograms of methylmycolates from *M. tuberculosis* ATCC 27294^T (lanes 1), *M. fortuitum* ATCC 6841^T (lanes 2), *M. alvei* CIP 103464^T (lanes 3), and *M. aurum* ATCC 23366^T (lanes 4). The mycolate types are indicated as follows: I, unsaturated mycolate; III, methoxymycolate; IV, ketomycolate; V, epoxymycolate; VI, ω-carboxymycolate and secondary alcohol; X, ω-1 methoxymycolate. (A) Solvent A (petroleum ether-diethyl ether [85:15, vol/vol]) was used for three runs. (B) Solvent B (dichloromethane).

TABLE 3. Distribution of mycolic acids in the type strains of rapidly growing mycobacteria

Species	Presence of mycolic acid type ^a :						
	I	II	III	IV	V	VI	VII
<i>M. agri</i>	+	+	+				
<i>M. aichiense</i>	+			+		+	
<i>M. alvei</i>	+						+
<i>M. aurum</i>	+			(+)		+	
<i>M. austroafricanum</i>	+	+		(+)		+	
<i>M. chelonae</i>	+	+					
<i>M. chitae</i>	+	(+)			+		
<i>M. chubuense</i>	+	+		(+)		+	
<i>M. diernhoferi</i>	+			+		+	
<i>M. duvalii</i>	+	+		+		+	
<i>M. fallax</i>	+						
<i>M. flavescens</i>	+			+		+	
<i>M. fortuitum</i>	+	(+)			+		
<i>M. gadium</i>	+			+		+	
<i>M. gilvum</i>	+	+		(+)		+	
<i>M. komossense</i>	+			+		+	
<i>M. morioakaense</i>	(+)			+		+	
<i>M. neoaurum</i>	+			(+)		+	
<i>M. obuense</i>	+	+		(+)		+	
<i>M. parafortuitum</i>	+	+		(+)		+	
<i>M. phlei</i>	+			(+)		+	
<i>M. porcinum</i>	+	(+)			+		+
<i>M. poriferae</i>	+			+		+	
<i>M. pulveris</i>	+	+		+		+	
<i>M. rhodesiae</i>	+			(+)		+	
<i>M. senegalense</i>	+	(+)			+		+
<i>M. smegmatis</i>	+	+			+		
<i>M. sphagni</i>	+			(+)		+	
<i>M. thermoresistibile</i>	+	(+)	+	+			
<i>M. tokaiense</i>	+			(+)		+	
<i>M. vaccae</i>	+	+		+		+	

^a Type I, α-mycolate; type II, α'-mycolate; type III, methoxymycolate; type IV, ketomycolate; type V, epoxymycolate; type VI, wax ester mycolate (ω-carboxymycolate and secondary alcohols); type VII, ω-1 methoxymycolate.

^b +, present; (+), a small amount is present.

FIG. 3. Molecular structure of methoxylated methylmycolate of *M. alvei* CIP 103464^T. Abbreviations: R, H or CH₃; R', H or CH₃; x, 13 to 18 when R is H or 15 when R is CH₃; y, 9 to 13; and z, 14 to 20 when R is H or 17 when R is CH₃.

and thiacetazone and is susceptible to isoniazid (10 mg/liter), kanamycin, and capreomycin.

Gas chromatography of the total fatty acid methyl esters reveals the presence of tuberculostearate. Pyrolytic C₂-C₃ cleavage gives intense peaks corresponding to docosanoate and tetracosanoate, indicating that characteristic α-branched, β-hydroxylated mycobacterial mycolates are present. Secondary alcohols are not detected. As determined by TLC, *M. alvei* contains α-mycolates and ω-1 methoxymycolates. Since as determined by gas chromatography *M. alvei* produces lipid profiles similar to those of the other rapidly growing nonphotochromogenic mycobacteria (18, 20, 25, 45), analysis of the mycolic acid pattern allows a clear distinction between *M. alvei* and the previously described *Mycobacterium* species. Recently, we have found that *M. porcinum*, *M. senegalense*, and some strains of *M. fortuitum* and "*Mycobacterium peregrinum*" also contain appreciable amounts of ω-1 methoxymycolate, but in these species this mycolate is always accompanied by an epoxy-

TABLE 4. Levels of DNA relatedness between strain CR-21^T (= CIP 103464^T) and reference strains belonging to selected mycobacterial species

Species	Source of DNA ^a		% of relative binding at 75°C ^b
	Strain	Other designation	
<i>M. alvei</i>	CR-21 ^T (= CIP 103464 ^T)		100 (0)
	CR-62		80 (0.5)
	CR-429		80 (0.2)
	CR-177		100 (0.1)
	CR-507		81 (0.4)
	CR-273		86 (0.0)
<i>M. agri</i>	CIPT 1320001 ^T	ATCC 27406 ^T	42 (7.9)
<i>M. chelonae</i>	CIPT 0420003 ^T	NCTC 946 ^T	9
	CIPT 801159		6
<i>M. chitae</i>	CIPT 1160001 ^T	ATCC 19627 ^T	17
<i>M. fallax</i>	CIPT 1390005 ^T	CIP 8139 ^T	8
<i>M. fortuitum</i>	CIPT 0410001 ^T	ATCC 6841 ^T	28 (8.4)
	CIPT 810539		26 (8.8)
	CIPT 27		25 (9.5)
	CIPT 0410020	ATCC 14467	18
<i>M. gordonae</i>	CIPT 0210008		21
<i>M. morioakaense</i>	CIPT 1470001 ^T	ATCC 43059 ^T	6
<i>M. phlei</i>	CIPT 1300001 ^T	ATCC 11758 ^T	22
<i>M. porcinum</i>	CIPT 1460001 ^T	ATCC 33776 ^T	49 (9.3)
<i>M. pulveris</i>	CIPT 1480001 ^T	ATCC 35154 ^T	14
<i>M. senegalense</i>	CIPT 1350002 ^T	NCTC 10956 ^T	37 (8.2)
<i>M. smegmatis</i>	CIPT 1330010 ^T	ATCC 19420 ^T	18
<i>M. triviale</i>	CIPT 0330001 ^T	ATCC 23292 ^T	5
<i>M. tuberculosis</i>	CIPT 0010002	ATCC 25177	5

^a CR, Collection of the Department of Tuberculosis, Hospital de la Santa Cruz y San Pablo, Barcelona, Spain; CIP, Collection Institut Pasteur, Paris, France; CIPT, Collection Institut Pasteur Tuberculose, Paris, France; ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, England.

^b The values in parentheses are ΔT_m values (in degrees Celsius).

mycolate (Table 3) (26, 27, 31). However, in *M. alvei* the ω -1 methoxymycolate is the only oxygenated compound (28).

Characteristics which differentiate *M. alvei* from other related mycobacteria. *M. alvei* is differentiated from *M. fallax* by its failure to form cords, by its positive arylsulfatase, thermostable catalase, β -glucosidase, β -galactosidase, penicillinase, trehalase, and urease activities, and by its ability to grow in Löwenstein-Jensen medium containing D-cycloserine (30 mg/liter) and rifampin (40 mg/liter).

M. alvei is easily differentiated from *M. fortuitum* and *M. chelonae* by its failure to grow on MacConkey agar without crystal violet, by its failure to degrade salicylate, and by its inability to grow in presence of picric acid (0.2%), ethambutol (2 mg/liter), kanamycin (20 mg/liter), capreomycin (40 mg/liter), and isoniazid (10 mg/liter).

M. alvei is differentiated from *M. senegalense* by its positive penicillinase and trehalase activities and by its ability to hydrolyze Tween 80 and to grow in presence of rifampin (40 mg/liter). Furthermore, *M. senegalense* grows on media containing mannitol as a sole carbon source and shows resistance to ethambutol (2 mg/liter) and isoniazid (10 mg/liter), properties which are negative in *M. alvei*.

This new taxon is differentiated from all other mycobacterial species by its unusual mycolate pattern.

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