Mycobacterium hodleri sp. nov., a New Member of the Fast-Growing Mycobacteria Capable of Degrading Polycyclic Aromatic Hydrocarbons

M. KLEESPIES,¹ R. M. KROPPESTEDT,² F. A. RAINEY,² L. E. WEBB,¹ AND E. STACKEBRANDT^{2*}

IBT3, Forschungszentrum Jülich GmbH, 52425 Jülich,¹ and DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, 38124 Braunschweig,² Germany

A bacterial strain isolated from a fluoranthene-polluted soil was characterized with respect to its metabolic and chemotaxonomic properties, and its phylogenetic position was determined. This bacterium exhibits all of the genus-specific properties of the genus *Mycobacterium* and clusters phylogenetically with the group of fast-growing mycobacteria. On the basis of its unique fatty acid pattern, the distinctness of its physiological properties and the uniqueness of the primary structure of its 16S ribosomal DNA, we propose that the new isolate should be assigned to a new species, *Mycobacterium hodleri*. This novel species is phylogenetically closely related to *Mycobacterium diernhoferi* and *Mycobacterium neoaurum*. The type strain of *M. hodleri* is strain EMI2 (= DSM 44183).

Members of the genera *Rhodococcus* (5) and *Mycobacterium* (8, 13) have been reported to be degraders of environmentally hazardous chemicals, including volatile aliphatic hydrocarbons and polyaromatic and chlorinated hydrocarbons. The members of these genera that are known to degrade one or more of these hazardous chemicals include *Mycobacterium aurum* (7) (which degrades vinyl chloride), *Mycobacterium chlorophenolicum* (6) (which degrades pentachlorophenol and other polychlorinated phenolic compounds), and *Rhodococcus rhodochrous* (24) (which degrades vinyl chloride, trichloroethylene and *cis*-1,2-dichloroethylene). Other *Rhodococcus* strains that mineralize vinyl chloride and trichloroethylene have been characterized (24). *Mycobacterium* sp. strain PYR-1 (= DSM 7251) has been thoroughly investigated (8) and has been found to degrade polycyclic aromatic hydrocarbons (PAHs) with up to four ring systems, including fluoranthene (13). On the basis of its physiological properties this strain has been found to be moderately closely related to *Mycobacterium austroafricanum* (30). As determined by 16S ribosomal DNA (rDNA) analysis, this strain clustered with *Mycobacterium vaccae* and *Mycobacterium aurum* (33, 39), but the 16S rDNA sequence of *M. austroafricanum* was not available for comparison. *Mycobacterium* sp. strains BB1 (= DSM 9487) (37) and PAH135 (39) are other PAH-metabolizing organisms (36) which are phylogenetically related to the cluster containing the rapidly growing mycobacteria; these strains are related to *Mycobacterium gilvum* (33) and *Mycobacterium aichiense* (39), respectively. In this paper we describe a new fluoranthene-degrading, rapidly growing isolate that represents a new *Mycobacterium* species in the *Mycobacterium diernhoferi*-*Mycobacterium neoaurum* cluster.

MATERIALS AND METHODS

Isolation. Strain EMI2^T (T = type strain) was isolated from soil contaminated with fluoranthene and benzo(a)pyrene. A 5-g portion of soil was incubated in mineral medium (pH 7.0) containing (per liter) 300 mg of (NH₄)₂HPO₄, 150 mg of KH₂PO₄, 20 mg of MgSO₄, 0.1 ml of a 1% FeCl₃·6H₂O solution, 1 ml of a trace element solution, medium 27 (3), and 2% (vol/vol) rapeseed oil. Fluoranthene

was added to the medium at a concentration of 50 mg liter⁻¹. The preparation was incubated with shaking for 28 days at 20°C. Subcultures were obtained by transferring 0.5-ml portions of the broth into 50-ml portions of the mineral medium containing 50 mg of fluoranthene per liter. Growth was detected after 2 weeks by observing the increase in the turbidity of the medium. Subsequent conventional isolation by dilution and transfer of single colonies onto solidified mineral medium containing 50 mg of fluoranthene per liter and 5 ml of Tween 80 per liter resulted in the recovery of a single isolate that was maintained by weekly transfer to mineral medium containing 50 mg of fluoranthene per liter.

Characterization of strain EMI2^T. Micromorphological properties were determined by phase-contrast microscopy. Gram staining, acid-alcohol fastness, colony morphology, the ability to grow at various temperatures (15, 18, 25, 28, 37, 42, and 45°C), pigment production, and photoreactivity were determined after 2 weeks of growth in Middlebrook 7H10 medium and on Trypticase soy broth agar supplemented with Tween 80 by using the proposed minimal standards for the genus *Mycobacterium* (21). All tests were carried out at 28°C. The catalase test was performed as described by Kubica and Pool (18). Nitrate reductase and Tween 80 hydrolysis were detected as described by Bönicke (1). The protocols used to determine growth on xylose, trehalose, mannitol, and sorbitol as sole sources of carbon were the protocols described by Silcox et al. (34). Additional carbon source utilization tests and qualitative enzyme assays were performed in standard microtiter plates (F-Forms; Greiner, Nürtingen, Germany) as described previously (11, 12) by using modifications described by Kirchof et al. (14) and Klatt et al. (15).

Oxidation of fluoranthene and a second PAH (anthracene, pyrene, or phenanthrene) was tested over a period of 7 days by using the liquid mineral medium described above supplemented with 50 mg of each compound per liter. The degradation products were determined by high-performance liquid chromatography (HPLC) by using an acetonitrile-H₃PO₄ gradient that consisted of 20% (vol/vol) acetonitrile and 80% (vol/vol) H₃PO₄ at zero time, 80% (vol/vol) acetonitrile and 20% (vol/vol) H₃PO₄ at 20 min, and 20% (vol/vol) acetonitrile and 80% (vol/vol) H₃PO₄ at 21 min; the flow rate was 1.0 ml min⁻¹. The concentration of fluoranthene was determined by using 85% (vol/vol) acetonitrile and 15% (vol/vol) H₃PO₄ at a flow rate of 1.0 ml min⁻¹.

Determination of chemotaxonomic properties. For chemical analyses the isolate was grown in liquid ISP medium 2 containing (per liter) 4 g of dextrose, 4 g of yeast extract, and 10 g of malt extract (pH 7.2) for 5 days at 28°C, harvested by centrifugation, washed, and freeze-dried. The G+C content of the DNA was determined by HPLC (25).

Analysis of cell wall diamino acids. An amino acid analysis of whole-cell hydrolysates was performed by using previously described procedures (36).

Extraction and analysis of isoprenoid quinones. Isoprenoid quinones were extracted and purified by using the small-scale integrated procedure of Minnikin et al. (28). Dried preparations were dissolved in 200 µl of isopropanol, and 1- to 10-µl portions were separated by HPLC without further purification. The menaquinones were separated by HPLC on a Lichrosorb (Merck) RP-18, 5-µm column at 40°C by using acetonitrile-isopropanol (65:35, vol/vol) as the solvent (17) and on a silver-loaded ion-exchange Nucleosil (Macherey & Nagel, Düren, Germany) SAB, 5-µm column at 50°C by using 100% methanol as the solvent (16).

Fatty acid analysis. For fatty acid analyses cells were grown on Middlebrook 7H10 medium enriched with Middlebrook OADC in petri dishes for 5 days at

* Corresponding author. Mailing address: DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany. Phone: 49 531 2616 352. Fax: 49 531 2616 418. Electronic mail address: erko@gbf-braunschweig.de.

TABLE 1. Physiological properties of isolate EMI2^T

Characteristic	Reaction
Utilization of ^a :	
D-Glucose.....	+
D-Galactose.....	+
L-Rhamnose.....	+
D-Ribose.....	+
D-Sucrose.....	-
D-Turanose.....	-
D-Arabinose.....	+
Inositol.....	+
N-Acetyl-D-glucosamine.....	+
Glucarate.....	-
Gluconate.....	+
D-Glucosaminic acid.....	-
Citrate.....	-
Caprate.....	-
4-Aminobutyrate.....	-
2-Hydroxavalerate.....	-
2-Oxoglutarate.....	+
Pimelate.....	-
Succinate.....	+
Tween 80.....	+
Benzoate.....	-
3-Hydroxybenzoate.....	-
4-Hydroxybenzoate.....	-
Phenylacetate.....	-
Quinate.....	+
L-Alanine.....	+
L-Aspartate.....	+
L-Leucine.....	+
L-Proline.....	+
L-Serine.....	+
L-Valine.....	+
Putrescine.....	+
Tyramine.....	-
Acetamide.....	-
Arylsulfatase activity ^b	ND
Urease activity ^b	+
Hydrolysis of ^c :	
CXY.....	+
CCH.....	-
CDP.....	-

^a Utilization was determined by the MTT (14) reduction method.

^b Enzymatic activity was detected by examining the hydrolysis of chromogenic substrates. ND, not detected.

^c Abbreviations: CXY, *p*-nitrophenyl- β -D-xyloside; CCH, *p*-nitrophenylphosphoryl-choline; CDP, 2-deoxythymidine-5'-*p*-nitrophenylphosphate.

28°C. Fatty acid methyl esters were prepared from 40 mg (wet weight) of cells as described previously (26), with minor modifications (19). The analyses were performed by using a model 5898A gas chromatograph (Hewlett-Packard Co.) connected to a 486 Vectra computer (Hewlett-Packard Co.). The Microbial Identification System software (Microbial ID, Newark, Del.) was used to control the system and to identify the fatty acids. The Microbial Identification System Library for mycobacteria was used to identify the mycobacterium on the basis of its fatty acids.

Extraction and analysis of mycolic acids. The mycolic acids of 50 mg of freeze-dried bacteria were analyzed as described previously (22, 27).

Analysis of 16S rDNA. Extraction of genomic DNAs and amplification of the 16S rDNAs of strain EMI2^T, *M. austroafricanum* ATCC 33464^T, and *Mycobacterium parafortuitum* DSM 43528^T were carried out as described previously (31). PCR products were sequenced directly by using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) and the manufacturer's protocol. The sequence reaction mixtures were electrophoresed by using an Applied Biosystems model 373A DNA sequencer.

Phylogenetic analysis. The 16S rDNA sequences which we determined were compared with 16S rDNA database for members of the order *Actinomycetales* consisting of the sequences deposited in the Ribosomal Database Project database (23). In order to compensate for multiple substitutions at any given site in the sequence, similarity values were corrected by the method of Jukes and Cantor (10). Dendrograms were constructed by the neighbor-joining method (4) and the distance matrix method of De Soete (2) by using the corrected dissimilarity values. To calculate bootstrap values, we analyzed 200 trees.

Nucleotide sequence accession numbers. The sequences of the type strains of *M. austroafricanum* and *M. parafortuitum* and strain EMI2^T have been deposited in the EMBL data library under accession numbers X93182, X93183, and X93184, respectively.

RESULTS AND DISCUSSION

Morphological properties. Cells of strain EMI2^T were gram positive, acid-alcohol fast, and nonmotile, did not form spores, and were rod shaped (length, 1.8 to 2.3 μ m; diameter, 1 μ m). Some cells occurred in V-forms and in palisades. Good growth was observed on Trypticase soy broth agar supplemented with Tween 80 at 25°C. No growth was observed at 15°C and at temperatures above 37°C. The colonies were smooth, but some rough colonies were also observed. A fatty acid analysis of two different colonies revealed that these colonies had identical fatty acid patterns. The scotochromogenic colonies were saffron yellow on Middlebrook medium and chrome yellow on Trypticase soy broth agar supplemented with Tween 80. Growth occurred within 4 or 5 days on both media.

Physiological properties. In mineral medium the aerobic isolate was able to grow on a variety of substrates, including glucose, Tween 80, and rapeseed oil, and 35% [3-¹⁴C]fluoranthene (32.5 μ g of fluoranthene per liter) was mineralized to ¹⁴CO₂ over a period of 120 h. A total of 29 metabolites were recognized by HPLC, but only one compound, *cis*-2,3-dihydroxy-2,3-dihydrofluoranthene, could be identified. In the

TABLE 2. Chemotaxonomic properties that show that strain EMI2^T is a member of the genus *Mycobacterium*^a

Taxon	No. of C atoms in mycolic acids	Fatty acid type ^b	Major menaquinone	Presence of ^c :				G+C content of DNA (mol%)
				PI	PE	TBSA	N-gly MA	
Strain EMI2 ^T	80	1B	MK-9(H ₂)	+	+	+	+	73
<i>Mycobacterium</i>	60-90	1B	MK-9(H ₂)	+	+	+	+	70-72
<i>Tsukamurella</i>	64-78	1B	MK-9	+	+	+	+	67-68
<i>Nocardia</i>	44-60	1B	MK-8(H _{4c})	+	+	+	+	64-72
<i>Rhodococcus</i>	34-64	1B	MK-8(H ₂)	+	+	+	+	63-73
<i>Gordonia</i>	48-66	1B	MK-8(H ₂)	+	+	+	+	63-69
<i>Dietzia</i>	34-38	1B	MK-8(H ₂)	-	+	+	-	73
<i>Corynebacterium</i>	22-36	1A	MK-8(H ₂)	+	- ^d	- ^e	-	51-67

^a The following properties are present in all taxa: *meso*-diaminopimelic acid is the diagnostic amino acid in the peptidoglycan, and arabinose and galactose are whole-cell sugars. Data from reference 32.

^b As defined by Kroppenstedt et al. (17).

^c Abbreviations: PI, phosphatidylinositol; PE, phosphatidylethanolamine; TBSA, tuberculostearic acid (10-methyl-octadecanoic acid); N-gly MA, *N*-glycolyl-muramic acid.

^d Phosphatidylethanolamine is present in some species (16).

^e Tuberculostearic acid is present in some species (16).

TABLE 3. Mycolic acids and pyrolysis esters of strain EMI2^T and members of the *M. parafortuitum* group^a

Taxon	Mycolic acid esters							Pyrolysis esters		
	α	α'	Methoxy	Keto	Epoxy	Wax-ester ^b	ω -1 ^c	C ₂₂	C ₂₄	C ₂₆
Strain EMI2 ^T	+	+	—	+	—	+	—	+	+	—
<i>M. aurum</i>	+	(+)	—	+	—	+	—	+	+	—
<i>M. austroafricanum</i>	+	(+)	—	+	—	+	—	+	+	—
<i>M. diernhoferi</i>	+	—	—	+	—	+	—	+	+	—
<i>M. neoaurum</i>	+	—	—	+	—	+	—	+	+	—
<i>M. parafortuitum</i>	+	+	—	+	—	+	—	+	+	—

^a Data from references 7, 9, and 22.^b Waxesters-mycolates.^c ω -1-Methoxymycolates.^d +, present; —, absent; (+), present only in some strains.

presence of fluoranthene strain EMI2^T was capable of cooxidizing several PAHs (at a concentration of 50 mg liter⁻¹), including phenanthrene (86% reduction), fluorene (60% reduction), pyrene (31% reduction), and anthracene (20% reduction); in the presence of these PAHs the concentration of fluoranthene was reduced by 83, 98, 81, and 69%, respectively. None of these other PAHs was degraded in the absence of fluoranthene.

The results of carbon source utilization and qualitative enzyme tests are shown in Table 1.

Chemotaxonomic properties. Our analysis of strain EMI2^T revealed a pattern of chemotaxonomic features that are typical of members of the genus *Mycobacterium* (Table 2). The cell walls were acid fast and contained *meso*-diaminopimelic acid as the only diamino acid. The sugars identified in whole-cell hydrolysates were mainly arabinose and galactose; small amounts of ribose and glucose were also found. Our analysis of a whole-cell methanolysate by thin-layer chromatography revealed a multipot pattern composed of long-chain alpha-mycolates, keto-mycolates, and two other spots identified as omega-carbomycolates and long-chain alcohols (wax esters). This combination of mycolic acids and the pattern of the pyrolysis products of the mycolic acids are shared by strain EMI2^T, *M. austroafricanum*, *M. parafortuitum*, and *M. neoaurum* (Table 3). The predominant isoprenoid quinone was menaquinone MK-9(H₂), which accounted for 85% of the total menaquinones; the remain-

TABLE 4. Whole-cell fatty acid compositions of isolate EMI2^T and related species for which data are available

Fatty acid	% of total fatty acids in:		
	Strain EMI2 ^T	<i>M. diernhoferi</i> DSM 43524 ^T	<i>M. neoaurum</i> DSM 44074 ^T
C _{14:0}	5.74	6.23	5.72
C _{15:0}	1.05		
C _{16:1 cis6}	1.49	2.17	3.48
C _{16:1 cis9}	2.42	0.77	5.23
C _{16:1 cis10}	6.07	5.65	
C _{16:0}	25.17	25.27	28.31
10Me-C _{16:0}	0.76		
C _{18:0} alcohol	13.93	18.00	15.07
C _{18:2}	0.63		
C _{18:1 cis9}	20.27	20.57	30.46
C _{18:0}	1.24	3.98	1.96
10Me-C _{18:0}	18.46	11.15	5.57
C _{20:0} alcohol	2.77	4.57	1.74

FIG. 1. Dendrogram based on fatty acid profiles showing the relationships between strain EMI2^T and some phylogenetically related *Mycobacterium* species.

ing 15% was MK-8(H₂). The phospholipid pattern revealed that phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol were present. The G+C content of the DNA was 73 mol%, as determined by the HPLC method. The qualitative and quantitative distributions of the fatty acids are shown in Table 4. Strain EMI2^T contained mainly straight-chain saturated and monounsaturated fatty acids that have chain lengths of 14 to 20 carbon atoms. Substantial amounts of 10-methyl-branched C_{18:0} fatty acids (18.5% of the total fatty acids) were also found. The occurrence of alcohols with chain lengths of 18 and 20 carbon atoms was a diagnostic feature of strain EMI2^T, and the fatty acid profile of this organism did not match the profile of any other rapidly growing mycobacterium presented in the Microbial Identification System Library for mycobacteria. Figure 1 is a dendrogram based on fatty acid profiles which shows the unique position of strain EMI2^T among related organisms.

Phylogenetic analysis. A comparison of the almost complete 16S rDNA sequence of isolate EMI2^T with the sequences of members of the genus *Mycobacterium* (29) revealed that strain EMI2^T is a member of the subgroup that contains the fast-growing mycobacteria. The binary similarity values determined

FIG. 2. 16S rDNA relatedness dendrogram showing the phylogenetic position of strain EMI2^T within the radiation of rapidly growing mycobacteria. Strain EMI2 is the type strain of *M. hodleri* sp. nov. The dendrogram was generated by using the algorithm of De Soete (2). The numbers are bootstrap values (expressed as percentages); only values greater than 20% are shown. Bar = 1% inferred sequence divergence.

TABLE 5. Physiological properties that distinguish *M. hodleri* from *M. diernhoferi*, *M. neoaurum*, *M. austroafricanum*, and *M. parafortuitum*^a

Species	Formation of pigment ^b	Growth at 42°C	Arylsulfatase activity (3 days)	Tween 80 hydrolysis	Nitrate reduction	Catalase activity	Utilization of:			
							Xylose	Trehalose	Mannitol	Sorbitol
<i>M. hodleri</i>	S	— ^c	ND	+	—	+	+	—	+	+
<i>M. diernhoferi</i>	N	—	—	—	+	—	+	—	+	—
<i>M. neoaurum</i>	S	v	+	v	v	—	v	+	+	—
<i>M. austroafricanum</i>	S	—	+	v	+	—	+	—	+	—
<i>M. parafortuitum</i>	P	v	v	—	v	+	v	—	+	—

^a Data from references 7, 20, and 38.^b S, scotochromogenic; N, nonchromogenic; P, photochromogenic.^c —, less than 15% of the strains are positive; +, more than 85% of the strains are positive; v, variable; ND, not determined.

for strain EMI2^T and these organisms range from 96.1 to 97.6%, and the binary similarity values determined for strain EMI2^T and the slowly growing mycobacteria range from 95.0 to 95.8% (data not shown). Isolate EMI2^T shares with the fast-growing mycobacteria a short helix at positions 451 to 482 (*Escherichia coli* numbering) (29, 35).

Strain EMI2^T shows the highest levels of 16S rDNA similarity with the type strains of *Mycobacterium madagascariense* (level of similarity, 97.6%), *M. neoaurum* (97.4%), and *M. diernhoferi* (97.3%). Phylogenetic trees based on a distance matrix analysis (2, 4) and a maximum-likelihood analysis (4) placed isolate EMI2^T with the *M. diernhoferi*-*M. neoaurum* subcluster. All of the algorithms which we used revealed similar patterns of relatedness among the fast-growing mycobacteria, and these patterns were also similar to the pattern based on the results obtained when the neighborliness method was used (29). According to Tsukamura et al. (38), the following species are members of the *M. parafortuitum* complex: *M. parafortuitum*, *M. aurum*, *M. neoaurum*, *M. diernhoferi*, and *M. austroafricanum*. We found that strain EMI2^T is not closely related to two of these species, *M. neoaurum* and *M. diernhoferi* (see above), and *M. aurum* appears to be only moderately closely related (Fig. 2). The 16S rDNA data also show that *M. austroafricanum* and *M. parafortuitum* are not members of the *M. diernhoferi*-*M. neoaurum* cluster (Fig. 2). Two strains of PAH-degrading mycobacteria whose 16S rDNAs have recently been analyzed (39) are not closely related to strain EMI2^T (Fig. 2); while strain PAH135 exhibits 96.6% sequence similarity with the type strain of *M. aichense*, the sequence of strain PYR-1 is identical to the sequence of the type strain of *M. austroafricanum*.

Differentiation of *Mycobacterium hodleri* sp. nov. from other mycobacteria. The results of our chemotaxonomic and phylogenetic analyses clearly indicate that strain EMI2^T is a member of a new species of the genus *Mycobacterium*. The phylogenetic position of this organism is in the subgroup of fast-growing mycobacteria that clusters adjacent to *M. diernhoferi* and *M. neoaurum*. Strain EMI2^T can be distinguished from *M. diernhoferi* by Tween 80 hydrolysis, an absence of nitrate reduction, and utilization of sorbitol. It differs from *M. neoaurum* by its inability to grow at 45°C, formation of catalase, lack of trehalose utilization, and utilization of sorbitol (Table 5). Strain EMI2^T differs from *M. austroafricanum* in nitrate reduction, catalase activity, and utilization of sorbitol and from *M. parafortuitum* in the mode of pigment production, Tween 80 hydrolysis, and utilization of sorbitol. The most reliable method for determining species affiliation is 16S rDNA sequence analysis, which clearly differentiates strain EMI2^T from related species (Fig. 2). The distinct differences (differences of more than 2.5%) are located mainly in the variable regions. The name *Mycobacterium hodleri* sp. nov. is proposed for this novel PAH-degrading organism.

Description of *Mycobacterium hodleri* sp. nov. *Mycobacterium hodleri* (hod' le.ri. L.gen. n. *hodleri*, of Hodler, referring to Christian Hodler, director of the Ministry of Science and Culture of the State of Lower Saxony, Germany, and a strong supporter of natural sciences). Gram positive, acid fast, non-spore forming, nonmotile, and rod shaped (length, 1.8 to 2.3 µm; diameter, 1 µm). Some cells occur in V-form and in palisades. Scotochromogenic, producing a saffron yellow pigment on Middlebrook agar and a chrome yellow pigment on Trypticase soy broth agar. Growth occurs at temperatures between 18 and 28°C. Aerobic and chemoorganotrophic. Catalase positive. Utilizes *N*-acetyl-D-glucosamine, L-alanine, D-arabitol, L-aspartate, D-galactose, gluconate, D-glucose, inositol, L-leucine, 2-oxoglutarate, L-proline, putrescine, quinate, D-ribose, L-rhamnose, L-serine, succinate, and L-valine. Fluoranthene and rapeseed oil are utilized. Capable of cooxidizing fluoranthene with several PAHs, including phenanthrene, fluorene, pyrene, and anthracene. Tween 80 is hydrolyzed.

Acetamide, 4-aminobutyrate, citrate, caprate, D-sucrose, glucarate, D-glucosaminic acid, 3-hydroxybenzoate, 4-hydroxybenzoate, 2-hydroxyvalerate, pimelate, phenylacetate, D-turanose, and tyramine are not utilized.

Long-chain mycolic acids present. The fatty acids are mainly straight-chain saturated (C_{14:0}, C_{16:0}) and monounsaturated (C_{16:1} *cis*-10 and C_{18:1} *cis*-9) acids. C_{18:0} and C_{20:0} alcohols and substantial amounts of tuberculostearic acid (10-methyl-branched C_{18:0}) are present. The predominant isoprenoid quinone is menaquinone MK-9(H₂). The G+C content of the DNA is 73 mol%. Isolated from a fluoranthene-contaminated site near Jülich, Germany. The type strain is strain EMI2, which has been deposited in the DSMZ-Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH as strain DSM 44183.

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REFERENCES

- Bönicke, R. 1961. Die Bedeutung der Acylamidasen für die Identifizierung und Differenzierung der verschiedenen Arten der Gattung *Mycobacterium*. Jahresber. Borstel 5:7-87.
- De Soete, G. 1983. A least squares algorithm for fitting additive trees to proximity data. Psychometrika 48:621-626.
- Deutsche Sammlung von Mikroorganismen. 1993. DSM catalogue of strains, 5th ed. Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany.
- Felsenstein, J. 1993. PHYLIP (phylogeny inference package), version 3.5c. University of Washington, Seattle.
- Finnerty, W. R. 1992. The biology and genetics of the genus *Rhodococcus*. Annu. Rev. Microbiol. 46:193-218.
- Häggeblom, M. M., L. J. Nohynek, N. J. Palleroni, K. Kronquist, E.-L. Nurmiäho-Lassila, M. S. Salkinoja-Salonen, S. Klatte, and R. Kroppen-

- stedt. 1994. Transfer of polychlorophenol-degrading *Rhodococcus chlorophenolicus* (Apajalahti et al. 1986) to the genus *Mycobacterium* as *Mycobacterium chlorophenolicum* comb. nov. *Int. J. Syst. Bacteriol.* **44**:485–493.
7. Hartmans, S., and J. A. M. deBont. 1992. Aerobic vinyl chloride metabolism in *Mycobacterium aurum* L1. *Appl. Environ. Microbiol.* **58**:1220–1226.
 8. Heitkamp, M. A., and C. E. Cerniglia. 1988. Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. *Appl. Environ. Microbiol.* **54**:1612–1614.
 9. Hinrikson, H. P., and G. E. Pfyffer. 1994. Mycobacterial mycolic acids. *Med. Microbiol. Lett.* **3**:49–57.
 10. Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules, p. 21–132. In H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, New York.
 11. Kämpfer, P., W. Dott, and R. M. Kroppenstedt. 1990. Numerical classification and identification of some nocardioform bacteria. *J. Gen. Appl. Microbiol.* **35**:309–331.
 12. Kämpfer, P., and R. M. Kroppenstedt. 1994. Enzymatic profiles of *Mycobacterium* spp. and their value for species differentiation. *Microbiology* **140**:209–214.
 13. Kelley, L. J., P. Freeman, F. E. Evans, and C. E. Cerniglia. 1993. Identification of metabolites from the degradation of fluoranthene by *Mycobacterium* sp. strain PYR-1. *Appl. Environ. Microbiol.* **59**:800–806.
 14. Kirchof, H., C. Maas, M. Runge, B. Franz, R. Schmidt, H. Quentmeier, and P. F. Mühlradt. 1992. Tetrazolium-[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction by mycoplasmas. *Int. J. Syst. Bacteriol.* **42**:506–508.
 15. Klatte, S., R. M. Kroppenstedt, and F. A. Rainey. 1994. *Rhodococcus opacus* sp. nov., an unusual nutritionally versatile *Rhodococcus* species. *Syst. Appl. Microbiol.* **17**:335–360.
 16. Kroppenstedt, R. M. 1985. Fatty acid and menaquinone analysis of actinomycetes and related organisms. *Soc. Appl. Bacteriol. Tech. Ser.* **20**:173–199.
 17. Kroppenstedt, R. M., F. Korn-Wendisch, V. F. Fowler, and E. Stackebrandt. 1981. Biochemical and molecular genetic evidence for transfer of *Actinoplanes armeniacus* into the family *Streptomycetaceae*. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C* **2**:254–262.
 18. Kubica, G. P., and G. L. Pool. 1960. Studies on the catalase activity of acid fast bacilli. I. An attempt to subgroup these organisms on the basis of their catalase activities at different temperatures and pH. *Am. Rev. Respir. Dis.* **81**:387–391.
 19. Kuykendall, L. D., M. A. Roy, J. J. O'Neill, and T. E. Devine. 1988. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int. J. Syst. Bacteriol.* **38**:358–361.
 20. Lefford, M. J. 1980. Mycobacterial culture collection. National Institutes of Health Publication 80-289. U.S. Department of Health and Human Services, Washington, D.C.
 21. Levy-Frebault, V. V., and F. Portaels. 1992. Proposed minimal standards for the genus *Mycobacterium* and for description of new slowly growing *Mycobacterium* species. *Int. J. Syst. Bacteriol.* **42**:315–323.
 22. 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.
 23. Maidak, B. L., N. Larsen, M. A. McCaughey, R. Overbeek, G. J. Olsen, K. Fogel, J. Blandy, and C. R. Woese. 1994. The Ribosomal Database Project. *Nucleic Acids Res.* **22**:3485–3487.
 24. Malachowsky, K. J., T. J. Phelps, A. B. Teboli, D. E. Minnikin, and D. C. White. 1994. Aerobic mineralization of trichloroethylene, vinylchloride, and aromatic compounds by *Rhodococcus* species. *Appl. Environ. Microbiol.* **60**:542–548.
 25. Mesbah, M., U. Premachandran, and W. B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**:159–167.
 26. Miller, L. T. 1982. A single derivatization method for bacterial fatty acid methyl esters including hydroxy acids. *J. Clin. Microbiol.* **16**:584–586.
 27. Minnikin, D. E., I. G. Hutchinson, A. B. Caldicott, and M. Goodfellow. 1980. Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J. Chromatogr.* **188**:221–233.
 28. Minnikin, D. E., A. G. O'Donnell, M. Goodfellow, G. Alderson, M. Athalye, K. Schaal, and J. H. Parlett. 1984. An integrated procedure for the extraction of isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**:233–241.
 29. Pitulle, C., M. Dorsch, J. Kazda, J. Wolters, and E. Stackebrandt. 1992. Phylogeny of rapidly growing members of the genus *Mycobacterium*. *Int. J. Syst. Bacteriol.* **42**:337–343.
 30. Rafii, F., W. R. Butler, and C. E. Cerniglia. 1992. Differentiation of rapidly growing, scotochromogenic, polycyclic-aromatic-hydrocarbon metabolizing strains of *Mycobacterium* sp. from other known *Mycobacterium* species. *Arch. Microbiol.* **157**:512–520.
 31. Rainey, F. A., M. Dorsch, H. W. Morgan, and E. Stackebrandt. 1992. 16S rDNA analysis of *Spirochaeta thermophila*: its phylogenetic position and implications for the systematics of the order *Spirochaetales*. *Syst. Appl. Microbiol.* **15**:197–202.
 32. Rainey, F. A., S. Klatte, R. M. Kroppenstedt, and E. Stackebrandt. 1995. *Dietzia*, a new genus including *Dietzia maris* comb. nov., formerly *Rhodococcus maris*. *Int. J. Syst. Bacteriol.* **45**:32–36.
 33. Rainey, F. A., and E. Lang. Unpublished data.
 34. Silcox, V. A., R. C. Good, and M. M. Floyd. 1981. Identification of clinically significant *Mycobacterium fortuitum* complex isolates. *J. Clin. Microbiol.* **14**:686–691.
 35. Stackebrandt, E., and J. Smida. 1988. The phylogeny of the genus *Mycobacterium* as determined by 16S rRNA sequences, and development of DNA probes, p. 244–250. In Y. Okami, T. T. Beppu, and H. Ogawara (ed.), *Biology of actinomycetes*. Japan Scientific Press, Tokyo.
 36. Stanek, J. L., and G. D. Roberts. 1974. Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. *Appl. Microbiol.* **28**:226–231.
 37. Tiem, B. A., B. Boldrien, and C. Fritsche. 1993. Degradation of phenanthrene, fluorene fluoranthene, and pyrene by a *Mycobacterium* species. *Appl. Environ. Microbiol.* **59**:1927–1930.
 38. Tsukamura, M., H. van der Meulen, and W. O. K. Grabow. 1983. Numerical taxonomy of rapidly growing, scotochromogenic mycobacteria of the *Mycobacterium parafortuitum* complex: *Mycobacterium austroafricanum* sp. nov. and *Mycobacterium diernhoferi* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* **33**:460–469.
 39. Wang, R. F., W. W. Cao, and C. E. Cerniglia. 1995. Phylogenetic analysis of polycyclic aromatic hydrocarbon degrading mycobacteria by 16S rRNA sequencing. *FEMS Microbiol. Lett.* **130**:75–80.