***Gordonia alkanivorans* sp. nov., isolated from tar-contaminated soil**

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Twelve bacterial strains isolated from tar-contaminated soil were subjected to a polyphasic taxonomic study. The strains possessed meso-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan, MK-9(H₂) as the predominant menaquinone, long-chain mycolic acids of the *Gordonia*-type, straight-chain saturated and monounsaturated fatty acids, and considerable amounts of tuberculostearic acid. The G+C content of the DNA was 68 mol%. Chemotaxonomic and physiological properties and 16S rDNA sequence comparison results indicated that these strains represent a new species of the genus *Gordonia*. Because of the ability of these strains to use alkanes as a carbon source, the name *Gordonia alkanivorans* is proposed. The type strain of *Gordonia alkanivorans* sp. nov. is strain HKI 0136^T (= DSM 44369^T).

Keywords: *Actinomycetales*, *Gordonia alkanivorans* sp. nov., tar-contaminated soil, degradation, alkanes

INTRODUCTION

The genus *Gordonia* belongs to the family *Gordoniaceae* of the suborder *Corynebacterineae* within the order *Actinomycetales* (Stackebrandt *et al.*, 1997). At present, this genus contains nine species: *Gordonia aichiensis*, *Gordonia amarae*, *Gordonia bronchialis*, *Gordonia hirsuta*, *Gordonia hydrophobica*, *Gordonia rhizosphaera*, *Gordonia rubropertincta*, *Gordonia sputi* and *Gordonia terrae*. Members of the genus *Gordonia* were distinguished from rapidly growing mycobacteria mainly by the lack of acid-fastness and the absence of aryl sulfatase, and from the genus *Nocardia* on the basis of a positive nitrate reduction, the ability to form acid from mannose, the utilization of sucrose as sole carbon source (Tsukamura, 1971) and the absence of a mycelium [with the exception of *G. amarae* which shows weakly branched hyphae (Klatte *et al.*, 1994)]. After the establishment of the genus *Gordonia* (*Gordona*) (Tsukamura, 1971), its status was controversial (Goodfellow & Alderson, 1977) until the distinctness of this taxon from neighbouring taxa was confirmed by combining phylogenetic and chemotaxonomic results (Stackebrandt *et*

al., 1988). The genus was emended (Stackebrandt *et al.*, 1988) to accommodate *Rhodococcus* species containing mycolic acids with 48–66 carbon atoms and MK-9(H₂) as the predominant menaquinone. *Rhodococcus aichiensis* and *Nocardia amarae* were reclassified as *Gordonia aichiensis* and *Gordonia amarae*, respectively (Klatte *et al.*, 1994; Ruimy *et al.*, 1994). Further species have been added recently: *G. hydrophobica* (Bendinger *et al.*, 1995), *G. hirsuta* (Klatte *et al.*, 1996) and *G. rhizosphaera* (Takeuchi & Hatano, 1998).

Members of the genus *Gordonia* are widely distributed, as generally described for other mycolic acid-containing actinomycetes (Goodfellow, 1992; Klatte *et al.*, 1996). *Gordonia* species were isolated from various soil samples of terrestrial and aquatic habitats, but also from biofilters for waste gas treatment (Bendinger *et al.*, 1995; Klatte *et al.*, 1996). Members of the genus *Gordonia* were cultivated from soil by prior treatment with alkali and from sputa of patients with lung cavities or bronchiectasis (Tsukamura, 1971, 1991; Tsukamura & Yano, 1985; Riegel *et al.*, 1994).

This study describes the taxonomic characterization of novel *Gordonia* strains by a polyphasic approach. Strains were isolated from tar- and phenol-contaminated soil of the former tar factory in Rositz (Eastern Thuringia, Germany) in the course of a screening program for n-alkane-degrading bacteria. These strains are characterized by their ability to degrade

Abbreviation: meso-A₂pm, meso-diaminopimelic acid.

The EMBL accession number for the 16S rDNA sequence of strain HKI 0136^T is Y18054.

hexadecane and differ from known *Gordonia* species in their mycolic acid patterns.

METHODS

Bacterial strains and cultivation conditions. Bacterial type strains used were *Corynebacterium glutamicum* DSM 20300^T, *Dietzia maris* DSM 43672^T, *G. aichiensis* DSM 43978^T, *G. amarae* DSM 43392^T, *G. bronchialis* DSM 43247^T (= NCTC 10667^T), *G. hirsuta* DSM 44140^T, *G. hydrophobica* DSM 44015^T, *G. rubropertincta* DSM 43197^T, *G. sputi* DSM 43896^T, *G. terrae* DSM 43249^T (= ATCC 25594^T), *Nocardia carnea* DSM 43397^T, *Rhodococcus rhodochrous* DSM 43241^T and *Tsukamurella paurometabola* DSM 20162^T.

Twelve bacterial strains HKI 0134, HKI 0135, HKI 0136^T (= DSM 44369^T), HKI 0144, HKI 0145, HKI 0146, HKI 0147, HKI 0148, HKI 0149, HKI 0150, HKI 0151 and HKI 0152 were (among many others) isolated from a soil sample taken from the contaminated area of the former tar factory in Rositz (Germany) at a depth of about 10 cm. The chemical characteristics of the soil sample were: 1.61 mg light volatile aromatic hydrocarbons kg⁻¹ such as benzene, toluene, ethylbenzene and xylenes; 18.6 g aliphatic hydrocarbons kg⁻¹, oils; 1.975 g polycyclic aromatic hydrocarbons kg⁻¹ (a total of 16 compounds, e.g. pyrene, fluorene, chrysene, phenanthrene, benzopyrene, benzofluoranthrene, naphthalene); and 334 mg phenol kg⁻¹. These data were provided by the tar factory in Rositz. The soil sample was collected in late April 1996 and stored at 4 °C until isolation of the strains in October 1996.

The selective enrichment culture technique used n-hexane as sole carbon source. For preparation of the cultivation medium, 5 g contaminated soil was extracted with 20 ml n-hexane. After extraction and filtration, 0.25 ml hexane/soil extract (containing many aromatic and aliphatic hydrocarbons) was mixed with 8 ml Brushnell-Haas-Bouillon (Difco) and the mixture was autoclaved. For the cultivation medium, 0.3 ml each vitamin solution, described for the HV agar (Hayakawa & Nonomura, 1987), S₁ solution (17% w/v KCl; 0.5% MgSO₄ · 7H₂O; 0.2% CaCO₃ in distilled water), S₂ solution (0.1% FeSO₄ · 7H₂O in distilled water) and 1 ml diluted soil/NaCl suspension (1 g soil and 10 ml 0.85% NaCl, shaken for 1 h at 28 °C, then diluted 1:10) were also added. After 5 d aerobic cultivation at 28 °C, the organisms were isolated by using the dilution plating technique on rich (R) medium (Yamada & Komagata, 1972) agar plates. General laboratory cultivation was performed at 28 °C on solidified or liquid R medium. For chemotaxonomic analysis, strains were cultivated for 24 h in liquid organic medium M 79 (Prauser *et al.*, 1997) or in tryptic soy broth (TSB; Difco) at 28 °C.

Morphological and physiological characteristics. Colony morphology was observed with a stereo microscope Olympus SZ 11. Cell morphology was determined by phase-contrast microscopy (Olympus BH-2 microscope) using an ocular X10 and an objective X100/1.25. Ultrastructure was demonstrated by the freeze-fracture technique. An electron micrograph of the replica was made with an electron microscope EM 902 (Carl Zeiss) at a primary magnification of 5700. The motility was studied by a hanging drop method. Cell dimensions were measured with a phase-contrast microscope (Olympus system microscope BX 50) with computing image analysis system VIS 550 4 H (Jentech). Gram-staining was performed according to Hucker's modi-

fication (Cowan & Steel, 1965) with reagents produced by bioMérieux except for the decolorizer which was substituted by 96% (v/v) ethanol. Acid-fastness was determined by Ziehl-Neelsen staining (Lanyi, 1987).

Tolerance to NaCl between 2 and 14% (w/v) was tested on R medium. The influence of temperature on growth was examined on R medium between 5 and 45 °C. Oxygen requirements were determined on R medium using the Generbag microaer and Generbag anaer incubation systems (bioMérieux). Acid production from carbohydrates was proven by the method of Hugh & Leifson (1953), as modified by Gledhill & Casida (1969). Utilization of organic acids was studied using the media described by Gordon & Mihm (1957) and Cowan & Steel (1965) by adding the sodium salts of the organic acids to a final concentration of 0.2%. In the tartrate-containing medium, the sodium salt was replaced by the potassium salt. Decomposition of adenine, hypoxanthine, xanthine and tyrosine was determined by using the method of Gordon *et al.* (1974). Nitrate reduction, urease activity, indole production, hydrogen sulphide production, hydrolysis of Tween 80, aesculin, gelatin, methyl red and Voges-Proskauer reactions were examined as described by Lanyi (1987). Reagents NIT 1 and NIT 2 (bioMérieux) were used for testing nitrate reduction. Indole production, methyl red and Voges-Proskauer reactions were examined by using Kovács' reagent, VP 1 and VP 2 (bioMérieux), respectively. Hydrogen sulphide production was demonstrated with Bacto-H₂S test strips (Difco). Catalase production and hydrolysis of casein and potato starch were tested by the methods of Gledhill & Casida (1969). Cytochrome oxidase was proven using DrySlide oxidase test strips (Difco). Hydrolysis of hippurate was examined as described by Cowan & Steel (1965). Susceptibility to antibiotics was tested by placing antibiotic discs (bioMérieux) on R medium agar plates seeded with a suspension of the strain.

In addition, the ability to utilize 95 carbon sources was tested by using GP-microplates of the Biolog identification system described by Bochner (1989) and Bochner & Savageau (1977). Utilization of different substrates for respiration (formation of NADH) was examined by strains after cultivation on Biolog Universal Growth Medium for 2 d at 28 °C. Enzyme activities of a 24-h-old culture on R medium were studied with the API ZYM system (bioMérieux). Culture samples (100 µl) were transferred onto the API ZYM strips and incubated at 37 °C for 4 h. Evaluation was carried out as recommended by the manufacturer.

Chemotaxonomic characterization. *meso*-Diaminopimelic acid (*meso*-A₂pm) in whole-cell hydrolysates (Hasegawa *et al.*, 1983) was identified by paper chromatography according to Rhuland *et al.* (1955). Cellular fatty acid methyl esters obtained from cells grown in TSB for 24 h at 28 °C by the method described by Korn-Wendisch *et al.* (1989) were separated by GC (Groth *et al.*, 1996) and identified as described previously (Schumann *et al.*, 1997). Mycolic acids were analysed by HPLC after derivatization with *p*-α-dibromoacetophenone as described by Butler *et al.* (1986). Menaquinones were analysed by HPLC as described by Collins *et al.* (1977) and Groth *et al.* (1996).

DNA base composition, DNA-DNA hybridization and ribotyping. DNA was isolated from 48-h-old cells grown in M 79 medium with the addition of 1% (w/v) glycine at 28 °C and with Puregene total-DNA-kit (Biozym). The G + C content was determined by HPLC of deoxyribonucleosides by using the method of Mesbah *et al.* (1989). For DNA-DNA

hybridization, previously described methods were used (Martin *et al.*, 1997). Ribotyping of strains HKI 0134, HKI 0136^T, HKI 0144, HKI 0147, HKI 0149 and HKI 0150 was performed as described by Vuorio *et al.* (1999). Analysis was done with the RiboPrinter Microbial Characterization system (Qualicon).

16S rDNA sequence determination and phylogenetic analysis. Genomic DNA extraction, PCR-mediated amplification of 16S rDNA, sequencing of the PCR products and determination of the phylogenetic position were described previously (Groth *et al.*, 1996). The phylogenetic dendrogram was constructed from evolutionary distance values by the neighbour-joining method (Rainey *et al.*, 1996).

Nucleotide sequence accession numbers. Accession numbers of sequences of type strains used for comparison are indicated in Fig. 3.

RESULTS

Morphological and cultivation characteristics

Twelve strains were isolated from tar-contaminated soil by enrichment culture techniques using hexadecane as sole carbon source. All isolates were highly similar to each other with respect to morphological and phenotypic properties. They formed circular and smooth colonies that were orange to orange-red coloured with shiny surfaces. Strains grew well on agar media and in liquid shaken cultures on R medium, M 79 or TSB. Occasionally, rough colonies were also observed. The diameter of colonies was 0.2–2.0 mm. On malt extract agar, the surface was slimy and bright. Cells were Gram-positive, non-acid-fast, non-motile, short rod-shaped or coccoid. In 24-h-old cultures, dimensions of rods were 0.5–0.9 µm by 1.0–2.3 µm. Cells occurred singly, in pairs or in small clusters, in short flexible chains and very often in a typical coryneform V-shape (Fig. 1a). In 48-h-old cultures, cells were shorter and rods were 0.8–1.0 µm by 0.7–

Fig. 2. Freeze-fracture electron micrograph of cells from a 48-h-old culture of strain HKI 0136^T grown in liquid organic medium M 79 at 28 °C. Bar, 1 µm.

1.3 µm (Fig. 1b). Rod–coccus growth cycles were observed at 48 h intervals. In stationary cultures, rods fragmented into coccoid elements. Freeze-fracture electron microscopy of strain HKI 0136^T revealed the belt-like zones surrounding the cells with different density and width (Fig. 2). The belts were bordered by band-like swellings on the plasma-sided fracture face which were formed in the process of cell fission.

Strains grew well aerobically on complex organic media at 28 °C (tolerance 13–40 °C). No growth occurred at 5 or 42 °C. On R medium, cells grew in the presence of up to 6% (w/v) NaCl; higher salt concentrations reduced the growth and no growth was observed at 12% (w/v) NaCl. Growth occurred under microaerophilic, but not under anaerobic conditions at 28 °C.

Physiological characteristics

All isolates and also the tested type strains of *G. amarae*, *G. hirsuta*, *G. hydrophobica*, *G. rubropertincta* and *G. terrae* were catalase-positive and oxidase-negative. Growth of all isolates and tested type strains in the presence of 2 and 4% (w/v) NaCl was positive; no growth occurred in the presence of 12 and 14% (w/v) NaCl. None of the strains grew at 5 or 45 °C, but all grew well at 28 °C. Their ability to utilize a narrow range of carbon sources was demonstrated by the classical method of Hugh & Leifson (1953) (Table 1) as well as by the microplate assay of the Biolog system (Table 2). In Table 1, the differentiating physiological properties of the twelve strains and type strains of selected species of the genus *Gordonia*, investigated in parallel tests, are listed. Acid was produced from D-fructose, while no acid was produced from L-

Fig. 1. Micrograph of strain HKI 0136^T grown in liquid organic medium M 79 for 24 h (a) and for 48 h (b) at 28 °C. Bar, 10 µm.

Table 1. Differentiating morphological characteristics and physiological properties of *Gordonia alkanivorans* and selected type strains of species of the genus *Gordonia*

Strains: 1, *G. alkanivorans* (12 strains); 2, *G. amarae*; 3, *G. hirsuta*; 4, *G. hydrophobica*; 5, *G. rubropertincta*; 6, *G. terrae*.
 —, Negative; +, positive; w, weakly positive.

Characteristic	1*	2	3	4	5	6
Colour of colonies	Orange to orange-red	Tannish to white	White to light yellow	Tannish to white	Pink to red	Red
Morphology	Rod-shaped	Filaments without branching	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped
Acid production from:						
D-Galactose	0	—	+	—	—	—
D-Glucose	0	—	+	—	+	—
Glycerol	67	+	+	—	+	—
Inulin	8	—	+	—	—	—
D-Mannitol	92	+	—	—	+	+
D-Mannose	0	—	+	—	—	—
L-Rhamnose	0	+	—	—	—	+
D-Ribose	17	—	+	—	—	+
Saccharose	100	+	+	—	+	+
Salicin	0	w	—	—	—	+
D-Sorbitol	92	—	—	—	+	+
Trehalose	8	+	+	—	—	+
Utilization of:						
cis-Aconitate	100	—	—	+	—	+
Benzoate	100	—	—	+	+	+
Citrate	100	—	—	+	+	+
Formate	100	w	—	+	w	+
Hexadecane	83	—	+	+	+	+
Hexadecene	83	—	—	+	+	—
Hippurate	0	—	—	+	—	—
Lactate	8	+	+	—	—	+
Malate	83	+	—	+	+	+
Succinate	100	+	—	+	+	+
Propionate	100	+	—	+	+	+
Decomposition of:						
Aesculin	0	+	—	—	—	+
Potato starch	100	—	—	—	+	—
Urea	100	+	—	—	+	+
Growth in presence of NaCl:						
6%	100	—	—	+	+	+
8%	83	—	—	+	+	+
10%	w	—	—	w	w	w
Growth on R medium at:						
13 °C	100	—	+	+	+	+
37 °C	100	—	+	+	+	+
40 °C	100	—	—	—	+	—
42 °C	0	—	—	—	+	—
Antibiotic susceptibility:						
Ampicillin (10 µg)	100	+	+	+	+	—
Erythromycin (15 µg)	8	+	+	+	+	+
Lincomycin (2 µg)	0	—	w	—	—	—
Nitrofurantoin (100 µg)	100	+	+	+	+	—
Oxacillin (5 µg)	17	+	w	—	—	—
Penicillin G (10 IU)	42	+	+	+	+	—
Polymyxin B (300 IU)	w	+	w	—	—	—
Sulfonamide (250 µg)	100	w	w	+	w	w

* Values are the percentages of the 12 strains tested that are positive.

Table 2. Differentiating characteristics of *G. alkanivorans* and selected species of the genus *Gordonia* determined by the Biolog system

Strains: 1, *G. alkanivorans* (12 strains); 2, *G. aichiensis*; 3, *G. amarae*; 4, *G. bronchialis*; 5, *G. hirsuta*; 6, *G. hydrophobica*; 7, *G. rubropertincta*; 8, *G. sputi*; 9, *G. terrae*. +, Positive; –, negative.

Carbon source	1*	2†	3	4	5	6	7	8	9
α -Cyclodextrin	50	–	–	+	–	–	–	–	–
β -Cyclodextrin	100	–	–	+	–	–	+	–	–
Dextrin	67	+	–	+	+	+	+	+	–
Glycogen	8	–	–	+	–	–	+	–	–
<i>N</i> -Acetylglucosamine	0	–	–	–	–	+	–	–	–
Amygdalin	0	–	–	–	–	+	–	–	–
D-Arabitol	0	–	–	–	–	–	+	+	–
Maltose	0	+	+	+	+	–	+	+	–
Maltotriose	0	–	–	+	–	–	–	–	–
D-Mannose	75	+	+	+	+	–	+	+	+
3-Methyl glucose	0	–	–	+	+	–	–	–	–
Palatinose	0	–	–	+	–	–	–	–	–
D-Raffinose	0	–	–	+	–	–	–	–	–
L-Rhamnose	0	–	–	–	–	–	–	–	+
D-Ribose	8	–	–	–	–	–	–	–	+
Salicin	0	+	–	+	–	–	–	+	+
D-Sorbitol	17	–	–	–	–	–	–	–	–
D-Trehalose	58	+	+	–	+	+	+	+	+
Turanose	58	+	–	+	+	–	+	+	+
D-Xylose	0	–	–	+	–	–	+	–	+
Acetic acid	100	–	+	–	+	+	+	–	+
α -Hydroxybutyric acid	73	–	–	–	–	+	+	+	+
β -Hydroxybutyric acid	100	+	–	–	–	+	+	+	–
α -Ketoglutaric acid	0	–	–	–	–	–	+	–	–
α -Ketovaleric acid	92	+	–	–	–	+	+	+	+
D-Lactic acid methyl ester	33	+	–	–	–	–	+	+	+
L-Lactic acid	0	–	–	+	–	–	–	–	–
L-Malic acid	0	–	–	–	–	+	–	–	–
Methyl succinate	0	–	–	+	–	–	–	–	–
Propionic acid	25	–	–	–	–	+	–	–	–
Succinamic acid	0	–	–	–	–	–	–	–	+
<i>N</i> -Acetyl-L-glutamic acid	100	+	–	+	+	+	+	+	+
Alaninamide	42	+	–	–	–	–	+	+	+
L-Alanine	0	–	–	–	–	–	+	–	–
Glycerol	33	+	–	–	–	–	+	+	–

* Values are the percentages of the 12 strains tested that are positive.

† Data from Database release 3.50 (Biolog).

arabinose, D-cellobiose, dextrin, lactose, maltose, D-raffinose, starch and D-xylose. The following substrates were used by some isolates as sole carbon source: glycerol, inulin, D-mannitol, D-ribose, D-sorbitol and trehalose (Table 1). All tested strains utilized acetate and pyruvate. Oxalate and tartrate were not utilized. Nitrate was reduced and Tween 80 was hydrolysed. Adenine, casein, gelatin, hypoxanthine, tyrosine and xanthine were not decomposed. All tested strains were positive for formation of H_2S , whereas they were negative for production of indole, the Voges-Proskauer test and the methyl red test. All strains listed in Table 1 were susceptible to the following antibiotics:

chloramphenicol (30 μ g), ciprofloxacin (5 μ g), gentamicin (10 μ g), kanamycin (30 μ g), neomycin (30 μ g), oxytetracycline (30 μ g), rifampicin (30 μ g) and streptomycin (10 μ g), but were resistant to lincomycin (2 μ g). All isolated strains grew on solid medium ISP 9 (Shirling & Gottlieb, 1966) with 1% (v/v) ethanol as the sole carbon source. Growth with 0.5% humic acid (Aldrich) was positive only in the presence of 0.1% glucose. Growth of tar soil isolates occurred on ISP 9 medium supplemented with 0.2% lignin (Aldrich), whereas growth with 0.5% cellulose (Aldrich) was poor. The isolates utilized 0.1% phenol in the presence of 0.1% glucose. In contrast, the type strain of *G.*

Table 3. Chemotaxonomic characteristics of strains isolated from tar-contaminated soil and of type strains of selected *Gordonia* species

Strain	G + C (mol %)	Menaquinone (peak area ratio)	Cellular fatty acids (%)									
			C12:0	C14:0	C16:0	C16:1	<i>t</i> ₁₇ [*]	C17:0	C17:1	C18:0	C18:1	<i>t</i> ₁₉ [†]
HKI 0144	68	9H ₂ , 8H ₂ , 7H ₂ (82:7:2)		3.0	31.8	23.0	1.2		1.1	1.4	25.0	12.3
HKI 0145	68	9H ₂ , 8H ₂ , 7H ₂ (82:6:2)	1.9	3.3	29.7	22.5	6.3				23.5	11.0
HKI 0146	68	9H ₂ , 8H ₂ , 9 (88:4:1)	6.4	5.5	30.7	28.9					16.9	11.5
HKI 0134	68	9H ₂ , 8H ₂ , 7H ₂ (82:4:2)		3.9	32.5	21.6		1.5		2.8	27.5	8.7
HKI 0135	68	9H ₂ , 8H ₂ , 7H ₂ (81:6:2)		2.8	33.8	23.5				1.5	25.4	11.9
HKI 0136 ^T	68	9H ₂ , 8H ₂ , 7H ₂ (83:6:2)		3.9	31.3	19.9				2.4	24.5	8.8
HKI 0148	67	9H ₂ , 8H ₂ , 7H ₂ (82:6:2)		2.4	33.0	24.7					26.6	13.2
HKI 0149	68	9H ₂ , 8H ₂ , 7H ₂ (81:6:2)	1.7	2.8	31.7	23.0					25.3	12.2
HKI 0150	68	9H ₂ , 8H ₂ , 7H ₂ (85:7:1)		4.7	36.1	23.7				2.3	26.3	6.9
<i>G. rubropertincta</i> DSM 43197 ^T	67–69‡	9H ₂ ‡		3.4	33.7	23.4					27.8	9.3
<i>G. terrae</i> DSM 43249 ^T	64–69‡	9H ₂ ‡	2.0	2.9	34.5	21.4					26.9	10.6
<i>G. amarae</i> DSM 43392 ^T	60–66§	9H ₂ §		4.1	34.7	24.1					33.1	
<i>G. hirsuta</i> DSM 44140 ^T	69	9H ₂		2.3	32.8	13.7				6.5	38.4	6.4
<i>G. hydrophobica</i> DSM 44015 ^T	69¶	9H ₂ ¶	1.1	2.5	33.6	20.0		1.2	3.0	1.1	26.5	8.3

* *t*₁₇, 10-Methyl hexadecanoic acid.† *t*₁₉, 10-Methyl octadecanoic acid.

‡ Data from Goodfellow (1986).

§ Data from Klatte *et al.* (1994).|| Data from Klatte *et al.* (1996).¶ Data from Bendinger *et al.* (1995).

rubropertincta grew well on phenol-containing media without glucose.

In Table 2, only those results of metabolic properties (Biolog analysis) which were neither positive nor negative for all isolated and type strains are indicated. All strains investigated were positive for utilization of the following substrates as sole carbon source: Tween 40 and 80, D-fructose, α -D-glucose, D-psicose, saccharose, methyl pyruvate and pyruvic acid. None of the tested strains utilized the following substrates: inulin, mannan, N-acetylmannosamine, L-arabinose, arbutin, cellobiose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, *m*-inositol, α -D-lactose, lactulose, D-mannitol, D-melezitose, D-melibiose, methyl α -D-galactoside, methyl β -D-galactoside, methyl α -D-glucoside, methyl β -D-glucoside, methyl α -mannoside, sedoheptulosan, stachyose, D-tagatose, xylitol, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, lactamide, D-malic acid, succinic acid, D-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-pyrogutamic acid, L-serine, putrescine, 2,3-butanediol, adenosine, 2'-deoxyadenosine, inosine, thymidine, uridine, AMP, TMP, UMP, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate and DL- α -glycerol phosphate. Only two isolates (17%) were able to utilize D-sorbitol. In contrast to the type strain of *G. rubropertincta*, isolated strains did not use glycogen, D-arabitol, maltose, D-xylose, α -ketoglutaric acid and L-alanine as sole carbon source, but α -cyclodextrin was used (Table 2). Activities of selected enzymes were analysed by the API ZYM method (bioMérieux). By

this semiquantitative method, 19 enzymic activities were examined. The isolated strains, as well as the type strains of *G. rubropertincta* and *G. terrae*, were: positive for the enzymes alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase and α -glucosidase; and weakly positive for the enzymes lipase (C14), valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β -glucosidase. Strains were negative for the enzymes chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

Chemotaxonomic characteristics

All isolates studied possessed *meso*-A₂pm, mycolic acids, MK-9(H₂) as predominating menaquinone, and straight-chain saturated and unsaturated cellular fatty acids with mainly 16 and 18 carbon atoms as well as tuberculostearic acid (10-methyl octadecanoic acid) (Table 3). HPLC analysis of mycolic acid *p*-bromophenacyl esters revealed the occurrence of three components with retention times between 21.4 and 23.3 min in all isolates examined. Strain HKI 0150 showed an additional minor component eluting after 20.5 min (Table 4). On average, retention times of type strains of *Gordonia* species were slightly higher (*G. terra*, *G. rubropertincta*) or significantly higher (*G. bronchialis*, *G. aichiensis*, *G. sputi*) than those of the isolates. The G + C composition of strain HKI 0148 was 67 mol%, while all other isolates showed a G + C value of 68 mol% (Table 3).

Table 4. HPLC-elution profiles of mycolic acid *p*-bromophenacyl esters of strains isolated from tar-contaminated soil and of selected type strains of the suborder *Corynebacterineae*

Strain	Overall no. of C atoms in mycolic acids		Retention time (min) of peaks			
<i>Corynebacterium glutamicum</i> DSM 20300 ^T	30–36*	18·13				
<i>Dietzia maris</i> DSM 43672 ^T	34–38†	18·63				
<i>Rhodococcus rhodochrous</i> DSM 43241 ^T	36–48‡		19·26	20·37	20·95	
HKI 0136 ^T	ND			21·55	22·63	23·22
HKI 0144	ND			21·45	22·63	23·22
HKI 0145	ND			21·47	22·63	23·22
HKI 0146	ND			21·48	22·38	23·22
HKI 0134	ND			21·52	22·51	23·27
HKI 0135	ND			21·46	22·62	23·21
HKI 0148	ND			21·50	22·48	23·25
HKI 0149	ND			21·50	22·50	23·26
HKI 0150	ND		20·52	21·49	22·68	23·26
<i>G. terrae</i> DSM 43249 ^T	58§			22·43	23·25	23·85
<i>G. rubropertincta</i> DSM 43197 ^T	58–59§			22·36	23·23	23·85
<i>G. bronchialis</i> DSM 43247 ^T	64§					24·57
<i>G. aichiensis</i> DSM 43978 ^T	62§					24·46
<i>G. sputi</i> DSM 43896 ^T	61–62§				23·98	24·60
<i>Nocardia carnea</i> DSM 43397 ^T	ND			23·24	23·85	
<i>Tsukamurella paurometabola</i> DSM 20162 ^T	68–76¶					26·54

ND, Not determined.

* Data from Collins *et al.* (1982).† Data from Rainey *et al.* (1995).‡ Data from Azuma *et al.* (1974).§ Data from Klatte *et al.* (1994).|| Mycolic acids of *N. carnea* similar to those of *G. amarae* (Lechevalier & Lechevalier, 1974) containing 46–54 carbon atoms (Klatte *et al.*, 1994).

¶ Data from Collins & Jones (1982).

16S rDNA sequence analysis

Partial 16S rDNA sequences of about 530 nt were determined for six isolated strains, HKI 0134, HKI 0136^T, HKI 0144, HKI 0147, HKI 0149 and HKI 0150. As all these sequences showed 100% identity, the almost complete 16S rDNA sequence, comprising 1490 nt (97·7% of the *Escherichia coli* sequence), was determined for one of these strains, strain HKI 0136^T. Comparison with the database of members of the class *Actinobacteria* revealed the highest degree of similarity with type strains of species of the genus *Gordonia* (96·2–99·1%). Strain HKI 0136^T is closely related to *G. rubropertincta* DSM 43197^T, sharing 99·1% sequence similarity. The phylogenetic dendrogram shown in Fig. 3 was constructed from evolutionary distance values by the neighbour-joining method. Low to moderate bootstrap values indicated that most branching points have little statistical significance. Information on the phylogenetic position of strain HKI

0136^T guided the selection of type strains for tests of phenotypic properties.

DNA–DNA homology

The DNA–DNA homology between strain HKI 0136^T and the type strain *G. rubropertincta* DSM 43197^T was 52%.

Ribotyping

Six isolates, HKI 0134, HKI 0136^T, HKI 0144, HKI 0147, HKI 0149, HKI 0150, as well as the type strains of *G. rubropertincta* and *G. terrae* were ribotyped with the restriction enzyme *EcoRI*. All strains possessed two bands of sizes 3 and 40–50 kb which are also present in species of *Mycobacterium* (Vuorio *et al.*, 1999). Strain-specific discrimination was done on the basis of the presence of several additional bands ranging between 0·5 and 18 kb (not shown). All isolates from tar-contaminated soil produced a fragment of

Fig. 3. Phylogenetic dendrogram based on the results of a 16S rDNA sequence comparison. Numbers at branching points refer to bootstrap values (500 trees resampled). Scale bar, 2 inferred nucleotide substitutions per 100 nucleotides.

8 kb which was absent in the type strains. These strains showed higher than 85% ribotype similarity among each other. Fragments of 15 and 18 kb were specific for *G. terrae* DSM 43249^T and *G. rubropertincta* DSM 43197^T. These two strains showed lower than 70% ribotype similarity to the isolates.

DISCUSSION

It was the aim of this study to identify the taxonomic position of isolates from contaminated soil on the basis of chemotaxonomic, physiological and genetic characteristics. Analysis of 16S rDNA, the presence of menaquinone type MK-9(H₂) and retention times of mycolic acid *p*-bromophenacyl esters that were similar to those identified in members of the genus *Gordonia* revealed that the isolates were members of this genus. As a 16S rDNA similarity value of 99.1%, determined for isolate HKI 0136^T and the type strain of *G. rubropertincta*, does not exclude membership to the same species (Stackebrandt & Goebel, 1994), DNA–DNA reassociation was carried out in order to determine the degree of relatedness at the genomic level. The value (52% DNA similarity) was significantly below the threshold value of about 70% recommended for the delineation of species (Wayne *et al.*, 1987). Ribotyping indicated that all isolates were more similar to each other than to their phylogenetic neighbours. The occurrence of different ribotyping patterns among strains of a species is a well-documented observation (Kloos *et al.*, 1997). The taxonomic distinctness of strain HKI 0136^T and the other tar isolates from *G. rubropertincta* DSM 43197^T was also verified at the chemotaxonomic level by differences in the retention times of mycolic acid derivatives. Although the precise number of carbon atoms was not determined, the differences in retention times between all isolates and the type strains of *Gordonia* species indicated differences in chain length.

As the number of carbon atoms of these type strains are known (Table 4), it can be concluded that the number of carbon atoms of the mycolic acids of the isolates ranges between about 55 and 57. Morphological, chemotaxonomic (Table 3) and 16S rDNA sequence data demonstrated that strain HKI 0136^T belongs to the genus *Gordonia*. On the basis of the moderate DNA–DNA reassociation value obtained for strain HKI 0136^T and its nearest phylogenetic neighbour, *G. rubropertincta* DSM 43197^T, the chain length of mycolic acids and the presence of a unique pattern of physiological and metabolic properties of strain HKI 0136^T and relatives (Tables 1 and 2), we propose a new species of *Gordonia*, *Gordonia alkanivorans* sp. nov.

Description of *Gordonia alkanivorans* sp. nov.

Gordonia alkanivorans (al.ka.ni'vo.rans. M.L. n. *alkanum* saturated aliphatic hydrocarbon; L. v. *vorare* to eat; L. adj. *alkanivorans* alkane-devouring).

Gram-positive, non-acid-fast, regular rod-shaped cells (0.5–0.9 × 1.0–2.3 µm) occurring singly, in pairs, small clusters, in V-shape arrangement or in short chains. Non-motile. Mycelium absent. Rod-coccus growth cycle. Colonies orange to orange-red. Colony morphology either convex and smooth or rough and flat with irregular margins. When grown on hexadecane or hexadecene as sole carbon source the orange colour fades. Catalase-positive and oxidase-negative. Acid production observed from fructose, glycerol, D-mannitol, saccharose and D-sorbitol, but not from D-glucose. The alkali salts acetate, aconitate, benzoate, citrate, formate, malate, succinate, propionate and pyruvate were used for growth, but lactate, oxalate and tartrate were not utilized. Nitrogenous compounds like ammonia, nitrate and urea were utilized. Tween 80 and potato starch were hydrolysed. Aesculin, casein, adenine, gelatin, hippurate, hypoxanthine, tyrosine and xanthine were not decomposed. Formation of H₂S was positive, whereas production of indole, the Voges–Proskauer test and the methyl red test were negative. Susceptible to the antibiotics ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), nitrofurantoin (100 µg), oxytetracycline (30 µg), rifampicin (30 µg), streptomycin (10 µg) and sulfonamide (250 µg), but weakly susceptible to erythromycin (15 µg), oxacillin (5 µg), penicillin G (10 IU), polymyxin B (300 IU). Resistant to lincomycin (2 µg). The following carbon substrates were not utilized: glycogen, D-arabitol, maltose, D-xylose, α-ketoglutaric acid and L-alanine, but α-cyclodextrin, β-cyclodextrin, dextrin, D-mannose, D-trehalose, turanose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, α-ketovaleric acid, N-acetyl-L-glutamic acid, Tween 40 and 80, D-fructose, α-D-glucose, D-psicose, saccharose, methyl pyruvate and pyruvic acid were utilized. The cell wall contains *meso*-A₂pm as the diagnostic diamino acid. The predominant isoprenologue is MK-

9(H₂); minor amounts of MK-8(H₂) are also present. Contains major amounts of straight-chain saturated and monounsaturated fatty acids and tuberculostearic acid. Mycolic acids with less than 60 carbon atoms are present. DNA G + C content is 68 mol%. Isolated from contaminated soil sampled from the area of a former tar factory in Rositz (Germany). The type strain is HKI 0136^T (= DSM 44369^T).

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