***Gordonia polyisoprenivorans* sp. nov., a rubber-degrading actinomycete isolated from an automobile tyre**

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A rubber-degrading bacterium (strain Kd2^T) was isolated from fouling tyre water inside a deteriorated automobile tyre. The strain was aerobic, Gram-positive, produced elementary branching hyphae which fragmented into rod/coccus-like elements and showed chemotaxonomic markers which were consistent with the classification of *Gordonia*, i.e. meso-diaminopimelic acid, N-glycolyl muramic acid, arabinose and galactose as diagnostic sugars, a fatty acid pattern composed of unbranched saturated and monounsaturated fatty acids with a considerable amount of tuberculostearic acid, and mycolic acids comprising 58–66 carbon atoms with two principal mycolic acids C₆₀ and C₆₂ counting for over 60%. Results of 16S rDNA analyses as well as chemotaxonomic results, led to the conclusion that *Gordonia* sp. strain Kd2^T (= DSM 44302^T) represents a new species within the genus *Gordonia* for which the name *Gordonia polyisoprenivorans* is proposed.

Keywords: *Gordonia polyisoprenivorans* sp. nov., polyphasic taxonomy, rubber degradation

INTRODUCTION

Since the reclassification of *Rhodococcus aichiensis* and *Nocardia amarae* (Klatte *et al.*, 1994b) in the genus *Gordonia*, this taxon is now a well-defined genus among the so-called CMN group (*Corynebacterium*, *Mycobacterium*, *Nocardia*), i.e. bacteria which are able to synthesize mycolic acids (high-molecular-mass alpha-branched 3-hydroxy fatty acids). The results of 16S rDNA sequence analyses have shown that the mycolic acid-producing bacteria, i.e. *Corynebacterium*, *Dietzia*, *Rhodococcus*, *Nocardia*, *Skermania*, *Corynebacterium*, *Tsukamurella* and *Mycobacterium*, form one homogeneous cluster among actinomycetes. Based on these results, the genera were combined into a new suborder of the order *Actinomycetales*, the *Corynebacterineae* (Stackebrandt *et al.*, 1997). Members of this taxon have many other chemotaxonomic markers in common, meso-diaminopimelic acid and arabinose

and galactose in whole cell hydrolysates, cell wall type IV according to Lechevalier & Lechevalier (1970), mostly N-glycolyl muramic acid in the peptidoglycan wall (Uchida & Aida, 1977), menaquinones with a partly saturated isoprenoid side chain (Kroppenstedt, 1982, 1985) and fatty acid patterns composed of unbranched saturated and unsaturated fatty acids plus tuberculostearic acid (TBSA) (Kroppenstedt, 1985). Although the overall chemical markers of members of the *Corynebacterineae* are roughly the same, the fine differences in the chemical markers can be used for genus and species differentiation (Klatte *et al.*, 1994a, 1994b, 1996). The best separation of *Gordonia* from the other taxa is obtained using a combination of the menaquinone type and the mycolic acid chain length. *Gordonia* synthesizes menaquinone MK-9(H₂) and mycolic acids of a carbon atom chain length ranging from C₅₀ to C₆₆ whereas *Corynebacterium* produces MK-8(H₂), some MK-9(H₂) and C₂₂–C₃₆, *Dietzia* MK-8(H₂) and C₃₄–C₃₈, *Rhodococcus* MK-8(H₂) and C₃₄–C₅₂, *Nocardia* MK-8(H₄, ω_{cyc1}) and C₄₄–C₆₀, *Tsukamurella* MK-9 and C₆₄–C₆₈, and *Mycobacterium* MK-9(H₂) and C₇₀–C₉₀. The homogeneity of this taxon has also been proved by 16S rDNA sequence data (Rainey *et al.*, 1995).

Abbreviations: MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; TBSA, tuberculostearic acid.

The EMBL accession number for the 16S rDNA sequence of strain Kd2^T is Y18310.

Originally, the *Gordonia* species, i.e. *Gordonia aichiensis*, *Gordonia bronchialis* and *Gordonia sputi*, have been regarded primarily as opportunistic pathogens because they have been found in sputum of humans suffering from pulmonary lesions (Tsukamura, 1971, 1978, 1982). Later, gordoniae were frequently isolated from the environment: *Gordonia rubropertincta* (Heferan, 1904) and *Gordonia terrae* from soil (Tsukamura, 1971); *Gordonia rhizosphaera* from mangrove rhizosphere (Takeuchi & Hatano, 1998); *Gordonia amarae* and *Gordonia* sp. from foams and in scumming activated sludge of sewage plants (Lechevalier & Lechevalier, 1974; Lemmer & Kroppenstedt, 1984; Sodell & Seviour, 1995); and *Gordonia hydrophobica* and *Gordonia hirsuta* from the packing material (tree bark compost) of biofilters used for the treatment of malodorous animal rendering emission (Bendinger *et al.*, 1995; Klatte *et al.*, 1996). The isolation of several rubber-degrading gordoniae strains from tyre water inside a deteriorated automobile tyre and from soil of a rubber tree plantation in Vietnam was previously reported (Linos & Steinbüchel, 1996, 1998). The aim of this study was to clarify the taxonomic position of one of these strains, *Gordonia* sp. strain Kd2^T (= DSM 44310^T).

Like the other taxa of the suborder *Corynebacterineae*, *Gordonia* plays an important role in bioremediation and biodegradation of pollutants in the environment. Some of the isolates have been used for the decontamination of polluted soils; others are used for different biotechnological processes in industry (Bell *et al.*, 1998). Before these organisms can be grown on a greater scale, a risk assessment for these strains has to be applied. A reliable taxonomy for this taxon is therefore essential to avoid any risk for humans and the environment.

METHODS

Bacterial strain and culture conditions. During a screening programme for rubber-degrading micro-organisms, strain Kd2^T was isolated from fouling tyre water inside a deteriorated automobile tyre on a farmer's field in Münster, Germany as described previously (Linos & Steinbüchel, 1998). The isolate was able to solubilize and mineralize natural rubber substrates but also grow on synthetic *cis*-1,4-polyisoprene after removal of antioxidants (added during manufacture against ageing) by organic solvent extraction. It grew adhesively on a layer of natural latex concentrate that was spread on mineral salt medium agar plate as a thin layer, but not when latex was dispersed into the agar at a concentration of 0.02 % (w/v). Cells occurred suspended in the medium when they were cultivated with squalene (*trans*-hexamer of isoprene), L-rhamnose or Standard I nutrient broth.

Gordonia polyisoprenivorans strain Kd2^T was deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen under accession number DSM 44302^T. For the determination of the colour, morphological studies and biochemical tests, strain Kd2^T was grown on solidified GYM agar medium [0.4 % (w/v) D-glucose, 0.4 % (w/v) yeast extract, 1 % (w/v) malt extract, 1.2 % (w/v) agar no. 1

(Oxoid)] at 28 °C. For biochemical tests, strain Kd2^T was harvested from GYM agar after 3 d. Morphological studies and colour examination were carried out after 1, 3 and 7 d. For analysis of the fatty acid and mycolic acid composition, strain Kd2^T was grown on TSB agar [3 % (w/v) Trypticase soy broth (BBL), 1.5 % (w/v) Bacto Agar (Difco)] for 4 d at 28 °C. For cell wall and quinone analysis, cells were grown in Trypticase soy broth for 4 d at 28 °C on a rotary shaker, harvested by centrifugation and washed twice with distilled water.

Macroscopic and microscopic studies. Macroscopic and microscopic studies and the staining procedures followed the instructions of Lefford (1980).

Biochemical characterization. Carbon source utilization and quantitative enzyme tests were performed in standard microtitration plates (F-form; Greiner) as described by Kämpfer *et al.* (1990) with the following modifications: carbon source utilization test medium Yeast-Nitrogen-Base (DIFCO), yeast extract (0.02 g l⁻¹), K₂HPO₄ (1.74 g l⁻¹), KH₂PO₄ (0.36 g l⁻¹); enzyme test medium, 0.05 M Tris/HCl buffer, 0.05 % (w/v) Casamino acids (DIFCO), 0.05 % (w/v) yeast extract (DIFCO). The panels were inoculated with standardized bacterial suspension of 0.5–1.0 McFarlane units in 0.9 % (w/v) NaCl, and then incubated for 24 h at 28 °C. After addition of 50 µl filter-sterilized indicator solution [4 mM MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)] and phenazine methosulfate (Sigma) to the auxanographic test and control wells, incubation continued for 24 h in the dark. The formation of the deep-blue coloured formazane was taken as an indication for carbon source utilization (Kirchhof *et al.*, 1992). Reading of the auxanographic test results was carried out photometrically by a Multiscan MCC340 MKII photometer (Flow Laboratories). Metabolism of a carbon source was considered positive if A₅₄₀ of the sample was greater than 0.129 (control). The results of the enzyme tests were evaluated visually.

Chemotaxonomic studies

Analysis of cell wall amino acids and sugars. The amino acid and sugar analysis of whole-cell hydrolysates followed the described procedures of Stanek & Roberts (1974).

Determination of the acyl type of the peptidoglycan. The murein acyl type was determined by a modification of the colorimetric method of Uchida & Aida (1977). In contrast to the original procedure, our whole-cell hydrolysate was neutralized by passing it through an ion-exchange column (Analytichem Bond Elut SCX; Varian).

Extraction and analysis of isoprenoid quinones and polar lipids. Isoprenoid quinones were extracted and purified using the small-scale integrated procedure of Minnikin (1984). Dried preparations were dissolved in 200 µl 2-propanol and 1–10 µl amounts were separated by HPLC without further purification. The menaquinones were separated by HPLC on Lichrosorb RP-18 at 40 °C using acetonitrile/2-propanol (65:35, v/v) as solvent (Kroppenstedt, 1982, 1985). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1977).

Extraction and analysis of fatty acids and mycolic acids. The fatty acid methyl esters were prepared from 40–80 mg wet cells (Miller, 1982). Half (0.3 ml) of the extract was passed through a silica gel column (Analytichem Bond Elut; Varian)

to trap the mycolic acids. The eluent was then used for GC analysis of the fatty acid methyl esters.

Trimethylsilylated derivatives of the mycolic acids were prepared by mixing the untreated half of the extract with 0.1 ml of a solution containing *n*-methyl-*n*-(trimethylsilyl)-heptafluorobutyramide and trimethylchlorosilane (10:1, v/v; Macherey & Nagel).

The mixtures of fatty acid methyl esters and silylated derivatives of the mycolic acid methyl esters were analysed by capillary GC using a Hewlett Packard model 5898A GC run by Microbial Identification Software (Microbial ID). For fatty acid methyl ester analysis, standard Microbial Identification System (MIS) conditions were used (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The trimethylsilylated derivatives of the mycolic acid esters were analysed by high-temperature GC with a model HP 5790A GC (Hewlett Packard) equipped with a flame-ionization detector and a 12 m type HT5 column (SGE); H₂ was the carrier gas at a flow rate of 30 ml min⁻¹. The oven temperature was increased from 210 to 400 °C at a rate of 10 °C min⁻¹. The final temperature was kept for 7 min. Peaks of the derivatives were identified by comparing their retention times with those of known standard mycolic acids (Klatte, 1994).

16S rDNA sequence determination. Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of PCR products were carried out using procedures described previously (Rainey *et al.*, 1996). Purified PCR products were sequenced using *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's protocol. Sequence reactions were electrophoresed using the Applied Biosystems 373A DNA sequencer. The 16S rDNA sequence was aligned manually with published sequences from representatives of the actinomycete sublines of descent included in the RDP (Maidak *et al.*, 1996) or obtained from EMBL.

Phylogenetic analysis. The ae2 editor (Maidak *et al.*, 1994) was used to align the 16S rDNA sequence of strain Kd2^T against the 16S rDNA sequences of the *Gordonia* type strains available from the public databases. Pairwise evolutionary distances were computed using the correction of Jukes & Cantor (1969). The least squares distance method of De Soete (1983) was used in the construction of the phylogenetic dendrogram from distance matrices.

Nucleotide sequence accession numbers. The strain designations and accession numbers of the reference strains used in the phylogenetic analyses are as follows: *G. aichiensis* DSM 43978^T, X80635; *G. amarae* DSM 43392^T, X80633; *G. bronchialis* DSM 43247^T, X79287; *G. hirsuta* DSM 44140^T, X93485; *G. hydrophobica* DSM 44015^T, X87340; *G. rhizosphaera* IFO 16068^T, AB004729; *G. rubropertincta* DSM 43197^T, X80632; *G. sputi* DSM 43896^T, X80634; *G. terrae* DSM 43249^T, X79286. The 16S rDNA sequence of strain Kd2^T has been deposited in the EMBL database under the accession number Y18310.

RESULTS AND DISCUSSION

Morphology

Strain Kd2^T produced very short elementary branching hyphae which disintegrated into rod- and cocci-like elements. It showed the typical rod-coccus growth cycle which is usually found among *Gordonia* and related taxa like *Rhodococcus* (Goodfellow, 1986,

1992; Goodfellow & Lechevalier, 1989). Strain Kd2^T exhibited creamy beige colonies which changed to pastel orange when cultivated in the presence of light. The cells were Gram-positive, slightly acid-fast and non-motile. No spores could be detected.

Biochemical characterization

The results of the physiological tests obtained from the microtitre plates revealed that strain Kd2^T was able to utilize more than half (19) of the 31 carbon sources by means of MTT reduction test, i.e. acetamide, L-alanine, D-arabitol, L-aspartate, benzoate, 4-hydroxybenzoate, citrate, D-galactose, gluconate, D-glucosaminic acid, *i*-inositol, L-proline, quinate, putrescine, L-rhamnose, sucrose, D-turanose and L-valine showed a positive reaction with MTT. The reaction with tyramine was doubtful.

No reduction was found with 4-aminobenzoate, 3-hydroxybenzoate, caprate, D-glucarate, L-leucine, 2-oxoglutarate, pimelate, phenylacetate, D-ribose, L-serine, succinate or 2-hydroxyvalerate.

None of the chromogenic substrates could be hydrolysed by strain Kd2^T, i.e. *p*-nitrophenyl-β-D-xyloside, *p*-nitrophenylphosphoryl choline and 2-desoxythymidine-5-*p*-nitrophenyl phosphate.

This utilization pattern did not match with any of the known *Gordonia* species (Table 3).

Chemotaxonomic characterization

The chemotaxonomic properties of Kd2^T were also consistent with their classification into the genus *Gordonia* (Klatte *et al.*, 1994a, b; Stackebrandt *et al.*, 1988). Whole cell hydrolysates of Kd2^T contained *meso*-diaminopimelic acid as the only diamino acid of the peptidoglycan and glucose and arabinose as major cell wall sugars. As expected for *Gordonia* and related taxa, the sugars of the peptidoglycan were glycolated. MK-9(H₂) was the only menaquinone of this strain. The polar lipids were composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidyl-inositol, phosphatidylinositol mannosides and some unspecified glycolipids. This pattern matched quite well with those reported by Minnikin *et al.* (1977) for *Gordonia*.

Kd2^T could be separated from species of the closely related genera: i.e. from *Corynebacterium*, which synthesizes the cell wall sugar *N*-acetylmuramic acid; from *Dietzia*, where phosphatidylethanolamine is missing in the polar lipid extract; from *Rhodococcus*, which synthesizes MK-8(H₂); from *Nocardia*, which produces the highly diagnostic menaquinone MK-8(H_{4,acycl}); and from *Tsukamurella*, which contains MK-9 (Klatte, 1994).

The fatty acid pattern was composed of straight-chain saturated and unsaturated fatty acids with 2% decanoic acid (C_{10:0}), 2% tetradecanoic acid (C_{14:0}), 29% palmitic acid (C_{16:0}), 12% palmitoleic acid

Table 1. Cellular fatty acids of *Gordonia polyisoprenivorans* and the type strains of the genus *Gordonia*

Main fatty acid components are shown. In addition, *G. polyisoprenivorans* contains 2% C_{10:0} and *G. hydrophobica* contains 2% *cis*-10 C_{15:1} and 10-methyl C_{17:0}. TBSA, Tuberculostearic acid (10-me C_{18:0}).

Strain	Cellular fatty acid composition (%)									
	C _{14:0}	C _{15:0}	<i>cis</i> -9 C _{16:1}	<i>cis</i> -10 C _{16:1}	C _{16:0}	<i>cis</i> -9 C _{17:1}	C _{17:0}	C _{18:1}	C _{18:0}	TBSA
<i>G. aichiensis</i> DSM 43978 ^T	2	1		13	34	1	1	17	2	25
<i>G. amarae</i> DSM 43392 ^T	1	1	3	15	29	2	1	24	1	21
<i>G. bronchialis</i> DSM 43247 ^T	1	1	11	16	23	2	1	10	1	20
<i>G. polyisoprenivorans</i> DSM 44302 ^T	2		1	12	29	1	1	21	4	29
<i>G. hirsuta</i> DSM 44140 ^T	2		1	16	30	1	1	30	3	17
<i>G. hydrophobica</i> DSM 44015 ^T	1	4		13	27	4	4	14	1	26
<i>G. rhizosphaera</i> IFO 16068 ^{T*}	3	4		4	27	7	13	27	11	3
<i>G. rubropertincta</i> DSM 43197 ^T	2	2		15	25	5	3	24		7
<i>G. sputi</i> DSM 43896 ^T	2	2			44	2	2	29		15
<i>G. terrae</i> DSM 43249 ^T	2	1		16	32	1	1	26	2	19

* Data from Takeuchi & Hatano (1998).

Table 2. Mycolic acids of *Gordonia polyisoprenivorans* and the type strains of the genus *Gordonia*

The main mycolic acid components are shown (designations indicate the chain lengths of the mycolic acids, i.e. no. of carbon atoms); data taken from Klatte *et al.* (1994b, 1996).

Strain	Mycolic acid composition (%)															
	C ₄₈	C ₅₀	C ₅₁	C ₅₂	C ₅₃	C ₅₄	C ₅₅	C ₅₆	C ₅₇	C ₅₈	C ₅₉	C ₆₀	C ₆₁	C ₆₂	C ₆₃	C ₆₄
<i>G. aichiensis</i> DSM 43978 ^T												14	11	47	11	17
<i>G. amarae</i> DSM 43392 ^T	5	30	3	40	4	18										
<i>G. bronchialis</i> DSM 43247 ^T												8	21	25	25	13
<i>G. polyisoprenivorans</i> DSM 44302 ^T										8	3	30	6	47	1	5
<i>G. hirsuta</i> DSM 44140 ^T												19	10	31	9	31
<i>G. hydrophobica</i> DSM 44015 ^T								12	11	30	16	26	5			
<i>G. rhizosphaera</i> IFO 16068 ^{T*}									TR	7	5	26	10	34	TR	6
<i>G. rubropertincta</i> DSM 43197 ^T						4	3	14	11	23	17	18	5			
<i>G. sputi</i> DSM 43896 ^T												8	12	48	14	8
<i>G. terrae</i> DSM 43249 ^T						5	1	27	4	36	3	19	1	4		

TR, Trace.

* Data from Takeuchi & Hatano (1998).

(C_{16:1}), 1% heptadecanoic acid (C_{17:0}), 4% stearic acid (C_{18:0}), 21% oleic acid (*cis*-9 C_{18:1}) and 29% TBSA (10-me C_{18:0}) as the main constituents. Kd2^T synthesized a homologous series of mycolic acids ranging from 58–64 carbon atoms with C₆₀ and C₆₂ being the two principal mycolic acids. The combination of the chemical markers found in Kd2^T was unique for species of the genus *Gordonia*. Therefore, Kd2^T could be classified in the genus *Gordonia* by chemotaxonomy (Table 1).

Although the fatty acid and mycolic acid patterns of Kd2^T are roughly the same in all gordoniae, there are fine qualitative and quantitative differences in the

patterns which are species-specific (Klatte, 1994). The mycolic acid chain-length of Kd2^T was in the range of the mycolic acids expected for *Gordonia* species. It fell into the mycolic acid group of *G. aichiensis*, *G. bronchialis*, *G. hirsuta* and *G. sputi* which show a mycolic acid chain length of about C₆₀–C₆₄ (Table 2). Kd2^T could be further distinguished from these species by quantitative differences in mycolic acids (Table 2).

16S rDNA sequence analysis

The almost complete 16S rDNA sequence of strain Kd2^T consisting of 1431 nt was compared to those of members of the genus *Gordonia*. These organisms were

Table 3. Physiological properties of *Gordonia polyisoprenivorans* and the type strains of the genus *Gordonia*

Strains: 1, *G. aichiensis* DSM 43978^T; 2, *G. amarae* DSM 43392^T; 3, *G. bronchialis* DSM 43247^T; 4, *G. polyisoprenivorans* DSM 44302^T; 5, *G. hirsuta* DSM 44140^T; 6, *G. hydrophobica* DSM 44015^T; 7, *G. rhizosphaera* IFO 16068^T; 8, *G. rubropertincta* DSM 43197^T; 9, *G. sputi* DSM 43896^T; 10, *G. terrae* DSM 43249^T. Assimilation of auxanographic substrates was detected photometrically by means of reduction of the redox dye MTT. +, $A_{540}(\text{test}) - A_{540}(\text{control}) > 0.129$; -, $A_{540}(\text{test}) - A_{540}(\text{control}) < 0.129$. All strains tested were positive for D-galactose and negative for pimelic acid, L-serine and 3-hydroxybenzoate. *G. polyisoprenivorans* gave a variable reaction with tyramine. No data were available for *G. rhizosphaera* for these substrates, except D-galactose which could not be used by this strain.

Compounds assimilated or cleaved	1	2	3	4	5	6	7*	8	9	10
N-Acetyl-D-glucosamine	-	-	+	+	-	-	ND	-	-	-
Glucarate	-	-	-	-	-	+	ND	+	-	+
Gluconate	-	-	+	+	-	-	-	+	-	-
D-Glucosaminic acid	-	-	-	+	-	-	ND	+	-	-
L-Rhamnose	-	-	+	+	-	-	+	+	-	+
D-Ribose	-	+	-	-	-	-	-	+	-	-
D-Sucrose	-	+	+	+	-	+	-	+	-	+
D-Turanose	-	+	+	+	-	+	ND	-	-	-
D-Arabitol	-	+	-	+	-	-	ND	+	+	+
i-Inositol	-	-	+	+	+	-	-	-	-	-
Citrate	+	+	-	+	-	+	+	-	+	+
2-Hydroxyvalerate	-	-	-	-	+	-	ND	-	-	-
2-Oxoglutarate	+	+	-	-	+	-	ND	-	+	+
Succinate	-	-	+	-	-	+	-	-	+	-
L-Alanine	+	-	+	+	-	-	ND	+	-	-
4-Aminobenzoate	+	-	-	-	-	-	ND	-	-	-
4-Aspartate	+	-	-	+	-	-	-	-	-	-
L-Leucine	-	-	+	-	-	+	+	-	-	-
L-Proline	-	+	-	+	-	+	+	-	-	-
L-Valine	-	+	+	+	-	+	+	-	-	-
Putrescine	-	+	-	+	-	-	ND	-	+	-
Acetamide	-	-	+	+	-	+	-	-	-	-
Benzoate	-	-	-	+	-	-	-	+	-	+
4-Hydroxybenzoate	-	+	+	+	-	-	ND	-	-	-
Phenylacetic acid	-	-	+	-	-	-	ND	+	-	-
Quinate	+	+	-	+	-	-	ND	-	-	-
p-Nitrophenyl-β-D-xyloside	-	-	-	-	-	-	ND	-	-	+
p-Nitrophenylphosphoryl choline	-	-	-	-	+	+	ND	-	-	-
2-Desoxythymidine-5-p-nitrophenyl phosphate	-	-	-	-	-	+	ND	-	-	-

ND, Not determined.

* Data from Takeuchi & Hatano (1998).

determined to be the closest phylogenetic neighbours. Strain Kd2^T showed highest 16S rDNA similarity values of 98.2% and 97.8% to the 16S rDNA sequences of *G. bronchialis* and *G. rhizosphaera*, respectively (Takeuchi & Hatano, 1998). Intra-genetic relationships revealed similarity values of 95.0–99.7%. The 16S rDNA data indicated that strain Kd2^T belongs to the genus *Gordonia* but is different from previously described species (Fig. 1).

Based on the phenotypic and genotypic data, it is concluded that the rubber-degrading strain merits

species status in the genus *Gordonia*. We therefore propose the name *Gordonia polyisoprenivorans* for the isolate Kd2^T (= DSM 44302^T).

Description of *Gordonia polyisoprenivorans* Linos, Steinbüchel, Spröer and Kroppenstedt sp. nov.

Gordonia polyisoprenivorans (poly.iso.preni'vor.ans. M.L. gen adj. *polyisoprenivorans* polyisoprene eating, referring to the ability to degrade polyisoprene).

Aerobic, Gram-positive, non-motile actinomycete

Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis showing the position of *Gordonia polyisoprenivorans* DSM 44302^T among members of the genus *Gordonia* and the phylogenetic neighbouring genera. Bar, 0.02 estimated nucleotide changes per position.

which forms a rudimentary mycelium which fragments into rod/coccoid-like elements. Colonies smooth, beige, pastel, orange when exposed to light. Able to utilize acetamide, L-alanine, D-arabitol, L-aspartate, benzoate, 4-hydroxybenzoate, citrate, D-galactose, gluconate, D-glucosaminic acid, *i*-inositol, L-proline, quinate, putrescine, L-rhamnose, sucrose, D-turanose and L-valine. No growth observed with 4-aminobenzoate, 3-hydroxybenzoate, caprate, D-glucarate, L-leucine, 2-oxoglutarate, pimelate, phenylacetate, D-ribose, L-serine, succinate and 2-hydroxyvalerate. Doubtful reaction with tyramine. No hydrolysis of the chromogenic substrates, i.e. *p*-nitrophenyl- β -D-xyloside, *p*-nitrophenylphosphoryl choline and 2-desoxythymidine-5-*p*-nitrophenyl phosphate. Whole-cell hydrolysates contain meso-diaminopimelic acid, arabinose and galactose (cell wall chemotype IV *sensu* Lechevalier & Lechevalier). Sugars of the peptidoglycan are glycolated. The predominant menaquinone is MK-9(H₂). The polar lipids are composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. The fatty acid pattern revealed C_{10:0} (2%), C_{14:0} (2%), C_{16:1} (12%), C_{16:0} (29%), C_{17:0} (1%), C_{18:1} (21%), C_{18:0} (4%), 10-

me C_{18:0} (29%). The principal mycolic acids have chain lengths of 60 and 62 carbon atoms. Able to solubilize and mineralize natural rubber substrates. Isolated from fouling tyre water inside a deteriorated automobile tyre on a farmer's field in Münster, Germany. Type strain: *Gordonia polyisoprenivorans* strain Kd2^T (= DSM 44302^T).

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