NOTE

***Gordonia westfalica* sp. nov., a novel rubber-degrading actinomycete**

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A *cis*-1,4-polyisoprene-degrading bacterium (strain Kb2^T) was isolated from foul water taken from the inside of a deteriorated automobile tyre found on a farmer's field in Westfalia, Germany. The strain was aerobic, Gram-positive, exhibited orange smooth and rough colonies on complex nutrient agar, produced elementary branching hyphae that fragmented into rod/coccus-like elements and showed chemotaxonomic markers which were consistent with its classification within the genus *Gordonia*, i.e. the presence of meso-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates (cell-wall chemotype IV), *N*-glycolylmuramic acid in the peptidoglycan wall, a fatty-acid pattern composed of unbranched saturated and monounsaturated fatty acids plus tuberculostearic acid, mycolic acids comprising 56–60 carbon atoms and MK-9(H₂) as the only menaquinone. The 16S rDNA sequence of strain Kb2^T was found to be most similar to the 16S rDNA sequences of the type strains of *Gordonia alkanivorans* (DSM 44369^T) and *Gordonia nitida* (KCTC 0605BP^T). However, DNA–DNA relatedness data showed that strain Kb2^T (= DSM 44215^T = NRRL B-24152^T) could be distinguished from these two species and represented a new species within the genus *Gordonia*, for which the name *Gordonia westfalica* is proposed.

Keywords: *Gordonia westfalica* sp. nov., polyphasic taxonomy, rubber degradation, biodegradation, *cis*-1,4-polyisoprene

The genus *Gordonia* belongs to the suborder *Corynebacterineae* within the order *Actinomycetales* (Stackebrandt *et al.*, 1997). This suborder, formerly called the CMN (*Corynebacterium*, *Mycobacterium* and *Nocardia*) group, comprises bacteria that are able to synthesize mycolic acids (i.e. high-molecular-weight α -branched 3-hydroxy fatty acids). There are two species within this suborder that have lost the ability to synthesize mycolic acids, *Corynebacterium amycolatum* and *Turicella otitidis*. The suborder *Corynebacterineae* encompasses 10 genera, namely *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Skermania*, *Tsukamurella*, *Turicella* and *Williamsia* (Chun *et al.*, 1996; Kämpfer *et al.*, 1999).

As well as mycolic acids, other chemotaxonomic markers are common within members of the *Corynebacterineae*, such as the type IV cell wall, according to Lechevalier & Lechevalier (1970), *N*-glycol-muramic acid in the peptidoglycan wall (Uchida & Aida, 1977), menaquinones with a partially saturated isoprenoid side chain (Kroppenstedt, 1982, 1985) and fatty-acid patterns composed of unbranched saturated and unsaturated fatty acid plus tuberculostearic acid (Kroppenstedt, 1985).

Members of the genus *Gordonia* exhibit a characteristic mycolic acid chain length ranging from C₅₀ to C₆₆; they also synthesize the menaquinone MK-9(H₂). The homogeneity of this genus has also been proven by 16S rDNA sequence data (Rainey *et al.*, 1995).

At the time of writing, the genus *Gordonia* comprised 14 validly described species (see Table 1). In contrast to the originally isolated strains of the genus which were described as opportunistic pathogens in humans

Abbreviation: MTT, tetrazolium-[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide].

The EMBL accession number for the 16S rDNA sequence of *Gordonia westfalica* DSM 44215^T is AJ312907.

Table 1. Physiological properties of *Gordonia westfalica* and the type strains of the genus *Gordonia*

Strains: 1, *G. westfalica* DSM 44215^T; 2, *G. aichiensis* DSM 43978^T; 3, *G. alkanivorans* DSM 44369^T; 4, *G. amarae* DSM 44392^T; 5, *G. amicalis* DSM 44461^T; 6, *G. bronchialis* DSM 43247^T; 7, *G. desulfuricans* DSM 44462^T; 8, *G. polyisoprenivorans* DSM 44302^T; 9, *G. hirsuta* DSM 44140^T; 10, *G. hydrophobica* DSM 44015^T; 11, *G. nitida* DSM 44499^T; 12, *G. rhizosphaera* DSM 44383^T; 13, *G. rubripertincta* DSM 43197^T; 14, *G. sputi* DSM 43896^T; 15, *G. terrae* DSM 43249^T. All strains were obtained from the DSMZ. Assimilation of auxanographic substrates was detected photometrically by means of reduction of the redox dye MTT. —, Difference in A_{540} (test) to A_{540} (control) < 0.129; +, difference in A_{540} (test) to A_{540} (control) > 0.129; CXY, *p*-nitrophenyl- β -D-xyloside; CCH, *p*-nitrophenylphosphoryl choline; CDP, 2-desoxythymidine-5-*p*-nitrophenyl phosphate; V, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Utilization of:															
D-Galactose	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Rhamnose	—	—	—	—	+	+	—	+	—	—	+	+	+	—	+
D-Ribose	+	—	+	+	+	—	+	—	—	—	+	—	+	—	—
D-Sucrose	+	—	+	+	+	+	+	+	—	+	+	+	+	—	+
D-Turanose	—	—	+	+	+	+	+	+	—	+	+	+	—	—	—
D-Arabitol	+	—	+	+	—	—	—	+	—	—	—	—	+	+	+
<i>D</i> -Inositol	—	—	+	—	—	+	—	+	+	—	+	+	—	—	—
<i>N</i> -acetyl-D-glucosamine	—	—	+	—	—	+	+	+	—	—	—	+	—	—	—
Glucarate	—	—	+	—	+	—	—	—	—	+	+	—	+	—	+
Gluconate	—	—	+	—	+	+	+	+	—	—	+	+	+	—	—
D-Glucosaminic acid	+	—	+	—	+	—	+	+	—	—	+	+	+	—	—
Caprate	+	+	+	ND	+	+	+	ND	ND	ND	—	—	V	+	—
Citrate	+	+	+	+	+	—	+	+	—	+	+	+	—	+	+
4-Aminobutyrate	—	+	—	ND	—	—	+	ND	ND	ND	—	—	—	—	—
2-Hydroxyvalerate	—	—	+	—	+	—	+	—	+	—	+	—	—	—	—
2-Oxoglutarate	+	+	+	+	+	—	+	—	+	—	+	+	—	+	+
Pimelate	—	—	+	—	+	—	+	—	—	—	—	—	—	—	—
Succinate	—	—	+	—	+	+	—	—	—	+	+	+	—	+	—
Benzoate	+	—	+	—	—	—	—	+	—	—	+	+	+	—	+
3-Hydroxybenzoate	—	—	+	—	—	—	+	—	—	—	+	+	—	—	—
4-Hydroxybenzoate	—	—	+	+	+	+	+	+	—	—	+	+	—	—	—
Phenylacetate	—	—	+	—	+	+	+	—	—	—	+	—	+	—	—
Quinate	—	+	—	+	—	—	—	+	—	—	—	+	—	—	—
L-Alanine	—	+	+	—	+	+	+	+	—	—	+	—	+	—	—
L-Aspartate	+	+	+	—	+	—	—	+	—	—	+	—	—	—	—
L-Leucine	—	—	+	—	+	+	+	—	—	+	—	—	—	—	—
L-Proline	—	—	+	+	—	—	+	+	—	+	—	—	—	—	—
L-Serine	—	—	—	—	+	—	—	—	—	—	+	—	—	—	—
L-Valine	—	—	—	+	—	+	—	+	—	+	+	—	—	—	—
Putrescine	—	—	+	+	+	—	+	+	—	—	—	—	—	—	—
Tyramine	—	—	+	ND	+	—	+	V	ND	ND	+	+	—	—	—
Acetamide	—	—	—	—	+	+	+	+	—	+	—	—	—	—	—
Hydrolysis of:															
CXY	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
CCH	—	—	+	—	+	—	+	—	+	+	+	—	—	—	—
CDP	—	—	—	—	—	—	+	—	—	+	—	—	—	—	—

(Tsukamura, 1971, 1978, 1982), most members of the genus *Gordonia* described nowadays represent environmental isolates that play an important role in bioremediation and the biodegradation of pollutants. Novel *Gordonia* strains have been isolated from the packing material of biofilters used for the treatment of malodorous rendering-plant emissions (Bendinger *et al.*, 1995, Klatte *et al.*, 1996), from the mangrove rhizo-

sphere (Takeuchi & Hatano, 1998), from automobile tyres (Linós *et al.*, 1999) and from tar- and phenol-contaminated soil (Kummer *et al.*, 1999), or they have been assigned as degraders of various recalcitrant substances, such as synthetic *cis*-1,4-polyisoprene (Linós *et al.*, 1999), benzothiophene (Kim *et al.*, 1999), dibenzothiophene (Kim *et al.*, 2000) and 3-ethylpyridine plus 3-methylpyridine (Yoon *et al.*, 2000).

The aim of this study was to clarify the taxonomic position of strain Kb2^T. The rubber-degrading properties of this strain have been previously reported in detail (Linos *et al.*, 2000; Berekaa *et al.*, 2000). A reliable classification for rubber-degrading micro-organisms has often been vague in the literature (Linos & Steinbüchel, 2001). Accurate knowledge concerning the taxonomic position of rubber-degrading actinomycetes permits a better understanding of the physiological peculiarities of these organisms and is also helpful when choosing or establishing suitable genetic tools to elucidate further biochemical questions – especially as members of this group are genetically barely accessible. Based on the phenotypic and genotypic data described below, we recognized the rubber-degrading strain Kb2^T as a new species of the genus *Gordonia*, for which the name *Gordonia westfalica* is proposed.

During a screening programme for rubber-degrading micro-organisms, strain Kb2^T was isolated from foul water held inside a deteriorated automobile tyre found on a farmer's field near Münster, Westfalia, Germany (Linos & Steinbüchel, 1998). As in the case of the already-described species *Gordonia polyisoprenivorans* (Linos *et al.*, 1999), Kb2^T was also able to solubilize and mineralize natural rubber substrates and synthetic *cis*-1,4-polyisoprene (Berekaa *et al.*, 2000). It grew on and adhered to a layer of natural latex concentrate that was spread on mineral salts medium agar plates as a thin layer, but it did not grow when latex was dispersed into the agar at a concentration of 0.02 % (w/v). Consequently, Kb2^T belongs to the group of adhesive rubber degraders that grow only in direct contact with the rubber substrate, thereby forming a biofilm on the surface and leading to disintegration of the solid rubber material during cultivation (Linos *et al.*, 2000). Cells occurred as aggregates in the medium when they were cultivated with squalene (a *trans*-hexamer of isoprene) and occurred as a suspension when grown with L-rhamnose or in Standard I nutrient broth [1.56 % (w/v) peptone, 0.28 % (w/v) yeast extract, 0.56 % (w/v) NaCl, 0.1 % (w/v) (+)-D-glucose]. For the determination of its colony colour, morphological traits and biochemical tests, strain Kb2^T was grown on solidified GYM agar [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 Kb2^T was harvested from GYM agar after 3 days. Morphological studies and colour examination were carried out after 1, 3 and 7 days. For analysis of the fatty-acid and mycolic-acid composition, strain Kb2^T was grown on TSB agar [3 % (w/v) Trypticase soy broth (BBL), 1.5 % (w/v) Bacto Agar (Difco)] for 4 days at 28 °C. For cell-wall and quinone analysis, cells were grown in Trypticase soy broth for 4 days at 28 °C on a rotary shaker, harvested by centrifugation and washed twice with distilled water.

Macroscopic and microscopic studies and the staining procedures used were as described by Lefford (1980); the carbon source utilization and quantitative enzyme

tests were performed in standard microtitre plates (Greiner) as described previously (Klatte, 1994; Linos *et al.*, 1999).

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

The isoprenoid quinones were extracted and purified using the small-scale integrated procedure of Minnikin *et al.* (1984) and analysed by HPLC, as described previously (Kroppenstedt, 1982, 1985). Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1977). 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 the Microbial Identification Software (Microbial ID). For fatty acid methyl ester analysis, standard Microbial Identification System conditions were used (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The trimethyl silylated derivatives of the mycolic acid esters were analysed by high-temperature GC with a model HP 5790A GC (Hewlett Packard), as described previously (Klatte, 1994).

The extraction of genomic DNA was performed as described in Ausubel *et al.* (1995). PCR-mediated amplification of the 16S rDNA and purification of the PCR products were carried out using procedures described previously (Rainey *et al.*, 1996). Purified PCR products were sequenced using a Thermo Sequenase fluorescence-labelled primer cycle-sequencing kit (Amersham Life Science), according to the manufacturer's instructions. Sequence reactions were electrophoresed by using a 4000L DNA sequencer (LI-COR). The 16S rDNA sequence generated was aligned manually with published sequences from representatives of the actinomycete sublines of descent included in the RDP library (Maidak *et al.*, 1996) or obtained from the EMBL database.

For DNA–DNA hybridization, DNA was isolated by chromatography on hydroxyapatite (Cashion *et al.*, 1977) and hybridization was performed as described by De Ley *et al.* (1970) with modifications (Escara &

Table 2. Cellular fatty acids of *Gordonia westfalica* (rough and smooth variants) and the type strains of the genus *Gordonia*

Strains: 1, *G. aichiensis* DSM 43978^T; 2, *G. alkanivorans* DSM 44369^T; 3, *G. amarae* DSM 43392^T; 4, *G. amicalis* DSM 44461^T; 5, *G. bronchialis* DSM 43247^T; 6, *G. desulfuricans* DSM 44462^T; 7, *G. hirsuta* DSM 44140^T; 8, *G. hydrophobica* DSM 44015^T; 9, *G. nitida* DSM 44499^T; 10, *G. polyisoprenivorans* DSM 44302^T; 11, *G. rhizosphaera* DSM 44383^T; 12, *G. rubripertincta* DSM 43197^T; 13, *G. sputi* DSM 43896^T; 14, *G. terrae* DSM 43249^T; 15, *G. westfalica* DSM 44215^T (rough); 16, *G. westfalica* DSM 44214 (smooth). All values are shown as a percentage of the total cellular fatty acids, and only those present at > 1% are shown. In addition to the fatty acids shown in Table 2, 10-methyl-heptadecanoic acid is found in *G. amicalis* (2%), *G. bronchialis* (7%), *G. hydrophobica* (4%), *G. nitida* (1%) and *G. rubripertincta* (1%), C_{19:1} is found in *G. rubripertincta* (3%) and *G. bronchialis* (3%), and C_{15:1} is found in *G. hydrophobica* (2%). C_{18:1}, Octadecanoic acid, oleic acid; TBSA, tuberculostearic acid.

Cellular fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C _{14:0}	2	4	1	2	1	2	2	1	3	2	3	2	2	2	2	3
C _{15:0}	1	1	1	3	1			4	1		4	2	2	1	1	1
C _{16:1 cis-7 16:1}			3	1	11	1	1		1	1					12	12
C _{16:1 cis-9 16:1}	13	16	15	14	16	15	16	13	13	12	4	15		16		
C _{16:0}	34	28	29	29	23	26	30	27	33	29	27	25	44	32	32	37
C _{17:1 cis-9}	1	2	2	6	2		1	4	3	1	7	5	2	1		
C _{17:0}	1	1	1	5	1	1	1	4	2	1	13	3	2	1	2	2
C _{18:1}	17	25	24	23	10	18	29	14	21	21	27	24	29	26	25	29
C _{18:0}	2	2	1	2	1	7	3	1	1	4	11			2	1	2
TBSA	25	20	21	13	20	30	17	26	19	29	3	17	15	19	17	13

Hutton, 1980; Huß *et al.*, 1983) using a Gilford System model 2527-R thermoprogrammer and plotter. Renaturation rates were calculated using the TRANSFER.BAS program (Jahnke, 1992).

The ae2 editor (Maidak *et al.*, 1994) was used to align the 16S rDNA sequence of strain Kb2^T against the 16S rDNA sequences of the *Gordonia* type strains available from the public databases. Pairwise evolutionary distances were computed using the correction algorithm of Jukes & Cantor (1969). The least-squares distance method of De Soete (1983) was used in the construction of the phylogenetic dendrogram from the distance matrices.

Strain Kb2^T produced very short elementary branching hyphae which disintegrated into rod/coccus-like elements. It showed the typical rod-coccus growth cycle usually found among members of the genus *Gordonia* and related genera, e.g. *Rhodococcus* (Goodfellow, 1989, 1992; Goodfellow & Lechevalier, 1989). Strain Kb2^T produced pastel orange colonies that were both smooth and rough upon cultivation on Standard I nutrient agar. The smooth colonies were able to produce rough colonies, whereas the rough colonies remained rough. There were only slight differences in the fatty-acid composition of the rough and smooth variants (Table 2), but there were no differences in their physiological test results. The cells were Gram-positive, slightly acid-fast and non-motile. No spores could be detected.

The results of the physiological tests, obtained by means of the tetrazolium-[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] (MTT) reduction test in microtitre plates, revealed that strain Kb2^T was

able to utilize nine of the 32 carbon sources tested. None of the chromogenic substrates tested could be hydrolysed by strain Kb2^T (see Table 1). This utilization pattern did not match with any of the known *Gordonia* species.

The chemotaxonomic properties of strain Kb2^T were also consistent with its classification into the genus *Gordonia* (Klatte *et al.*, 1994; Stackebrandt *et al.*, 1988). Whole-cell hydrolysates of Kb2^T contained *meso*-diaminopimelic acid as the only diamino acid of the peptidoglycan, and arabinose plus galactose as the major cell-wall sugars (cell-wall chemotype IV, according to Lechevalier & Lechevalier, 1970). As expected for *Gordonia* spp. and related taxa, the sugars of the peptidoglycan were glycolated. MK-9(H₂) was the only menaquinone found in this strain. The polar lipids were composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and some unspecified glycolipids. This pattern matched quite well with those reported by Minnikin *et al.* (1977) for *Gordonia*.

The fatty-acid pattern was composed of straight-chain saturated and unsaturated fatty acids plus tuberculostearic acid. Although this pattern is roughly the same in all members of the genus *Gordonia*, there are fine qualitative and quantitative differences that are species specific (Klatte, 1994) or even variant specific, as in the case of the smooth and rough variants of strain Kb2^T (as shown in Table 1).

Strain Kb2^T also synthesized a homologous series of mycolic acids ranging from C₅₆ to C₆₀, with C₅₆, C₅₈ and C₆₀ being the three principal mycolic acids. This chain length was in the range of the mycolic acids

Fig. 1. Phylogenetic dendrogram, obtained by distance-matrix analysis, showing the position of *Gordonia westfalica* DSM 44215^T among members of the genus *Gordonia*. Bar, 0.01 substitutions per nucleotide position.

expected for *Gordonia* species (C₄₆–C₆₆). It fell into the group of *Gordonia hydrophobica*, *Gordonia rubripertincta* and *Gordonia terrae*, which show a mycolic acid chain length of about C₅₄–C₆₀.

The almost complete 16S rDNA sequence of strain Kb2^T, consisting of 1478 nt, was compared to the members of the genus *Gordonia*. These organisms were determined to be the closest phylogenetic neighbours of Kb2^T. The 16S rDNA sequence of strain Kb2^T showed highest similarity with the 16S rDNA sequences of *Gordonia nitida* (Yoon *et al.*, 2000) and *Gordonia alkanivorans* (Kummer *et al.*, 1999) (99.8 and 99.7%, respectively). Intrageneric relationship data revealed similarity values ranging between 95.0 and 99.7% for Kb2^T. The 16S rDNA data indicated that strain Kb2^T belongs to the genus *Gordonia*, but gave no reliable evidence about the status of a novel species within this genus. However, a phylogenetic tree based on the 16S rDNA sequences shows the position of strain Kb2^T among other *Gordonia* species and another member of the *Corynebacterineae*, *Williamsia murale* (Fig. 1).

DNA relatedness studies performed between strain Kb2^T and *G. nitida* KCTC 0605BP^T and between

strain Kb2^T and *G. alkanivorans* DSM 44369^T resulted in re-association values of 46.0 and 60.6%, respectively. From these data it became evident that strain Kb2^T belonged to a distinct genomic species.

Based on the phenotypic and genotypic data, it is concluded that the rubber-degrading strain, which was isolated in Westfalia, Germany, merits species status within the genus *Gordonia*. We therefore propose the name *Gordonia westfalica* for strain Kb2^T (= DSM 44215^T = NRRL B-24152^T).

Description of *Gordonia westfalica* sp. nov.

Gordonia westfalica (west.fa'li.ca. M.L. fem. adj. *westfalica* from Westfalian, referring to the origin of the species, Westfalia, a geographical area in Germany).

Aerobic, Gram-positive, non-motile actinomycete, which forms a rudimentary mycelium that fragments into rod/coccus-like elements. Colonies pastel orange. Colony morphology of the type strain rough and flat with irregular margins. Upon primary isolation, convex colonies with smooth surface. Cells occur in aggregates when growing with squalene and in suspension when growing with L-rhamnose. The physio-

logical properties, as determined by MTT, can be found in Table 1. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, arabinose and galactose (cell-wall chemotype IV *sensu* Lechevalier & Lechevalier). Sugars of the peptidoglycan are glycolated. The only menaquinone is MK-9(H₂). The polar lipids are composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. The fatty-acid patterns of the rough and smooth variants are shown in Table 2. The principal mycolic acids have chain lengths of 56, 58 and 60 carbon atoms. Able to degrade and mineralize natural rubber substrates and synthetic *cis*-1,4-polyisoprene. Isolated from foul water held inside a deteriorated automobile tyre found on a farmer's field near Münster, Westfalia, Germany. The type strain is Kb2^T (= DSM 44215^T = NRRL B-24152^T).

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