Gordonia kroppenstedtii sp. nov., a phenol-degrading actinomycete isolated from a polluted stream

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A phenol-degrading actinomycete, strain NP8-5^T, was isolated from a polluted stream in Gumi, Korea. The strain was aerobic, Gram-positive-staining, non-motile and non-spore-forming, displayed a rod-coccus growth cycle, exhibited white opaque colonies on complex media and showed chemotaxonomic markers that were consistent with its classification in the genus *Gordonia*. Phylogenetic analysis, based on 16S rRNA gene sequencing, also showed that strain NP8-5^T belonged to the genus *Gordonia*, sharing the highest levels of sequence similarity with *Gordonia arii* IFM 10211^T, *G. hydrophobica* DSM 44015^T and *G. sinesedis* NCIMB 13802^T (96.4, 96.0 and 95.9 %, respectively) and forming a separate lineage within this genus. Combined phylogenetic and phenotypic data supported the conclusion that strain NP8-5^T represents a novel species of the genus *Gordonia*, for which the name *Gordonia kroppenstedtii* sp. nov. is proposed. The type strain is NP8-5^T (=KCTC 19360^T =DSM 45133^T).

At the time of writing, the suborder *Corynebacterineae* within the order *Actinomycetales* comprises 13 genera of mycolic-acid-containing actinomycetes. There are two exceptional species, *Corynebacterium amycolatum* and *Turicella otitidis*, which are known to lack mycolic acids (Goodfellow & Maldonado, 2006; Pascual *et al.*, 1995; Soddell *et al.*, 2006). As well as mycolic acids, other chemotaxonomic markers are common in members of the *Corynebacterineae*, such as *meso*-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates, menaquinones with a partly saturated isoprenoid side chain and a fatty acid pattern composed of unbranched saturated and unsaturated fatty acids plus tuberculostearic acid. Fine differences in chemotaxonomic markers can be used for genus and species differentiation within this suborder (Goodfellow & Maldonado, 2006; Linos *et al.*, 1999; Soddell *et al.*, 2006).

Members of the genus *Gordonia* were originally regarded as opportunistic pathogens that were isolated from sputum of humans suffering from pulmonary diseases, e.g. *Gordonia bronchialis*, *Gordonia aichiensis* and *Gordonia sputi*. Since

then, a number of species have been isolated as a result of their abilities to degrade xenobiotics, environmental pollutants or slowly biodegradable natural polymers, e.g. benzothiophene and dibenzothiophene by *Gordonia desulfuricans* and *Gordonia amicalis*, alkanes by *Gordonia alkanivorans*, malodorous animal emissions such as aldehydes and ketones by *Gordonia hydrophobica* and *Gordonia hirsuta*, 3-ethylpyridine and 3-methylpyridine by *Gordonia nitida*, polyisoprenoids and synthetic *cis*-1,4-polyisoprene by *Gordonia polyisoprenivorans* and *Gordonia westfalica*, nitrile compounds by *Gordonia namibiensis* and hydrocarbons such as liquid paraffin by *Gordonia paraffinivorans* (Kim *et al.*, 2003; Arenskötter *et al.*, 2004). At the time of writing, the genus *Gordonia* comprises 25 recognized species. Recently, *Gordonia nitida* was reported to be a later synonym of *Gordonia alkanivorans* (Arenskötter *et al.*, 2005).

During the screening of phenol-degrading bacteria from a polluted stream near the Gumi industrial complex in Korea, strain NP8-5^T was recovered from white colonies on R2A agar (Difco) at 25 °C. On the basis of preliminary 16S rRNA gene sequence comparisons, the strain was found to belong to the genus *Gordonia*, but it showed low levels of 16S rRNA gene sequence similarity (<97 %) to all of the recognized species of this genus. To elucidate the taxonomic status of strain NP8-5^T further, it was subjected to a polyphasic investigation.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NP8-5^T is AM883151.

Chemotaxonomic characteristics of strain NP8-5^T and closely related *Gordonia* type strains are available as supplementary material with the online version of this paper.

A sediment sample from a stream was initially enriched with 100 p.p.m. (1.06 mM) phenol and the enriched culture was serially diluted in 0.85 % NaCl solution. Aliquots of each serial dilution were spread on R2A agar and incubated at 25 °C for 7 days. A white colony, designated NP8-5^T, was isolated. The isolate was sub-cultured onto R2A agar at 30 °C for 4 days. For most experiments, strain NP8-5^T was cultivated on tryptic soy agar (TSA) or broth (Difco) at 30 °C for 3 days. For analysis of fatty acid and mycolic acid compositions, strain NP8-5^T was cultivated on TSA at 30 °C for 4 days. *Gordonia araii* KCTC 19354^T, *G. bronchialis* KCTC 3076^T, *G. hydrophobica* KCTC 9814^T, *G. namibiensis* KCTC 9952^T, *G. sihwensis* KCTC 19385^T, *G. sinesedis* KCTC 19353^T and *G. westfalica* KCTC 9954^T, grown under identical conditions, were used as reference strains for fatty acid and mycolic acid analyses and for other phenotypic tests.

The Gram reaction was performed as described by Gerhardt *et al.* (1994). Cell morphology and motility were observed under a light microscope ($\times 1500$ magnification; Nikon Optiphot-2) with cells grown for 1–7 days. Oxidase activity was tested using Bactident-Oxidase strips (Merck) and catalase activity was tested using 3 % H₂O₂. Growth was investigated at 5, 10, 15, 20, 25, 30, 37 and 42 °C, in the presence of 1, 2, 3, 5, 6.5 and 10 % NaCl and at pH 5–11 in increments of 1 pH unit. The following biological buffers were used to adjust the pH: Na₂HPO₄/NaH₂PO₄ for pH 5–7 and Na₂CO₃/NaHCO₃ for pH 8–11 (Gomori, 1955; Bates & Bower, 1956). Hydrolysis of casein and starch was tested on casein agar and starch agar (Difco). Carbon source utilization and quantitative enzyme tests were performed in standard microtitre plates as described previously (Bendinger *et al.*, 1995; Linos *et al.*, 1999). Acid production tests and additional physiological tests were performed using API 20E and API 20NE galleries according to the instructions of the manufacturer (bioMérieux).

Fatty acid methyl esters and mycolic acid trimethylsilyl ethers were prepared and analysed as described by Klatte *et al.* (1994) by using the standard microbial identification system (MIDI) for automated GC analyses (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by using TLC on Kieselgel 60 F₂₅₄ plates (20 × 20 cm, 0.5 mm thickness; Merck) using petroleum ether/diethyl ether (9:1, v/v) as the solvent. Quinones were identified using reversed-phase HPLC analysis, as described by Shin *et al.* (1996). Amino acid and sugar analyses of whole-cell hydrolysates followed the procedures of Stanek & Roberts (1974). The murein acyl type was determined by the colorimetric method of Uchida *et al.* (1999). Polar lipids were extracted, examined by two-dimensional TLC and identified by using published procedures (Minnikin *et al.*, 1977).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR

product were carried out according to Rainey *et al.* (1996). The 16S rRNA gene sequence was aligned with published sequences retrieved from EMBL by using CLUSTAL X (Thompson *et al.*, 1997) and edited via the BioEdit program (Hall, 1999). Phylogenetic trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods; distances were estimated by the method of Jukes & Cantor (1969) by using MEGA version 3.0 (Kumar *et al.*, 2004). The resultant topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resampled datasets. DNA G + C contents were determined by HPLC, following hydrolysis, as described by Tamaoka & Komagata (1984) and non-methylated λ DNA (Sigma) was used as a standard.

Strain NP8-5^T was an aerobic, catalase-positive, non-spore-forming actinomycete and displayed the typical rod-coccus growth cycle that is usually found among members of the genus *Gordonia* and related taxa, e.g. *Rhodococcus* (Goodfellow & Maldonado, 2006). On TSA, the colonies were white, rough, umbonate and irregular, whereas upon primary isolation on R2A agar, they were white, glossy, convex and circular.

Analysis of the almost-complete 16S rRNA gene sequence (1463 nt) of strain NP8-5^T indicated its relationship with the genus *Gordonia*; the sequence similarity ranged from 93.9 to 99.7 % among the type strains of recognized species of this genus. Strains NP8-5^T showed the highest 16S rRNA gene sequence similarity, with values of 96.4 and 96.0 %, to *G. araii* IFM 10211^T and *G. hydrophobica* DSM 44015^T, respectively, and showed 95.9 % similarity to each of *G. sinesedis* NCIMB 13802^T, *G. bronchialis* DSM 43247^T, *G. namibiensis* NAM-BN063A^T, *G. sihwensis* DSM 44576^T and *G. westfalica* KB2^T. The levels of 16S rRNA gene sequence similarity with all *Gordonia* type strains were 94.1–96.4 %, below the level that is indicative of relatedness at the species level (Stackebrandt & Goebel, 1994). Also, strain NP8-5^T occupied a quite distinct position in the phylogenetic tree, forming a stable branch with all recognized *Gordonia* species within the suborder *Corynebacterineae* (Fig. 1), which supported the conclusion that the organism merits recognition as the type strain of a novel species within the genus *Gordonia*.

The physiological, biochemical and chemotaxonomic characteristics of strain NP8-5^T are given in the species description and those that distinguish the organism from closely related *Gordonia* type strains are listed in Table 1. Strain NP8-5^T had a fatty acid pattern similar to those of related taxa, except for the lack of 17:1 ω 8c (Supplementary Table S1, available in IJSEM Online), and synthesized a series of mycolic acids that ranged from 56 to 60 carbon atoms in length, with 58, 59 and 60 as the three principal mycolic acids. This chain-length was in the range expected for *Gordonia* species (48–70), and the pattern was most similar to that of *G. hydrophobica* (Supplementary Table S1).

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, constructed with the neighbour-joining method, showing the position of strain NP8-5^T among all recognized *Gordonia* species and representative species within the suborder *Corynebacterineae*. Percentages at nodes are levels of bootstrap support ($\geq 50\%$) based on 1000 resamplings. Filled circles indicate nodes that are also found with the maximum-parsimony algorithm. Bar, 1 substitution per 100 nucleotide positions.

On the basis of phylogenetic and phenotypic data, strain NP8-5^T represents a novel species of the genus *Gordonia*, for which the name *Gordonia kroppenstedtii* sp. nov. is proposed.

Description of *Gordonia kroppenstedtii* sp. nov.

Gordonia kroppenstedtii (krop.pen.sted'ti.i. N.L. gen. n. *kroppenstedtii* of Kroppenstedt, in honour of Reiner Michael Kroppenstedt, a German microbiologist, for his enormous contributions to the taxonomy of actinomycetes).

Cells are aerobic, Gram-positive-staining, non-motile, non-spore-forming rods and display a rod-coccus growth cycle. Catalase-positive but oxidase-negative. Growth occurs at 10–30 °C (optimum, 25–30 °C) and at pH 6–9 (optimum, pH 6–7). Growth occurs in the presence of 1–6.5 % NaCl, but not with 10 %. Colonies are white, opaque and polymorphic on complex media. Indole and H₂S are not produced. Nitrate is not reduced. Aesculin is hydrolysed, but casein, gelatin, starch, *p*-nitrophenyl β -D-xyloside, *p*-nitrophenyl phosphorylcholine and 2-deoxythymidine-5'-*p*-nitrophenyl phosphate are not. β -Galactosidase (weakly) and urease are produced, but arginine dihydrolase, ornithine decarboxylase and lysine

decarboxylase are not. Voges–Proskauer test is positive. Acid is produced from amygdalin and L-arabinose, but not from D-glucose, *myo*-inositol, D-mannitol, melibiose, L-rhamnose, D-sorbitol or sucrose. L-Alanine, 4-aminobutyrate, caprate, glucarate, 2-hydroxyvalerate, *myo*-inositol, L-leucine, phenylacetate, L-proline, D-ribose, L-serine, succinate, sucrose, turanose and L-valine are utilized as sole carbon sources, but acetamide, *N*-acetyl-D-glucosamine, D-arabitol, L-aspartate, benzoate, citrate, D-galactose, gluconate, D-glucosaminic acid, 3-hydroxybenzoate, 4-hydroxybenzoate, 2-oxoglutarate, pimelate, putrescine, quinate, L-rhamnose and tyramine are not. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, arabinose and galactose. Peptidoglycan sugars are glycolated. Predominant menaquinone is MK-9(H₂). Polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides. Major fatty acids are 16:1 ω 7*c*, 16:0, 18:1 ω 9*c* and 10-methyl 18:0. Principal mycolic acids have a chain-length of 58–60 carbon atoms. The DNA G + C content of the type strain is 68.5 mol%.

The type strain, NP8-5^T (=KCTC 19360^T =DSM 45133^T), was isolated from a polluted stream in Gumi, Korea.

Table 1. Physiological and biochemical characteristics that differentiate strain NP8-5^T from type strains of closely related *Gordonia* species

Strains: 1, *G. kroppenstedtii* sp. nov. NP8-5^T; 2, *G. sinesedis* KCTC 19353^T; 3, *G. araii* KCTC 19354^T; 4, *G. hydrophobica* KCTC 9814^T; 5, *G. bronchialis* KCTC 3076^T; 6, *G. namibiensis* KCTC 9952^T; 7, *G. silhwensis* KCTC 19385^T; 8, *G. westfalica* KCTC 9954^T. Data were taken from Kim *et al.* (2003), Linos *et al.* (1999, 2002) and this study. +, Positive; (+), weakly positive; –, negative; pNP, *p*-nitrophenyl.

Characteristic	1	2	3	4	5	6	7	8
Colony colour*	WH	BE	WH–BE	WH–T	BR	O	WH–T	PO
Nitrate reduction	–	–	+	+	+	–	+	+
β -Galactosidase	(+)	–	+	–	(+)	–	–	–
Oxidase	–	–	–	+	–	–	(+)	–
Urease	+	+	–	–	+	+	–	+
Growth with:								
6.5 % (w/v) NaCl	+	+	–	+	+	+	+	+
10 % (w/v) NaCl	–	+	–	+	–	+	+	(+)
Utilization of:								
D-Galactose	–	–	–	+	+	+	+	–
L-Rhamnose	–	–	–	+	+	–	–	–
D-Ribose	+	+	–	+	–	+	–	+
Sucrose	+	+	–	+	+	+	+	+
Turanose	+	+	–	–	+	–	+	–
D-Arabitol	–	–	–	–	–	+	+	+
<i>myo</i> -Inositol	+	+	–	+	+	–	–	–
<i>N</i> -Acetyl-D-glucosamine	–	+	–	–	+	–	+	–
Glucarate	+	–	–	+	–	–	–	–
Gluconate	–	–	–	–	+	+	+	–
D-Glucosaminic acid	–	–	–	–	–	–	+	+
Caprate	+	+	+	–	+	–	+	+
Citrate	–	–	–	+	–	–	+	+
4-Aminobutyrate	+	–	–	–	–	–	+	–
2-Hydroxyvalerate	+	–	–	+	–	–	+	–
2-Oxoglutarate	–	–	–	+	–	–	+	+
Pimelate	–	–	–	–	–	–	+	–
Succinate	+	+	–	+	+	+	–	–
Benzoate	–	–	–	–	–	–	–	+
4-Hydroxybenzoate	–	–	–	–	+	+	–	–
Phenylacetate	+	–	–	–	+	–	+	–
Quinate	–	–	–	–	–	+	–	–
L-Alanine	+	+	–	+	+	+	–	–
L-Aspartate	–	–	–	–	–	–	–	+
L-Leucine	+	+	–	+	+	–	+	–
L-Proline	+	–	–	+	–	–	+	–
L-Serine	+	–	–	+	–	+	+	–
L-Valine	+	+	–	+	+	–	+	–
Tyramine	–	+	+	–	–	+	–	–
Acetamide	–	–	–	+	+	–	–	–
Hydrolysis of:								
Aesculin	+	–	+	+	–	+	–	+
Gelatin	–	(+)	–	–	(+)	–	–	–
Starch	–	+	–	–	+	–	–	–
pNP β -D-xyloside	–	–	+	–	–	–	–	–
pNP phosphorylcholine	–	+	–	+	–	–	+	–
2-Deoxythymidine-5'-pNP phosphate	–	–	–	+	–	–	+	–
DNA G + C content (mol%)	68.5	69.2	68.4	69.0	65.9	67.8	65.8	67.6

*BE, Beige; BR, brown; O, orange; PO, pastel orange; T, tan; WH, white.

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References

- Arenskötter, M., Bröker, D. & Steinbüchel, A. (2004). Biology of the metabolically diverse genus *Gordonia*. *Appl Environ Microbiol* **70**, 3195–3204.
- Arenskötter, M., Linos, A., Schumann, P., Kroppenstedt, R. M. & Steinbüchel, A. (2005). *Gordonia nitida* Yoon *et al.* 2000 is a later synonym of *Gordonia alkanivorans* Kummer *et al.* 1999. *Int J Syst Evol Microbiol* **55**, 695–697.
- Bates, R. G. & Bower, V. E. (1956). Alkaline solutions for pH control. *Anal Chem* **28**, 1322–1324.
- Bendinger, B., Rainey, F. A., Kroppenstedt, R. M., Moormann, M. & Klatte, S. (1995). *Gordonia hydrophobica* sp. nov., isolated from biofilters for waste gas treatment. *Int J Syst Bacteriol* **45**, 544–548.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Gomori, G. (1955). Preparation of buffers for use in enzyme studies. *Methods Enzymol* **1**, 138–146.
- Goodfellow, M. & Maldonado, L. A. (2006). The families Dietziaceae, Gordoniaceae, Nocardiaceae and Tsukamurellaceae. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 3, pp. 843–888. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kim, K. K., Lee, C. S., Kroppenstedt, R. M., Stackebrandt, E. & Lee, S. T. (2003). *Gordonia sihwensis* sp. nov., a novel nitrate-reducing bacterium from a wastewater-treatment bioreactor. *Int J Syst Evol Microbiol* **53**, 1427–1433.
- Klatte, S., Rainey, F. A. & Kroppenstedt, R. M. (1994). Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordonia* as *Gordonia aichiensis* comb. nov. and *Gordonia amarae* comb. nov. *Int J Syst Bacteriol* **44**, 769–773.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Linos, A., Steinbüchel, A., Spröer, C. & Kroppenstedt, R. M. (1999). *Gordonia polyisoprenivorans* sp. nov., a rubber-degrading actinomycete isolated from an automobile tyre. *Int J Syst Bacteriol* **49**, 1785–1791.
- Linos, A., Berekaa, M. M., Steinbüchel, A., Kim, K. K., Spröer, C. & Kroppenstedt, R. M. (2002). *Gordonia westfalica* sp. nov., a novel rubber-degrading actinomycete. *Int J Syst Evol Microbiol* **52**, 1133–1139.
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Pascual, C., Lawson, P. A., Farrow, J. A. E., Gimenez, M. N. & Collins, M. D. (1995). Phylogenetic analysis of the genus *Corynebacterium* based on 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 724–728.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method; a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shin, Y. K., Lee, J.-S., Chun, C. O., Kim, H.-J. & Park, Y.-H. (1996). Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^T. *J Microbiol Biotechnol* **6**, 68–69.
- Soddell, J. A., Stainsby, F. M., Eales, K. L., Kroppenstedt, R. M., Seviour, R. J. & Goodfellow, M. (2006). *Millisia brevis* gen. nov., sp. nov., an actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* **56**, 739–744.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. *Appl Microbiol* **28**, 226–231.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Uchida, K., Kudo, T., Suzuki, K. & Nagase, T. (1999). A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49–56.