Gordonia paraffinivorans sp. nov., a hydrocarbon-degrading actinomycete isolated from an oil-producing well

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The taxonomic position of an actinomycete, strain HD321^T, isolated from an oil-producing well of Daqing oilfield, was clarified using a polyphasic taxonomic approach. The strain possessed cell-wall chemotype IV, MK-9(H₂) as the predominant menaquinone, relatively long-chain mycolic acids (52–62 carbon atoms) of the *Gordonia* type, straight-chain saturated and monounsaturated fatty acids and tuberculostearic acid. The G + C content of the DNA was 66 mol%. 16S rDNA analyses as well as chemotaxonomic and physiological properties indicated that strain HD321^T represents a novel species within the genus *Gordonia*, for which the name *Gordonia paraffinivorans* sp. nov. is proposed; the type strain is HD321^T (=AS 4.1730^T=DSM 44604^T).

The genus *Gordonia* belongs phylogenetically to the sub-order *Corynebacterineae*, the mycolic acid group within the order *Actinomycetales* (Stackebrandt *et al.*, 1997). The species of *Gordonia* show considerable metabolic diversity and are capable of degrading toxic environment-contaminating compounds, as exemplified by the isolation of strains possessing hydrocarbon-oxidizing, rubber-degrading, aromatic-desulphurizing and 3-ethylpyridine-degrading pathways (Kummer *et al.*, 1999; Linos *et al.*, 2002; Kim *et al.*, 1999; Gilbert *et al.*, 1998; Rhee *et al.*, 1998; Yoon *et al.*, 2000). From the point of view of bioremediation, the genus *Gordonia* is a very important taxon. Some species have been used for decontamination of polluted soils and waters (Bell *et al.*, 1998). The members of the genus *Gordonia* are widely distributed in various environments such as soil, activated sludge, biofilm and the mangrove rhizosphere (Tsukamura, 1971; Lechevalier & Lechevalier, 1974; Bending *et al.*, 1995; Takeuchi & Hatano, 1998; Linos *et al.*, 1999; Kummer *et al.*, 1999). There are no reports of *Gordonia* species in the producing-well water of oilfields, which has low levels of nutrients, consisting mainly of straight-chain and aromatic hydrocarbons.

In the course of an investigation of the microflora in the Daqing oilfield formation, we isolated several hydrocarbon-degrading strains from well-bore water samples (Nazina *et al.*, 2000). A hydrocarbon-degrading bacterium, designated strain HD321^T, appeared to represent a novel taxon

based on preliminary investigations of its phenotypic and phylogenetic characteristics. Here, we describe the physiological, chemotaxonomic and phylogenetic characteristics of strain HD321^T and propose a novel species of the genus *Gordonia*, *Gordonia paraffinivorans* sp. nov., for this strain.

Strain HD321^T was isolated from a water sample collected in late May 1996 from a producing well of Daqing oilfield, China. The water sample was inoculated into 100 ml minimal salts medium containing (l⁻¹) 1.0 g NH₄Cl, 0.5 g KH₂PO₄, 0.75 g Na₂HPO₄·12H₂O, 0.2 g MgSO₄·7H₂O, 0.02 g CaCl₂ and 10 ml trace-element solution (Lee *et al.*, 1991), supplemented with 5 ml liquid paraffin. This medium was incubated at 37 °C on a rotary shaker at 250 r.p.m. After the liquid paraffin was emulsified by growth of micro-organisms, a portion of the cultivation medium was diluted and plated on nutrient agar containing (l⁻¹) 10 g tryptone, 3 g beef extract, 5 g NaCl and 15 g agar. Strain HD321^T was isolated on nutrient agar and showed orange-red colonies on glucose potato agar (GPA).

Cell morphology was determined by phase-contrast and electron microscopy. Mobility was determined by the hanging-drop method. The optimal conditions for growth were determined in nutrient broth with 0–14% NaCl, pH 5–10 and at 4–60 °C. Carbon source utilization was tested in the medium described previously (Kämpfer *et al.*, 1990). Controls were grown without substrate addition. The ability to grow in the presence of 0.1% oleic acid and 0.001% zinc chloride was tested as described previously (Kim *et al.*, 1999). The methods used for biochemical tests were described previously (Smibert & Krieg, 1981). The isomeric form of diaminopimelic acid in the cell wall was

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analysed using TLC according to the method of Komagata & Suzuki (1987). The sugars of the cell wall were analysed as described previously (Saddler *et al.*, 1991). Menaquinones were extracted and purified from freeze-dried cells using the method of Collins (1985) and determined by reversed-phase HPLC. Fatty acid analysis was performed using standard methods and compared to the database of fatty acids in the MIDI Sherlock Microbial Identification System (Microbial ID). Mycolic acid was determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). Genomic DNA of the strain was prepared by the method of Marmur (1961) and the purity was checked spectrometrically. The G + C content of the DNA was determined by the thermal denaturation method (Marmur & Doty,

1962). The methods used for PCR amplification of 16S rRNA gene, sequencing of the PCR products and determination of the phylogenetic position were described previously (Zhang *et al.*, 2002). The reference strains used in sequence comparison are shown in Fig. 1.

The novel strain HD321^T had morphological and biochemical properties that were consistent with its classification in the genus *Gordonia* (Stackebrandt *et al.*, 1988). The strain was a Gram-positive, non-acid-fast, non-motile, rod-shaped and strictly aerobic bacterium. Cells (0.3–0.5 × 1–2 µm) often occurred singly or in a typical coryneform V-shape. Catalase and urease were positive. Oxidase was negative. Gelatin and cellulose were not hydrolysed. Strain HD321^T grew well at 30–37 °C and very slowly at 20 and 45 °C. Growth

Table 1. Phenotypic characteristics that separate strain HD321^T from the type strains of other *Gordonia* species

Strains: 1, HD321^T; 2, *Gordonia aichiensis* DSM 43978^T; 3, *Gordonia alkanivorans* DSM 44369^T; 4, *Gordonia amarae* DSM 43392^T; 5, *Gordonia amicalis* DSM 44461^T; 6, *Gordonia bronchialis* DSM 43247^T; 7, *Gordonia desulfuricans* DSM 44462^T; 8, *Gordonia hirsuta* DSM 44140^T; 9, *Gordonia hydrophobica* DSM 44015^T; 10, *Gordonia namibiensis* DSM 44568^T; 11, *Gordonia nitida* DSM 44499^T; 12, *Gordonia polyisoprenivorans* DSM 44302^T; 13, *Gordonia rhizosphaera* NBRC 16068^T; 14, *Gordonia rubripertincta* DSM 43197^T; 15, *Gordonia sputi* DSM 43896^T; 16, *Gordonia terrae* DSM 43249^T; 17, *Gordonia westfalica* DSM 44215^T. Data were taken from this study and from Brandão *et al.* (2001), Linos *et al.* (2002) and Yoon *et al.* (2000).

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Colony colour*	O/R	P/O	P/O	T/W	R	B	P	W/LY	T/W	O	O	O	P/O	O/R	P	P/O	PO
Assimilation of:																	
D-Galactose	+	+	–	–	+	–	+	+	+	+	–	+	–	+	+	+	–
D-Arabitol	–	–	–	+	–	–	–	–	–	+	–	+	–	+	+	+	+
D-Cellobiose	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–
L-Rhamnose	–	–	–	–	+	+	–	–	–	ND	–	+	+	+	–	+	–
D-Ribose	–	–	–	+	+	–	+	–	–	+	+	–	–	+	–	–	+
Sucrose	+	–	+	+	+	+	+	–	+	–	+	+	–	+	–	+	+
Succinate	–	–	+	–	+	+	–	–	+	+	–	–	–	–	+	–	–
Glycerol	+	+	+	+	+	+	+	+	+	+	+	–	ND	+	+	+	ND
L-Alanine	+	+	–	–	+	+	+	–	–	–	+	+	+	+	–	–	–
L-Leucine	+	–	+	–	+	+	+	–	+	–	–	–	–	–	–	–	–
L-Proline	+	–	+	+	–	–	+	–	+	–	+	+	–	–	–	–	–
L-Valine	+	–	–	+	–	+	–	–	+	–	–	+	–	–	–	–	–
Decomposition of:																	
Hypoxanthine	–	–	–	+	–	–	+	–	+	+	–	–	–	+	+	+	ND
Xanthine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	ND
Tyrosine	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	ND
Starch	–	–	+	+	+	+	+	+	–	–	–	+	+	+	+	+	ND
Tween 80	+	+	+	–	–	–	+	+	+	–	+	+	–	–	–	–	ND
Uric acid	–	+	–	+	–	+	+	–	+	+	+	+	+	+	+	+	ND
Hydrolysis of:																	
Aesculin	+	–	–	+	–	–	–	–	+	+	+	–	–	–	+	+	ND
Allantoin	+	–	–	+	–	–	–	–	+	+	–	–	–	–	–	–	ND
Arbutin	–	+	–	+	–	–	–	–	+	–	–	–	–	+	+	+	ND
Urea	+	+	+	+	–	+	+	–	–	+	+	+	–	+	+	+	ND
Nitrate reduction	–	+	+	–	–	+	+	+	+	–	–	–	–	–	+	+	ND

*B, Brown; LY, light yellow; O, orange; P, pink; PO, pastel orange; R, red; T, tan; W, white.

ND, Not determined.

occurred in medium containing 0.5–7 % (w/v) NaCl and at pH 5.5–9.5. Some other biochemical properties useful for identifying and differentiating strain HD321^T and type strains of other species of the genus *Gordonia* are represented in Table 1 and in the description of the species.

The chemotaxonomic properties of strain HD321^T were consistent with the chemotaxonomic markers of the genus *Gordonia* (Chun *et al.*, 1997; Takeuchi & Hatano, 1998; Stackebrandt *et al.*, 1988). The cell wall of strain HD 321^T contained *meso*-diaminopimelic acid as the only diamino acid and arabinose and galactose as the major cell-wall sugars, indicating that the wall chemotype was type IV (Lechevalier & Lechevalier, 1970). The predominant menaquinone was MK-9(H₂). The fatty acid profile of strain HD321^T was composed of C_{16:0} (25.8 %), tuberculostearic acid (10-methyl-C_{18:0}) (22.4 %), C_{18:1ω9c} (20.5 %), summed feature 4 (C_{16:1ω7c} and/or i-C_{15:0} 2-OH) (11.5 %), C_{18:0} (9.3 %), C_{14:0} (2.7 %), C_{17:0} (2.2 %), C_{17:1ω8c} (1.6 %) and C_{20:0} (1.2 %). Mycolic acids ranged from 52 to 62 carbon atoms (2.8 % C₅₂, 6.7 % C₅₄, 25.2 % C₅₆, 2.5 % C₅₇, 41 % C₅₈, 1 % C₅₉, 19.1 % C₆₀, 1.7 % C₆₂), with C₅₆ and C₅₈ being the principal mycolic acids. The G + C content of the DNA was 66.0 mol%. Fine qualitative and quantitative differences in fatty acid patterns and mycolic acid chain lengths could distinguish strain HD321^T from type strains of other species of the genus *Gordonia*.

The 16S rDNA of strain HD321^T was amplified by PCR and an almost-complete nucleotide sequence (1472 bp) was determined by direct sequencing. The phylogenetic tree showed that strain HD321^T was in the cluster comprising members of the genus *Gordonia* and formed a monophyletic clade (Fig. 1). The 16S rDNA sequence similarities between strain HD321^T and the type strains of other *Gordonia* species with validly published names were 95.3–97.6 %. The highest level of sequence similarity (97.6 %) was to *Gordonia amicalis* and *Gordonia rubripertincta*. These values and the results shown in the phylogenetic tree indicate that strain HD321^T represents a novel member of the genus *Gordonia* (Stackebrandt & Goebel, 1994). Because of the ability of the strain to use paraffin as a carbon source, the name *Gordonia paraffinivorans* sp. nov. is proposed.

Description of *Gordonia paraffinivorans* sp. nov.

Gordonia paraffinivorans (pa.raff.fi.ni.vo'rans. N.L. n. *paraffina* paraffin; L. part. adj. *vorans* devouring; N.L. part. adj. *paraffinivorans* paraffin-devouring, referring to the ability to degrade paraffin).

Aerobic, Gram-positive, non-motile, short rod-shaped bacterium (0.3–0.5 × 1–2 µm); orange-red colonies formed on GPA. No spore formation is observed. Oxidase-negative. Catalase- and urease-positive. Sucrose, maltose, glucose, D-fructose, D-galactose, D-mannose, inulin, dextrin, L-proline, L-alanine, L-leucine, glutamate and glycerol are used as sole sources of carbon for energy and growth. Does not

Fig. 1. Phylogenetic tree based on 16S rDNA sequences showing the position of strain HD321^T, *Gordonia* species and representatives of mycolic-acid-containing actinomycete taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 0.02 substitutions per nucleotide position.

utilize L-arabinose, D-raffinose, L-rhamnose, D-ribose, lactose, D-cellobiose, L-sorbose, D-xylose, sorbitol, D-mannitol, D-arabitol, aesculin, L-malic acid, succinic acid, L-lysine, oxalate, citrate or tartrate. Acids are not produced from glucose, sucrose, D-mannose, D-galactose or glycerol. Hypoxanthine, xanthine, tyrosine, starch, arbutin, uric acid, gelatin, cellulose and Tween 20 are not degraded. Aesculin, allantoin and Tween 80 are hydrolysed. Growth occurs in the presence of oleic acid (0.8 %, w/v) and zinc chloride (0.001 %, w/v). Positive for formation of H₂S; negative for production of indole and methyl red test. Nitrate is not reduced to nitrite. Cell wall contains *meso*-diaminopimelic acid, arabinose and galactose (cell-wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). The predominant menaquinone is MK-9(H₂). Mycolic acids have 52–62 carbon atoms, with C₅₆ and C₅₈ being the principal mycolic acids. The major fatty acids are C_{16:0}, C_{18:1ω9c} and 10-methyl-C_{18:0} (tuberculostearic acid). The G + C content of the DNA of the type strain is 66 mol%.

The type strain, strain HD321^T (=AS 4.1730^T=DSM 44604^T), was isolated from a producing-well water sample from Daqing oilfield, China.

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