Gordonia neofelifaecis sp. nov., a cholesterol side-chain-cleaving actinomycete isolated from the faeces of Neofelis nebulosa

Yuchang Liu, Fanglan Ge, Guiying Chen, Wei Li, Pingmei Ma, Guangxiang Zhang and Lihua Zeng

College of Life Science, Sichuan Normal University, Chengdu, 610061, PR China

A cholesterol side-chain-cleaving bacterial strain, AD-6\textsuperscript{T}, was isolated from fresh faeces of a clouded leopard (Neofelis nebulosa) and was studied using a polyphasic taxonomic approach. 16S rRNA gene sequence analysis showed that the novel strain formed a distinct subline within the genus Gordonia, its closest neighbours being the type strains of Gordonia cholesterolivorans, Gordonia sihwensis and Gordonia hydrophobica, with sequence similarity values of 98.2, 97.8 and 97.6\%, respectively. The gyrB gene sequence of strain AD-6\textsuperscript{T} exhibited similarities of 77–91\% with those of the type strains of recognized species of the genus Gordonia, being most similar to the type strains of G. sihwensis, G. hydrophobica and Gordonia hirsuta (91, 87 and 84\% similarity, respectively). The results of whole-cell fatty acid analyses and DNA–DNA relatedness data readily distinguished the new isolate from its nearest neighbours. Strain AD-6\textsuperscript{T} is therefore considered to represent a novel species of the genus Gordonia, for which the name Gordonia neofelifaecis sp. nov. is proposed. The type strain is AD-6\textsuperscript{T} (=NRRL B-59395\textsuperscript{T} = CCTCC AB-209144\textsuperscript{T}).

The genus Gordonia, first described by Tsukamura (1971), belongs to the suborder Corynebacterineae (Stackebrandt et al., 1997). At the time of writing, the genus comprises 31 recognized species. In recent years, members of the genus Gordonia have attracted much interest for their potential use in industrial and environmental biotechnology. Many species of the genus Gordonia are able to degrade toxic environmental pollutants, such as rubber by Gordonia westfalica (Linos et al., 2002), benzo thiophene and dibenzo thiophene by Gordonia desulfuricans and Gordonia amicalis (Kim et al., 1999, 2000), hydrocarbons by Gordonia paraffinivorans and Gordonia alk a nivorans (Kummer et al., 1999; Xue et al., 2003), phenol by Gordonia kroppenstedtii (Kim et al., 2009) and cholesterol by Gordonia cholesterolivorans (Drzyzga et al., 2009). Moreover, members of the genus Gordonia play an important role in bioremediation (Franzetti et al., 2009). Species of the genus Gordonia are widely distributed in various environments such as soil, activated sludge, biofilms, industrial wastewater, oil-producing wells, estuaries (le Roes et al., 2008) and the mangrove rhizosphere (Takeuchi & Hatano, 1998), and some are human pathogens (Kageyama et al., 2006). There are no reports of the isolation of species of the genus Gordonia from the faeces of healthy animals.

The aim of the present study was to clarify the taxonomic position of an actinomycete that transformed cholesterol to androsta-1,4-diene-3,17-dione (ADD) by side-chain cleavage. The novel strain was isolated from fresh faeces of a clouded leopard, Neofelis nebulosa, a medium-sized feline which is mainly restricted to southern China and mainland south-east Asia. Fresh faecal samples were collected directly from the anus of a healthy specimen of Neofelis nebulosa maintained at Chengdu Zoo, China, and were suspended in 10 ml minimal medium (MM, containing 0.1 % NaNO\textsubscript{3}, 0.025 % KH\textsubscript{2}PO\textsubscript{4}, 0.025 % MgSO\textsubscript{4}.7H\textsubscript{2}O, 0.0001 % FeSO\textsubscript{4} and 0.1 % cholesterol, pH 7.0). The suspension was shaken (200 r.p.m.) at 30 °C for about 3 days. Fresh isolation medium was inoculated with 200 μl of the culture and incubated under the same conditions. As previously described, four subcultures were prepared to dilute the faecal material (Ye et al., 2008). The bacterial suspension was then spread onto agar plates of the minimal medium in 200 μl volumes and incubated at 30 °C for 2–3 days. For screening bacteria capable of transforming cholesterol into ADD and AD (androsta-4-en-3,17-dione), well-developed colonies on agar plates were cultured in 10 ml liquid MM at 30 °C for 48 h with shaking. The bacterial culture liquid was then extracted by adding an equal volume of ethyl
acetate and was analysed by TLC on silica gel 60F-254 plates with benzene/ethyl acetate (10:1, v/v) as the solvent system, at room temperature. The ADD and AD yielded by cholesterol side-chain cleavage were detected as dark, UV-absorbing spots under UV illumination and were further identified by spectroscopic techniques, including 1H NMR, 13C NMR, IR and time-of-flight MS (this work will be reported elsewhere). One colony that was able to degrade the side chain of cholesterol was isolated and subcultured on a Luria–Bertani (LB) agar plate for 2 days at 30°C; this strain was designated AD-6T. On this medium the strain formed beige colonies. The organism was subjected to a study utilizing a polyphasic taxonomic approach, which showed that it represented a novel species of the genus Gordonia.

Extraction of chromosomal DNA, amplification of the 16S rRNA gene by PCR (employing a pair of universal 16S rRNA gene primers) (Hiraishi, 1992; Hiraishi et al., 1994) and purification of the PCR products were carried out according to the procedures described by Rainey et al. (1996). The purified PCR products were sequenced with an ABI PRISM 3730 DNA sequencer. A 16S rRNA gene sequence (1417 nt; 92% of the 16S rRNA gene sequence of Escherichia coli) was obtained from strain AD-6T. A BLAST search showed that the levels of 16S rRNA gene sequence similarity between strain AD-6T and the type strains of 13C NMR, IR and time-of-flight MS (this work will be reported elsewhere). One colony that was able to degrade the side chain of cholesterol was isolated and subcultured on a Luria–Bertani (LB) agar plate for 2 days at 30°C; this strain was designated AD-6T. On this medium the strain formed beige colonies. The organism was subjected to a study utilizing a polyphasic taxonomic approach, which showed that it represented a novel species of the genus Gordonia.

Analysis of the gyrB gene sequence of strain AD-6T (1219 bp) indicated that it belonged to the genus Gordonia, with sequence similarities ranging from 77 to 91% with those of recognized species of the genus. Strain AD-6T formed a distinct phyletic line in the neighbour-joining tree based on gyrB gene sequences (Fig. 2). The strain was most closely related to the type strains of G. sihwensis (91% gyrB gene sequence similarity), G. hydrophobica (87%) and G. hirsuta (84%), which corresponded to 110, 164 and 190 nt differences out of 1219 nt, respectively. The result supported the phylogenetic analysis based on 16S rRNA gene sequences.

DNA–DNA hybridization analysis was performed between strain AD-6T, G. sihwensis DSM 44576T and G. cholesterivorans DSM 45229T. Genomic DNA extraction was carried out by using the procedures described by Rainey et al. (1996). The renaturation rates of genomic fragments from pairs of strains were determined spectrophotometrically with a model 1601 UV spectrophotometer equipped with a thermoelectric cell temperature controller (Shimadzu) according to previously described methods (De Ley et al., 1970; Huß et al., 1983).

Strain AD-6T had morphological properties that were consistent with its classification in the genus Gordonia (Stackebrandt et al., 1988). Cells were Gram-positive, non-motile, non-spore-forming, aerobic rods (0.3–0.6 × 0.5–2.1 μm). Colonies on LB agar were beige and rough with irregular margins.

The results of physiological tests revealed that strain AD-6T was able to utilize a set of carbon sources that distinguished it from closely related type strains within the genus.
Gordonia (Table 1). The chemotaxonomic properties of strain AD-6T were also consistent with its classification in the genus Gordonia (Stackebrandt et al., 1988). TLC and HPLC patterns of mycolic acids from strain AD-6T were similar to those of Gordonia amarae DSM 43392T (data not shown). MK-9(H2) was the predominant menaquinone.

The major fatty acids of strain AD-6T were C16:1 (18 %), C16:0 (43 %), C18:1ω9c (10 %) and 10-methyl C18:0 (13 %) (see Supplementary Table S1 in IJSEM Online). Although this pattern, containing major amounts of C16:0 and tuberculostearic acids, was similar to those of other species of the genus Gordonia, there were some obvious differences between strain AD-6T and closely related species. Strain AD-6T contained a relatively large amount of fatty acid C16:1ω9c, whereas the type strains of G. sihwensis, G. cholesotrolivorans and G. hydrophoba showed a complete absence of this fatty acid (Supplementary Table S1). Strain AD-6T also contained C18:1ω9c, a fatty acid present as a major component only in G. rhizosphera IFO 16068T and G. alkaniivorans KCTC 0605BP T (Takeuchi & Hatano, 1998; Drzyzga et al., 2009). Although fatty acid C18:1ω9c has been detected by others in G. sihwensis DSM 44576T, it was not detected under the cultivation conditions used in this study. The type strains of most other recognized species of the genus Gordonia lack this fatty acid (Stackebrantd et al., 1988; Kim et al., 1999, 2000; Drzyzga et al., 2009).

DNA–DNA relatedness studies performed between strain AD-6T and G. sihwensis DSM 44576T and between strain AD-6T and G. cholesterolivorans DSM 45229T resulted in reassociation values of 45.5 ± 4.0 and 55.7 ± 4.0 %, respectively (values are means ± SD for four replications). From

**Fig. 1.** Unrooted neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences of strain AD-6T (1417 nt) and its closest relatives. Numbers at nodes are bootstrap percentages (from 1000 replications). Only values above 50% are given. Asterisks indicate branches of the tree that were also recovered using minimum-evolution and maximum-parsimony methods. Bar, 0.005 substitutions per nucleotide position. An expanded version of this tree including the type strains of all recognized species of the genus Gordonia is available in Supplementary Fig. S1.

**Fig. 2.** Unrooted neighbour-joining tree based on *gyrB* gene sequences of strain AD-6T (1219 bp) and its closest phylogenetic neighbours. Numbers at nodes are bootstrap percentages (from 1000 replications). Only values above 50% are given. Asterisks indicate clades that were conserved in the minimum-evolution and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.
Gordonia neofelifaecis

Gram-positive, aerobic, slightly acid-fast, non-motile rods

Description of gordonia neofelifaecis

Gordonia neofelifaecis (ne.o.fel.i.faec.is. N.L. fem. n. Neofelis a zoological genus name which encompasses the clouded leopard Neofelis nebulosa; L. n. faex faeces faeces; N.L. gen. n. neofelisfeci of the faeces of a clouded leopard).

Gram-positive, aerobic, slightly acid-fast, non-motile rods (0.3–0.6 × 0.5–2.1 μm). Colonies are beige and rough with irregular margins. Colonies are 3.0–5.0 mm in diameter after 3 days at 30 °C on LB and TSA plates. No soluble pigment is produced. When grown in liquid MM medium containing 1 g cholesterol l⁻¹, 87% of the cholesterol is transformed to ADD and 3% to AD. The optimum temperature for growth is 30 °C. Utilizes 4-aminobutyrate, D-galactose, L- leucine, L-serine and L-rhamnose as carbon sources, but not benzoate, caprate, citrate, gluconate, glucosaminic acid, phenylacetate or L-valine. MK-9(H2) is the predominant menaquinone. Major fatty acids are C₁₆:1 C₁₆:0, C₁₈:1ω9c and 10-methyl C₁₈:0.

The type strain, AD-6ᵀ ( = NRRL B-59395ᵀ = CCTCC AB-209144ᵀ), was isolated from fresh faeces of a specimen of Neofelis nebulosa maintained at Chengdu Zoo, China.

Acknowledgements

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Table 1. Differential carbon source utilization patterns between strain AD-6ᵀ and the type strains of closely related species of the genus Gordonia

<table>
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<tr>
<th>Carbon source</th>
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<th>2</th>
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<th>4</th>
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<tr>
<td>4-Aminobutyrate</td>
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<td>Benzoate</td>
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<td>Caprate</td>
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<td>+</td>
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<tr>
<td>Citrate</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>-</td>
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<td>Glucarate</td>
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<td>Gluconate</td>
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<td>D-Glucosaminic acid</td>
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<tr>
<td>L-Leucine</td>
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<td>Phenylacetate</td>
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<td>L-Valine</td>
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these data it was evident that strain AD-6ᵀ represented a species distinct from G. sihwensis and G. cholesterolivorans.

Based on the genotypic and phenotypic data presented, it was concluded that strain AD-6ᵀ represents a novel species of the genus Gordonia, for which the name Gordonia neofelifaecis sp. nov. is proposed.

References


