**Gordonia amicalis** sp. nov., a novel dibenzothiophene-desulphurizing actinomycete

Seung Bum Kim,¹ Roselyn Brown,¹ Christopher Oldfield,² Steven C. Gilbert,² Sergei Iliarionov³ and Michael Goodfellow¹

Author for correspondence: Michael Goodfellow. Tel: +44 191 222 7706. Fax: +44 191 222 5228. e-mail: m.goodfellow@ncl.ac.uk

The taxonomic position of a dibenzothiophene-desulphurizing soil actinomycete was established using a polyphasic taxonomic approach. The organism, strain IEGM⁷, was shown to have chemical and morphological properties typical of members of the genus *Gordonia*. The tested strain formed a distinct phylectic line within the evolutionary radiation occupied by the genus *Gordonia*, with *Gordonia alkanivorans* DSM 44369⁷, *Gordonia desulfuricans* NCIMB 4081⁶ and *Gordonia rubropertincta* DSM 4319⁷ as the most closely related organisms. Strain IEGM⁷ has a range of phenotypic properties that distinguish it from representatives of all of the validly described species of *Gordonia*. It was also sharply distinguished from the type strains of *Gordonia desulfuricans* and *Gordonia rubropertincta* on the basis of DNA–DNA relatedness data. The combined genotypic and phenotypic data show that strain IEGM⁷ merits recognition as a new species of *Gordonia*. The name proposed for the new species is *Gordonia amicalis*; the type strain is IEGM⁷ (≡ DSM 44461⁷ = KCTC 9899⁷).

**Keywords:** *Gordonia*, polyphasic taxonomy, dibenzothiophene, desulphurization

---

**INTRODUCTION**

The genus *Gordonia* (formerly *Gordona*) has had a short, but eventful, taxonomic history. It was proposed by Tsukamura (1971), subsumed within the genus *Rhodococcus* (Tsukamura, 1974; Goodfellow & Alderson, 1977) and re-established by Stackebrandt et al. (1988) for actinomycetes classified as *Rhodococcus bronchialis*, *Rhodococcus rubropertinctus* and *Rhodococcus terrae*. The genus belongs to the mycolic acid group of actinomycetes, that is, to the suborder *Corynebacterineae* (Stackebrandt et al., 1997), which form a distinct phylectic line that also encompasses the genera *Corynebacterium*, *Diezizia*, *Mycobacterium*, *Nocardioides*, *Rhodococcus*, *Skermania*, *Tsukamurella* and *Williamsia* (Chun et al., 1996; Goodfellow et al., 1998a, b, 1999). Members of these taxa can be separated from one another by using a battery of biochemical, chemical and morphological features (Goodfellow et al., 1999).


---

**Abbreviation:** meso-Å, pm; meso-2,6-diaminopimelic acid.

The GenBank accession number for the 16S rDNA sequence of strain IEGM⁷ is AF101418.
et al., 1994; Riegel et al., 1994; Takeuchi & Hatano, 1998; Kim et al., 1999; Kummer et al., 1999). Little is known about the biological properties of gordoniae. However, they are a potentially rich source of metabolic diversity, as exemplified by the isolation of strains possessing hydrocarbon-oxidizing and aromatic desulphurizing pathways (Gilbert et al., 1998; Rhee et al., 1998; Finkel'shtein et al., 1999; Kummer et al., 1999; Linos et al., 1999).

The aim of the present study was to determine the taxonomic relationships of a putatively novel member of the genus *Gordonia*, strain IEGM, which uses dibenzothiophene as a sole source of sulphur. Genotypic and phenotypic data show that the strain should be recognized as a new species of *Gordonia*, for which the name *Gordonia amicalis* sp. nov. is proposed.

**METHODS**

**Isolation, maintenance and cultivation.** Strain IEGM was isolated by enrichment culture from garden soil collected in Perm, Russia. Ten grams of soil was shaken at 27 °C for 7 d in a flask containing 100 ml mineral medium (Pfennig, 1965). Inocula from the enrichment culture were spread over the surface of corresponding mineral agar plates, which were incubated at 27 °C for 10 d, at which point the characteristic pink colonies were seen. A representative colony was streaked onto a beef-extract agar plate (Atlas, 1993), which was incubated at 27 °C for 4 d. The purified strain was maintained on modified Bennett’s agar slopes (Jones, 1949) at 30 °C and as a suspension in 20% (v/v) glycerol at −20 °C. Biomass for the chemotaxonomic and molecular systematic investigations was prepared by growing the strain in shake flasks of modified Bennett’s broth for 7 d at 30 °C. Cells for the chemical studies were washed in distilled water, whereas those used for the molecular systematic studies were washed in NaCl/EDTA buffer (0.1 M EDTA, pH 8.0, 0.1 M NaCl).

**Phenotypic characterization.** The strain was examined for a range of phenotypic characteristics (notably those shown in Table 1) and for the presence of menaquinones, mycolic acids, whole-organism sugars and the isomers of 2,6-diaminopimelic acid (Apm), as described previously (Kim et al., 1999). Mass spectra of the purified mycolic esters were obtained with an Autospec M instrument (Micromass) operating in electron-impact mode with an ionizing voltage of 70 eV, and a mass detection range of 50–580 m/z. The dominant menaquinones, which were identified by their mass spectra, were found to be predominantly composed of 4–8 isoprene units.

**Small-subunit rDNA sequencing.** Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified PCR products were carried out using the procedure of Kim et al. (1998). The resultant 16S rDNA sequence was aligned manually with the corresponding sequences of the type strains of *Gordonia* species and representatives of the other members of the subclass *Corynebacterineae* retrieved from the EMBL/GenBank/DDBJ databases by using the CLUSTAL programs of Fitch and Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining methods (Saitou & Nei, 1987), as described previously (Kim et al., 1999). The PHYLIP package (Felsenstein, 1993) was used for all of the analyses. Bootstrap analyses (Felsenstein, 1985) of the neighbour-joining data were carried out as described by Kim et al. (1999).

**RESULTS AND DISCUSSION**

The chemotaxonomic and morphological properties of strain IEGM are consistent with its assignment to the genus *Gordonia* (Stackebrandt et al., 1988; Goodfellow et al., 1998a, 1999). The organism is aerobic, Gram-positive, slightly acid–alcohol-fast, mycelial and forms red colonies on various complex media, including modified Bennett’s, ISP medium 2 and peptone–glucose–yeast-extract agars. It contains meso-Apm, arabinose and galactose in whole-organism hydrolysates (wall chemotype IV sensu Lechevalier & Lechevalier, 1970), predominant amounts of dihydrogenated menaquinones with nine isoprene units [MK-9 (H4)], mycolic acids with 50–58 carbon atoms and has DNA with a G+C value of 64.2 mol%.

Comparison of the nearly complete 16S rDNA sequence (1448 nucleotides) of the tested strain with the corresponding sequences of representatives of the suborder *Corynebacterineae* shows that it forms a monophyletic clade, in all three analyses, with the type strains of *G. alkanivorans*, *G. desulfuricans* and *G. rubropertincta* (Fig. 1). These relationships are also supported by the high nucleotide-similarity values between strain IEGM and *G. alkanivorans* DSM 44369T (98.4% similarity, which corresponds to 23 nucleotide differences), *G. desulfuricans* NCIMB 40816T (98.5% similarity, which corresponds to 21 nucleotide differences) and *G. rubropertincta* DSM 43197T (99.0% similarity, which corresponds to 14 nucleotide differences). The separation of the latter two strains is also supported by DNA–DNA relatedness data (Kim et al., 1999); strain IEGM showed DNA relatedness values of 31.8% and 37.3% with the type strains of *G. desulfuricans* and *G. rubropertincta*, respectively. The type strains of *G. alkanivorans* and *G. rubropertincta*, which share a 99.1% 16S rDNA similarity value (which corresponds to 13 nucleotide differences), show a DNA–DNA relatedness value of 52% (Kümmer et al., 1999). All of these values are well below the 70% cut-off point recommended by Wayne et al. (1987) for the recognition of genomic species. The mean 16S rDNA similarity value between the tested strain and the other members of the *Gordonia* clade was 97.4%. 16S rDNA similarity values such as those considered above have been reported between representatives of validly described species of *Gordonia* (Kim et al., 1999).
Table 1. Phenotypic characteristics separating the test strain from the type strains of other validly described Gordonia species

<table>
<thead>
<tr>
<th>Character</th>
<th>Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Colour of colony</td>
<td>Red</td>
</tr>
<tr>
<td>Biochemical tests:</td>
<td></td>
</tr>
<tr>
<td>Asesculin hydrolysis</td>
<td>–</td>
</tr>
<tr>
<td>Allantoins hydrolysis</td>
<td>–</td>
</tr>
<tr>
<td>Arbutin hydrolysis</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
</tr>
<tr>
<td>Urease hydrolysis</td>
<td>–</td>
</tr>
<tr>
<td>Decomposition of (% w/v):</td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine (0.5)</td>
<td>–</td>
</tr>
<tr>
<td>Starch (1)</td>
<td>+</td>
</tr>
<tr>
<td>Tributyrin (0.5)</td>
<td>–</td>
</tr>
<tr>
<td>Tyrosine (0.5)</td>
<td>–</td>
</tr>
<tr>
<td>Urea (0.5)</td>
<td>–</td>
</tr>
<tr>
<td>Xanthine (0.4)</td>
<td>–</td>
</tr>
<tr>
<td>Growth on sole carbon sources (4%) w/v:</td>
<td></td>
</tr>
<tr>
<td>Arbutin (1)</td>
<td>+</td>
</tr>
<tr>
<td>n-Cellobiose (1)</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol (1)</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-d-glucosamine (0.5)</td>
<td>–</td>
</tr>
<tr>
<td>Adipic acid (0.5)</td>
<td>+</td>
</tr>
<tr>
<td>Betaine (0.5)</td>
<td>–</td>
</tr>
<tr>
<td>Oxalic acid (0.1)</td>
<td>–</td>
</tr>
<tr>
<td>Propan-1-ol (0.5)</td>
<td>–</td>
</tr>
<tr>
<td>Sodium fumarate (1)</td>
<td>+</td>
</tr>
<tr>
<td>Growth in the presence of (% w/v):</td>
<td></td>
</tr>
<tr>
<td>Oleic acid (0.1)</td>
<td>+</td>
</tr>
<tr>
<td>Zinc chloride (0.001)</td>
<td>+</td>
</tr>
<tr>
<td>G+C content (mol%)*</td>
<td>64</td>
</tr>
</tbody>
</table>

ND: Not determined.

* Data taken from previous studies (Bendinger et al., 1995; Klatte et al., 1994, 1996; Riegel et al., 1994; Stackebrandt et al., 1988; Takeuchi & Hatano, 1998; Kim et al., 1999; Kummer et al., 1999; Linos et al., 1999).

The tested strain was also examined for a set of phenotypic properties (Table 1) known to be of value in gordonial systematics (Kim et al., 1999). It is evident from this table that strain IEGM1 can be distinguished from representatives of all of the validly described Gordonia species by using a combination of phenotypic properties. It is clear from both genotypic and phenotypic data, therefore, that strain IEGM1 forms a distinct centre of taxonomic variation within the genus Gordonia. Consequently, it is proposed that this organism be recognized as a new species – *Gordonia amicalis*.

**Description of Gordonia amicalis sp. nov.**

*Gordonia amicalis* (am.i.cal/is. L. adj. amicalis pertaining to friendship, reflecting the collaborative nature of the study).

Aerobic, Gram-positive, slightly acid–alcohol-fast actinomycete which forms short rods and coccoid elements. Red colonies are formed on modified Bennett’s, ISP 2 and peptone–yeast-extract agars. Neither aerial hyphae nor diffusible pigments are produced. Adipic acid, arbutin, glycerol, sodium fumarate, sodium propionate and sodium salicylate (but not N-acetyl-d-glucosamine, d-arabinose, betaine, d-cellobiose, sodium oxalate or 1-propanol) are used as sole sources of carbon for energy and growth. The organism grows in the presence of oleic acid (0.8%, v/v) and zinc chloride (0.001%, w/v), within the temperature range 10–40 °C and within the pH range 5.5–10. Starch is degraded but hypoxanthine, tributyrin, Tween 80, tyrosine and xanthine are not degraded. Nitrate is not reduced. Aesculin, allanton, arbutin and urea are not hydrolysed. Cells contain major amounts of meso-A<sub>4</sub>pm, arabinose and ga-
lactose. The predominant menaquinone is MK-9(H<sub>4</sub>), though substantial amounts of MK-8(H<sub>4</sub>) are present. The mycolic acids have 50–58 carbon atoms. The G+C content of the DNA is 64–2 mol%, as determined using the HPLC procedure. The organism was isolated from a garden soil in the vicinity of Perm, Russia. The type strain is strain IEGM<sup>T</sup> (DSM 44461<sup>T</sup> = DSM 44461<sup>T</sup> = KCTC 9899<sup>T</sup>).

Gordonia amicalis strain IEGM<sup>T</sup> is able to grow in mineral media containing dibenzoithiophene as the sole source of sulphur and in the presence of an organic compound as a source of carbon and energy, using the same pathway as Rhodococcus erythropolis strain IGTS8 (Oldfield et al., 1997, 1998). It is unable to grow using benzoithiophene as a sole source of sulphur (C. Oldfield & S. C. Gilbert, unpublished results). The presence of the dibenzoithiophene-desulphurization pathway in the G. amicalis strain is consistent with the emerging status of the gordoniae as a source of metabolic diversity rivalling that of the rhodococci. Other gordoniae possessing desulphurization pathways include Gordonia sp. strain CYSK1 (Rhee et al., 1998) and G. aichiensis strain 51 (Finkel'stein et al., 1999), both of which are reported to desulphurize dibenzoithiophene, and G. desulfuricans strain 213E, which desulphurizes benzoithiophene (Gilbert et al., 1998; Kim et al., 1999). In addition, Kummer et al. (1999) isolated G. alkaniivorans strain HK1<sup>T</sup> (DSM 44369<sup>T</sup>), which is capable of growth using an alkane as the sole source of carbon and energy, while Linos et al. (1999) described G. polyisoprenivorans strain Kd2<sup>T</sup>, a rubber-degrading bacterium isolated from an automobile tyre.

**ACKNOWLEDGEMENTS**

M. Goodfellow and C. Oldfield gratefully acknowledge support from the BBSRC (grant no. TO6520); S. B. Kim was supported by a Chevening-MOST scholarship. The
authors are also indebted to Fiona Stainsby, who provided some of the phenotypic data for the *G. alkanivorans* and *G. polysoprenovorans* strains.

**REFERENCES**


Tsukamura, M. (1982). Numerical analysis of the taxonomy of nocardiae and rhodococci. Division of Nocardia asteroides sensu stricto into two species and description of Nocardia paratuberculosis sp. nov. Tsukamura, (formerly the Kyoto-I group of Tsukamura), Nocardia nova sp. nov. Tsukamura, Rhodococcus aichiensis sp. nov. Tsukamura, Rhodococcus chu-buensis sp. nov. Tsukamura, and Rhodococcus obuensis sp. nov. Tsukamura. Microbiol Immunol 26, 1101–1119.
