Gordonia desulfuricans sp. nov., a benzothiophene-desulphurizing actinomycete

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The taxonomic position of two actinomycetes isolated from soil was established using a polyphasic approach. The organisms, designated 213E and 213F, were found to have chemical and morphological properties consistent with their assignment to the genus Gordonia. Nearly complete sequences of the 16S rDNA genes of the two strains were determined following the isolation and direct sequencing of the amplified genes. The tested strains were found to have identical 16S rDNA sequences and formed a phylogenetic line within the evolutionary radiation occupied by the genus Gordonia that was most closely related to Gordonia rubropertincta DSM 43197T. However, DNA–DNA relatedness data showed that strain 213E and Gordonia rubropertincta DSM 43197T belonged to distinct genomic species. Strains 213E and 213F also shared an identical phenotypic profile which distinguished them from representatives of validly described Gordonia species. The combined genotypic and phenotypic data show that strains 213E and 213F merit recognition as a new species of Gordonia. The name proposed for the new species is Gordonia desulfuricans, for which the type strain is 213E (= NCIMB 40816T).

Keywords: Gordonia, polyphasic taxonomy, mycolic acids, benzothiophene, desulphurization

INTRODUCTION

The application of the polyphasic taxonomic concept has led to far reaching changes in the taxonomy of the mycolata, that is, the mycolic acid containing actinomycetes (Chun et al., 1996; Goodfellow et al., 1998; Kämpfer et al., 1999). These organisms are currently assigned to nine taxa, namely, the genera Corynebacterium, Dietzia, Gordonia, Mycobacterium, Nocardia, Rhodococcus, Skermania, Tsukamurella and Williamsia, members of which can be separated from one another using a combination of biochemical, chemical and morphological properties. Representatives of the genera Gordonia, Nocardia, Rhodococcus and Tsukamurella have also been distinguished from one another by two-dimensional gel electrophoresis of ribosomal protein AT-L30 (Ochi, 1992), by DNA amplification and restriction endonuclease analysis (Steingrube et al., 1997) and by taxon-specific 16S rDNA–RNA signature nucleotides (Stackebrandt et al., 1997).

The genus Gordonia (formerly Gordona) was proposed by Tsukamura (1971), fell into abeyance but was re-established by Stackebrandt et al. (1988) for actinomycetes classified as Rhodococcus bronchialis, Rhodococcus ruber and Rhodococcus terrae. Gordoniae are aerobic, Gram-positive to Gram-variable, non-motile, catalase-positive actinomycetes which generally form short rods and cocci. They are usually partially acid–alcohol-fast. Rough brownish, pink or orange to red colonies are formed on glucose yeast extract and Sauton’s agars. The organism has an oxidative carbohydrate metabolism, is arylsulphatase-negative, sensitive to lysozyme and forms mycobactins. The peptidoglycan is of the Alγ type, contains meso-diaminopimelic acid (meso-A,pm) as the diamino acid and muramic acid with N-glycolyl residues. The polysaccharide fraction of the wall is rich in arabinose.
and galactose. The wall envelope contains mycolic acids with 44–66 carbon atoms with up to four double bonds; fatty acid esters released on Py-GC of mycolic acids have 16–18 carbon atoms. Major proportions of straight-chain, saturated, monounsaturated and 10-methyl (tuberculostearic) cellular fatty acids are also formed. Cells contain diphosphatidylglycerol-, phosphatidylethanolamine- and phosphatidylinositol mannosides as major phospholipids and have dihydrogenated menaquinones with nine isoprene units as the predominant isoprenologue. The range of G+C content of the DNA is 63–69 mol%. Gordoniae have been isolated from a variety of habitats including activated sludge foam, biofilms, mangrove rhizosphere and sputa of patients with bronchiectasis and cavitary pulmonary tuberculosis. The type species is *Gordonia bronchialis*.


The tested organisms, strains 213ET and 213F, were isolated in a screening program designed to yield micro-organisms capable of desulphurizing benzo thiophene (Gilbert et al., 1998). The aim of the present investigation was to clarify the relationships of these strains using the polyphasic taxonomic approach. It was evident from the resulting genotypic and phenotypic data that the organisms should be recognized as a new species of *Gordonia* for which the name *Gordonia desulfuricans* is proposed.

**METHODS**

**Isolation of strains 213ET and 213F.** The tested strains were isolated by enrichment culture in glycerol *Rhodococcus* broth supplemented with benzo thiophene, using as inoculum a soil sample collected in the vicinity of an oil shale spoil heap near a disused mine located at West Calder, West Lothian, Scotland, UK (Gilbert et al., 1998).

**Maintenance and cultivation of organisms.** Strains 213ET (NCIMB 40816T) and 213F (NCIMB 40817) were maintained on modified Bennett's agar (Jones, 1949) or on yeast extract/malt extract/agar (ISP medium 2; Diço) plates at 30 °C and as cell suspensions in 20% (w/v) of glycerol at −20 °C. The tested strains were also examined together with the type strains of all of the validly described species of *Gordonia* for the phenotypic properties shown in Table 1. The cells for the chemical studies were washed in distilled water and freeze-dried; those needed for the molecular systematic investigations were washed in NaCl/EDTA buffer (0.1 M EDTA, pH 8.0, 0.1 M NaCl) and stored at −20 °C until needed.

**Phenotypic characterization.** The ability of the tested strains to use eight carbon compounds (Table 1) as sole sources of carbon for energy and growth was examined using Stevenson's basal medium (Stevenson, 1967). The organisms were also examined for their capacity to grow in the presence of 0.1% oleic acid and 0.001% zinc chloride using peptone/glucose/yeast extract agar (PGYE; Bacto peptone, 5 g; glucose, 5 g; yeast extract, 5 g; agar, 15 g; distilled water, 1 l; pH 7.0) as the basal medium. The biochemical tests and the degradation of starch, tributyrin and Tween 80 were carried out following the procedures of Williams et al. (1983), and the degradation of hypoxanthine, tyrosine, xanthine and uric acid were examined on ISP 2 plates. All of the tests were read after incubation for 2 weeks at 30 °C. The colonial properties of strains 213ET and 213F were examined on PGYE plates incubated at 30 °C. Smears from these plates were Gram-stained using Hucker's modification (Society for American Bacteriologists, 1957) and examined for micromorphological features. Smears were also stained using a modification of the Zielh–Neelsen method (Gordon, 1967).

**Chemotaxonomy.** The isomeric form of the diaminopimelic acid of the tested strains were determined by TLC of whole-organism hydrolysates (Staneck & Roberts, 1974). Standard procedures were also used for the extraction and analysis of menaquinones (Minnikin et al., 1984) and mycolic acids (Minnikin et al., 1980). Mass spectra of the purified mycolic esters were taken on an Autospec M instrument (Micromass, Warrington, UK) operating in electron impact mode with an ionizing voltage of 55 eV and a probe temperature of 150–300 °C.

**Small subunit rDNA sequencing.** Isolation of chromosomal DNA, PCR amplification and direct sequencing of purified PCR products were carried out as described previously (Kim et al., 1998). The resultant 16S rDNA sequences of strains 213ET and 213F were aligned manually with the corresponding sequences of the type strains of the validly described *Gordonia* species, and representatives of other mycolata genera, retrieved from the Ribosomal Database Project (Maidak et al., 1997) and EMBL/GenBank/DDBJ databases by using the AL16S program of Chun (1995). The resultant dataset contained information on 1448 nucleotide positions for 19 gordoniae and related mycolata genera. Evolutionary trees were inferred by using the phylip package (Felsenstein, 1981), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms. Evolutionary distances for the least-squares, neighbour-joining and neighbour-joining methods were generated according to Jukes & Cantor (1969). The phylip package (Felsenstein, 1993) was used for all of the analyses. The resultant unrooted tree topologies were evaluated by carrying out bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method data based on 1000 resamplings using the seqboot and consense programs in the phylip package (Felsenstein, 1993). The root position of the unrooted tree based on the
neighbour-joining method was estimated by using four outgroup organisms (Arthrobacter globiformis, accession no. M23411; Bacillus subtilis, K00637; Escherichia coli, J01695; Nocardia asteroides, Z36934), as described by Swofford & Olsen (1990).

**DNA relatedness.** DNA–DNA hybridization was carried out by the identification service of DSMZ (Braunschweig, Germany). DNA was isolated by chromatography on hydroxypatitie (Cashon et al., 1977) and hybridization was performed as described by De Ley et al. (1970) with modifications (Escara & Hutton, 1980; Huß et al., 1983) using a Gilford System model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were calculated using the TRANSFER.BAS program (Jahnke, 1992).

**DNA base composition.** Chromosomal DNA from the tested strains was digested, dephosphorylated and analysed by the HPLC method (Tamaoka, 1984), as described by Kim et al. (1998).

**RESULTS AND DISCUSSION**

Strains 213ET and 213F have chemical and morphological properties which are consistent with their classification in the genus *Gordonia* (Stackebrandt et al., 1988; Goodfellow et al., 1998). The organisms are aerobic, Gram-positive, amycelial, non-motile, and form pink colonies on modified Bennett's agar. They contain meso-A₈pm, 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(H₂)], mycolic acids with 56–62 carbon atoms and have DNA with a G+C ratio of 64±1 % mol. The strains have identical 16S rDNA nucleotide sequences which place them in the genus *Gordonia*.

Comparison of nearly complete 16S rDNA sequences (1448 nucleotides) of the tested strains with the corresponding sequences of representative mycolata strains shows that they form a monophyletic clade with *Gordonia rubropertincta* DSM 43197T in the least-squares, maximum-likelihood and neighbour-joining trees (Fig. 1). These relationships are also supported by the high nucleotide similarity values between the tested strains and *Gordonia rubropertincta* (98.7 % similarity which corresponds to 19 nucleotide differences) and by the high bootstrap value (76 %) obtained in the neighbour-joining analysis. However, strain 213ET and *Gordonia rubropertincta* DSM 43197T share a DNA–DNA relatedness value of 36±8 % and hence belong to distinct genomic species (Wayne et al., 1987). Strains 213ET and 213F also showed close relationships with *Gordonia bronchialis* DSM 43247T (98.3 % similarity which corresponds to 25 nucleotide differences) and *Gordonia alkanivorans* (98.2 %, which corresponds to 27 nucleotide differences). The mean 16S rDNA similarity value between the tested strains and members of the *Gordonia* clade was 97.6 %.

16S rDNA nucleotide similarity values such as those cited above have been reported for several validly described *Gordonia* species. *Gordonia bronchialis* DSM 43247T and *Gordonia terrae* DSM 43249T, for example, share a nucleotide similarity value of 98.3 % which corresponds to 25 nucleotide differences; members of these species show DNA–DNA relatedness values within the range 16–21 % (Zakrzewska-Czerwinska et al., 1988). Similarly, *Gordonia aichiensis* DSM 43978T and *Gordonia sputi* DSM 43896T share a 16S rDNA nucleotide similarity value of 99.7 % (which corresponds to four nucleotide differences) and DNA–DNA relatedness values within the range 38–40 % (Goodfellow et al., 1978; Klatte et al., 1994).

The tested organisms and the type strains of all of the validly described species of *Gordonia* were examined for a number of phenotypic properties found to be of differential value in an extensive numerical taxonomic study of mycolata strains (Toboli, 1995). Strains 213ET and 213F gave identical responses in the carbon utilization, degradation and tolerance tests (Table 1). It is also evident from this table that these strains can be distinguished from representatives of the validly described *Gordonia* species using a combination of phenotypic properties.

It is apparent from the genotypic and phenotypic data that strains 213ET and 213F form a distinct centre of taxonomic variation within the genus *Gordonia*. It is, therefore, proposed that these organisms be recognized as a new species. *Gordonia desulfuricans* is proposed for this taxon.

**Description of Gordonia desulfuricans** sp. nov.

*Gordonia desulfuricans* (de.sul.fu.'ri.cans. L. pref. de from; L. n. sulfur sulphur. M.L. part. adj. desulfur-icans reducing sulphur compounds).

Aerobic, Gram-positive, slightly acid–alcohol-fast actinomycete which forms short rods and coccoid elements. Rough pinkish colonies are formed on modified Bennett’s and peptone glucose yeast extract agars. Neither aerial hyphae nor diffusible pigments are produced. Adipic acid, d-arabinose, arbutin, glycerol, l-propanol, sodium propionate and sodium salicylate are used as sole sources of carbon for energy and growth but not N-acetyl-d-glucosamine, betaine, d-cellobiose, sodium fumarate or sodium oxalate. Growth occurs in the presence of adenine (0.25 %), oleic acid (0.8 %) and zinc chloride (0.001 %) but not in the presence of picric acid (0.3 %). Cells contain major amounts of meso-A₈pm, arabinose and galactose. The predominant menaquinone is MK-9(H₂) though minor amounts of MK-8(H₂) are present. The mycolic acids have 56–62 carbon atoms with up to three double bonds. The G+C content of the DNA is 64.1 mol %, as determined by the HPLC procedure. The organism was isolated from a soil sample collected in the vicinity of an oil shale spoil heap near a disused mine located at West Calder, West Lothian, Scotland, UK. The type strain is strain 213ET (NCIMB 40816T). Strain 213F
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Gordonia aichiiensis DSM 43978\(^T\)  
Gordonia spuri DSM 43896\(^T\)  
Gordonia bronchialis DSM 43247\(^T\)  
Gordonia rhizosphera IFO 16688\(^T\)  
Gordonia spuri DSM 43896\(^T\)  
Gordonia bronchialis DSM 43247\(^T\)  
Gordonia rhizosphera IFO 16688\(^T\)  
Gordonia rubropertincta DSM 43197\(^T\)  
Gordonia terrae DSM 43249\(^T\)  
Gordonia amarae DSM 43392\(^T\)  
Gordonia hirsuta DSM 44140\(^T\)  
Gordonia hydrophobica DSM 44015\(^T\)  
Skermania piniformis IFO 15059\(^T\)  
Nocardia asterales ATCC 33057  
Directus marui DSM 43672\(^T\)  
Tsukamurella parometae DSM 20162\(^T\)  
Rhodococcus Rhodochrous DSM 43241\(^T\)  
Coronarctaricum glaucoma NCIMB 10025\(^T\)

**Fig. 1.** Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rDNA sequences of gordoniae and related mycolic acid containing taxa. Asterisks indicate branches of the tree that were also formed using the least squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993), and maximum-parsimony (Kluge & Farris, 1969) treeing algorithms. The numbers at the nodes indicate the level of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values more than 50\% are given. The scale bar indicates 0.01 substitutions per nucleotide position. \(^T\), Type strain.

(NCIMB 40817) gave identical results to strain 213ET\(^T\) in both the genotypic and phenotypic tests.

**Significance of desulphurization in gordoniae**

*Gordonia desulfuricans* strains 213ET\(^T\) and 213F, which were isolated for their ability to grow on benzothiophene as a sole source of sulphur, have a unique kind of sulphur-scavenging pathway whereby benzothiophene is desulphurized to 2-(2'-hydroxyphenyl)-ethan-1-al and an inorganic sulphur compound, probably sulphite (Gilbert et al., 1998). This reaction corresponds to the dibenzothiophene desulphurization pathway first identified in *Rhodococcus* sp. strain IGTS8 (now classified as a strain of *Rhodococcus erythropolis*; M. Goodfellow and others, unpublished). Strain IGTS8 desulphurizes dibenzothiophene (DBT) to 2-hydroxybiphenyl and sulphite (Oldfield et al., 1997), as do a large number of subsequent isolates, nearly all of which have been classified in the genus *Rhodococcus*. In both cases the organic product, which is basically the carbon skeleton of the original organosulphur molecule, is not further metabolized (Kayser et al., 1993; Oldfield et al., 1997; Gilbert et al., 1998).

Desulphurization pathways are of prime importance in the development of microbial fuel desulphurization technologies, as a cheap and environmentally friendly alternative to chemical processes. Strains such as 213ET\(^T\) and 213F may find particular application in the desulphurization of diesel fuel, which contains a high proportion of benzothiophenes (McFarland et al., 1998).

The discovery of desulphurization pathways in gordoniae, together with a report of a *Gordonia* sp. with the DBT desulphurization phenotype (Rhee et al., 1998), highlights the potential importance of gordoniae as a source of metabolic diversity. Much attention has been given to rhodococci in this context (see e.g. Warhurst & Fewson, 1994), but not to the closely related gordoniae or tsukamurellae. This is not surprising since, except in taxonomic terms, these genera remain little studied; nevertheless it now seems that members of these taxa will, like rhodococci, be found to have novel enzymic capabilities for the
Table 1. Phenotypic characteristics separating the test strains from other validly described Gordonia species

<table>
<thead>
<tr>
<th>Character</th>
<th>Strain:</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
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<tr>
<td>Colony colour</td>
<td>Pink</td>
</tr>
<tr>
<td>Biochemical tests:</td>
<td></td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>−</td>
</tr>
<tr>
<td>Allantoin hydrolysis</td>
<td>−</td>
</tr>
<tr>
<td>Arbutin hydrolysis</td>
<td>−</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
</tr>
<tr>
<td>Urea hydrolysis</td>
<td>+</td>
</tr>
<tr>
<td>Decomposition of (% w/v):</td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine (0.4)</td>
<td>+</td>
</tr>
<tr>
<td>Starch (1)</td>
<td>+</td>
</tr>
<tr>
<td>Tributyrin (0.1)</td>
<td>−</td>
</tr>
<tr>
<td>Tween 80 (1)</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine (0.5)</td>
<td>−</td>
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<tr>
<td>Lactic acid (0.5)</td>
<td>+</td>
</tr>
<tr>
<td>Xanthine (0.4)</td>
<td>−</td>
</tr>
<tr>
<td>Growth on sole carbon sources (% w/v):</td>
<td></td>
</tr>
<tr>
<td>Arbutin (1)</td>
<td>−</td>
</tr>
<tr>
<td>D-Cellobiose (1)</td>
<td>+</td>
</tr>
<tr>
<td>Glycerol (1)</td>
<td>−</td>
</tr>
<tr>
<td>N-Acetyl-D-glucosamine (0.1)</td>
<td>−</td>
</tr>
<tr>
<td>Adipic acid (0.1)</td>
<td>+</td>
</tr>
<tr>
<td>Betaine (0.1)</td>
<td>+</td>
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<tr>
<td>Oxalic acid (0.1)</td>
<td>−</td>
</tr>
<tr>
<td>Propan-1-ol (0.1)</td>
<td>+</td>
</tr>
<tr>
<td>Sodium fumarate (0.1)</td>
<td>−</td>
</tr>
<tr>
<td>Growth in the presence of (% v/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>Mycolic acids* (mol%)*</td>
<td>56-62</td>
</tr>
</tbody>
</table>

* 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).

REFERENCES


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