Gordonia defluvii sp. nov., an actinomycete isolated from activated sludge foam

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Three strains of non-motile, Gram-positive, filamentous actinomycetes, isolates J4T, J5 and J59, initially recognized microscopically in activated sludge foam by their distinctive branching patterns, were isolated by micromanipulation. The taxonomic positions of the isolates were determined using a polyphasic approach. Almost-complete 16S rRNA gene sequences of the isolates were aligned with corresponding sequences of representatives of the suborder Corynebacterineae and phylogenetic trees were inferred using three tree-making algorithms. The organisms formed a distinct phylectic line in the Gordonia 16S rRNA gene tree. The three isolates showed 16S rRNA gene sequence similarities within the range 96–97·2 % with their nearest phylogenetic neighbours, namely Gordonia bronchialis DSM 43247T and Gordonia terrae DSM 43249T. Strain J4T was shown to have a chemotaxonomic profile typical of the genus Gordonia and was readily distinguished from representatives of the genus on the basis of Curie-point pyrolysis mass spectrometric data. The isolates shared nearly identical phenotypic profiles that distinguished them from representatives of the most closely related Gordonia species. It is evident from the genotypic and phenotypic data that the three isolates belong to a novel Gordonia species. The name proposed for this taxon is Gordonia defluvii sp. nov.; the type strain is J4T (=DSM 44981T =NCIMB 14149T).

Most activated sludge systems suffer intermittently from the serious operational disorder known as foaming or scumming, whereby a stable foam or scum develops on the surface of aeration tanks (Soddell, 1999). The reasons for the appearance of such foams are not clear, but their stable nature is imparted by the presence of large numbers of bacteria in the foam layer. In many cases, these foam-associated bacteria are mycolic acid-containing actinomycetes that belong to the suborder Corynebacterineae Stackebrandt et al. 1997. It is generally accepted that mycolic acids render cells sufficiently hydrophobic to allow them to accumulate on the surfaces of aeration tanks (Seviour & Blackall, 1999).

Initially, Nocardia amarae (now Gordonia amarae Klatt et al. 1994) was implicated in foaming (Lechevalier & Lechevalier, 1974), but it is apparent from culture-dependent and -independent approaches that diverse populations of mycolic acid-containing actinomycetes are involved. Isolates representing the genera Millisia (Soddell et al., 2006) and Skermania (Blackall et al., 1989; Chun et al., 1997) have been recovered only from activated sludge foams. Novel species isolated from foams include members of the genera Nocardia (Yamamura et al., 2005) and Tsukamurella (Nam et al., 2003, 2004). However, it is clear that many additional mycolic acid-containing taxa associated with foam need to be formally described (Soddell & Seviour, 1998; Stainsby et al., 2002). Such studies are important, as attempts to control the appearance and persistence of foams are unlikely to succeed until the taxonomic diversity and functional roles of the causal organisms are understood (Goodfellow et al., 1996, 1998; Stainsby et al., 2002).

Three isolates with a cellular morphology intermediate between the right-angled branching pattern typical of G. amarae Klatt et al. 1994 and the ‘pine-tree-like’ morphotype of Skermania piniformis Chun et al. 1997 were isolated, by micromanipulation, in Australia from activated sludge foams at Brimbank Park, Victoria (isolates J4T and J5) and Craigieburn, Victoria (isolate J59), as described by Soddell & Seviour (1998). These authors considered that the strains might form a novel species on the basis of an extensive numerical taxonomic survey of Skermania and related strains, a proposition underpinned by the results of the present investigation.
Extraction of chromosomal DNA and PCR amplification and sequencing of 16S rRNA genes of the three isolates were carried out as described previously (Soddell et al., 2006). The resultant 16S rRNA gene sequence data were compared with corresponding results taken from the RDP database for representatives of genera classified in the suborder Corynebacterineae. Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms. An evolutionary distance matrix was generated for the neighbour-joining algorithm using the Jukes & Cantor (1969) distance matrix was generated for the neighbour-joining tree-making algorithms. An evolutionary radiation occupied by the genus Gordonia (data not shown). It is evident from Fig. 1 that the isolates are loosely associated with the type strains of G. amarae (96.2–96.4 % similarity) and G. hirsuta (96.0–96.2 % similarity), though these relationships are not supported by a high bootstrap value. Indeed, the isolates are not closely related to any of the Gordonia type strains; their closest relatives are Gordonia bronchialis D3M 42347T (96.9–97.2 % similarity) and Gordonia terrae D3M 42349T (96.7–97.1 % similarity). Given the low values, it was not necessary to carry out DNA–DNA pairing experiments between isolate J4T and its closest phylogenetic neighbours, especially since similarity values well above these levels have been reported between representatives of several species of Gordonia with validly published names. The type strains of Gordonia alkaniavorans and Gordonia rubripertincta, for example, share 99.1 % 16S rRNA gene sequence similarity and have a DNA–DNA relatedness of 52 % (Kummer et al., 1999), a value well below the 70 % cut-off point recommended by Wayne et al. (1987) for the recognition of genomic species.

Strain J4T was the subject of chemotaxonomic studies designed to determine whether it had chemical markers consistent with its classification in the genus Gordonia. Standard methods were used for the extraction and analysis of fatty acids ( MIDI system; http://www.midi-inc.com), isoprenoid quinones (Minnikin et al., 1984), muramic acid type (Uchida et al., 1999), mycolic acids (Minnikin et al., 1984), polar lipids (Minnikin et al., 1984) and sugars (Schaal, 1985). The isolate contained meso-diaminopimelic acid, arabinose and galactose (wall chemotype IV sensu Lechevalier & Lechevalier, 1970), N-glycolated muramic acid, dihydrogenated menaquinones with nine isoprene units [MK-(9H2)] as the predominant isoprenologue with small amounts of MK-(8H2) and diposphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol dimannosides, phosphatidylethanolamine and an unidentified glycolipid as major polar lipids (phospholipid type 2 sensu Lechevalier et al., 1977). It also contained major proportions of oleic (C18:1; 35-2 % of the total fatty acid composition), palmitic (C16:0; 21-3 %), palmitoleic (C16:1; 17-5 %) and tuberculostearic (9-0 %) acids and minor proportions of myristic (3-7 %), margaric (2-7 %), eicosenoic (2-7 %), stearic (2-4 %), heptadecanoic (2-2 %), nonadecenoic (2-1 %) and pentadecyclic (1-2 %) acids. The organism possessed mycolic acids, the methyl esters of which had the same Rf value after thin-layer chromatography as those of G. bronchialis D3M 42347T. All of these properties are in line with the assignment of the strain to the genus Gordonia (Goodfellow & Maldonado, 2006).

The DNA G+C content was determined for strain J4T; isolation and purification of the DNA was carried out after Pitcher et al. (1989). The base composition of the resultant DNA preparation was determined using the reversed-phase

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**Fig. 1.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing that isolates J4T, J5 and J59 form a distinct phyletic line in the Gordonia 16S rRNA gene tree. Asterisks indicate branches that were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50 % are given. Bar, 0-01 substitutions per nucleotide position. The outgroup strain used to root the tree (not shown) was Turlcilla otitidis 234/92T (GenBank accession no. X73976).
HPLC procedure described by Tamaoka (1994). The molar $G⁺C$ ratio calculated after Mesbah et al. (1989) was 63.1 mol%.

Curie-point pyrolysis mass spectrometry (PyMS) has been used to good effect to distinguish between closely related organisms, including members of mycolic acid-containing taxa (Goodfellow et al., 1997). A standard PyMS procedure (Goodfellow et al., 1998) was used to determine relationships between isolate J4$^T$ and representatives of the genus *Gordonia*. It is apparent from Fig. 2 that strain J4$^T$ can be readily distinguished from the *Gordonia* marker strains, including the type strains of *G. amarae*, *G. bronchialis*, *G. hirsuta* and *G. terrae*. In addition, all three isolates can be distinguished from representatives of these and other phylogenetically close species using a combination of phenotypic properties (Table 1) and by their characteristic micromorphology.

It is clear from the genotypic and phenotypic data that isolates J4$^T$, J5 and J59 merit recognition within a novel species in the genus *Gordonia*. The name *Gordonia defluvii* sp. nov. is proposed for this taxon.

**Description of *Gordonia defluvii* sp. nov.**

*Gordonia defluvii* (de.flu‘vi.i. L. gen. n. *defluvii* of sewage).

The description is taken from this and the earlier study of Soddell & Seviour (1998). Aerobic, Gram-positive, slightly

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**Table 1. Phenotypic characteristics that separate isolates J4$^T$, J5 and J59 from representatives of closely related *Gordonia* species**

<table>
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<th>Characteristic</th>
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<tr>
<td>Colour of colony*</td>
<td>NP</td>
<td>T/W</td>
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<td>W/LW</td>
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<td>Biochemical tests</td>
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<td>Aesculin hydrolysis</td>
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<td>Allantoin hydrolysis</td>
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<td>Nitrate reductase</td>
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<td>Urea hydrolysis</td>
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<td>Degradation of: (%, w/v)</td>
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<td>Hypoxanthine (0-4)</td>
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<td>Tween 80 (1)</td>
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<td>Uric acid (0-5)</td>
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<td>Xanthine (0-4)</td>
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<td>Growth on sole carbon sources (%), w/v</td>
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<td>D-Cellobiose (1)</td>
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<td>+</td>
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<td>Glycerol (1)</td>
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<td>Betaine (0-1)</td>
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<td>Sodium fumarate (1)</td>
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*B*, Brown; *LY*, light yellow; *NP*, non-pigmented; *O*, orange; *P*, pink; *R*, red; *T*, tan; *W*, white.
acid–alcohol-fast, non-motile, non-spore-forming, slowly growing actinomycete that shows acute-angled and right-angled branching. Non-pigmented colonies with filamentous margins and abundant aerial hyphae are formed on glucose-yeast extract agar. Diffusible pigments are not produced. The organism is catalase- and phosphatase-positive but does not produce m- or p-nitrophenol oxides. Esterase lipase (C₈), leucine aminopeptidase and β-glucosidase are produced but not alkaline phosphatase, chymotrypsin, α-fucosidase, α- or β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, x-mannosidase, trypsin or ovaline aminopeptidase. Degrades Tween 20 but not adenine, casein or elastin. Pyruvate is used as a sole carbon source but not arabinose, fructose, galactose, maltose, mannose, melezitose, rhamnose, salicin or sorbitol (all at 0-1 %, w/v) or acetamide, benzamide, benzoate, butyrate, citrate, p-cresol, gluconate, m- or p-hydroxybenzoic acid, lactate, malate, octanoate, oxalate, pimelate, propionate, sebacate, succinate, tartrate, testosterone or tyrosine (all at 0-01 %, w/v). Grows from pH 5 to 8 and between 15 and 30 °C, albeit weakly at these latter temperatures. Does not grow in the presence of crystal violet (0-001 %), phenol (0-01 %, w/v), phenol ethanol (0-03 %, v/v), sodium azide (0-01 %, w/v), sodium chloride (5 %, w/v) or penicillin (10 IU). Additional phenotypic properties are shown in Table 1. Chemotaxonomic properties are typical of the genus *Gordonia*. The G+C content of the genomic DNA is 63-1 mol%.

The type strain, DSM 44981<sup>T</sup> = NCIMB 14149<sup>T</sup>, was isolated from a foam sample taken from an activated sludge plant at Brimbank Park, Victoria, Australia.

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**References**


