Gordonia malaquae sp. nov., isolated from sludge of a wastewater treatment plant

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The taxonomic status of a bacterial isolate from the sludge of a wastewater treatment plant was characterized by using a polyphasic taxonomic approach. Chemotaxonomic investigations revealed the presence of cell-wall chemotype IV, short-chain mycolic acids that co-migrated with those extracted from members of the genus Gordonia, fatty acids C₁₆ : ₀ and C₁₈ : ₀ (found by pyrolysis gas chromatography) and a dihydrogenated menaquinone with nine isoprene units [MK-9(H₂)] as the predominant menaquinone. The genus assignment was confirmed by 16S rRNA gene sequencing. Comparative analysis of the 16S rRNA gene sequence showed that the novel isolate constitutes a hitherto unknown subsline within the genus Gordonia, displaying 95.9 to 97.6 % gene sequence similarity to the recognized species of the genus. The novel isolate was distinguished from the type strains of phylogenetically related species by using a set of phenotypic features. The genotypic and phenotypic data show that the new strain merits classification as a novel species of the genus Gordonia, for which the name Gordonia malaquae sp. nov. is proposed. The type strain is IMMIB WWCC-22¹ (= DSM 45064¹ = CCUG 53555¹).

The genus Gordonia belongs to the suborder Corynebacterineae (Stackebrandt et al., 1997). The genus Gordonia has attracted much interest in recent years for a variety of reasons. In contrast to the originally isolated strains of the genus, which were described as opportunistic pathogens in humans (Tsukamura, 1971, 1978, 1982), most members of the genus Gordonia described recently represent environmental isolates that play an important role in bioremediation and the biodegradation of pollutants (Bendinger et al., 1995; Klatte et al., 1996; Kim et al., 2000; Linos et al., 2002; Kageyama et al., 2006; Soddell et al., 2006). At the time of writing, the genus Gordonia includes 22 species with validly published names. The aim of this study was to clarify the taxonomic position of strain IMMIB WWCC-22¹ which was isolated from the sludge of a wastewater treatment plant. Based on phylogenetic and phenotypic evidence, it is proposed that this new isolate be classified as a novel species of the genus Gordonia.

Strain IMMIB WWCC-22¹ was isolated from the sludge of a wastewater treatment plant located in Taichung Industrial Park, Taichung city, Taiwan. The organism was cultivated on Columbia agar supplemented with 5 % sheep blood agar and brain–heart infusion (BHI) agar to determine its chemotaxonomic properties. Pigment production was determined by growing the strain at 27 °C for 7 days; observations were made at 24 h intervals. Air-dried smears were stained by the Gram method in order to determine the Gram stain and cell morphology. The Ziehl-Neelsen method was used to determine acid-fastness. Growth temperatures were determined by incubating the strain at 27, 37 and 42 °C. The physiological properties of the novel strain were determined by using tests to determine the hydrolysis of complex substrates as described previously (Gordon, 1966, 1967; Gordon & Mihm, 1957) as well as tests to determine carbon source utilization according to Yassin et al. (1995). The isomeric form of the dianamipimel acid was determined by the method of Becker et al. (1964) and whole-cell sugars were determined according to Lechevalier (1968). Lipids were extracted using acid methanolysis and mycolic acids were detected with TLC as described by Minnikin et al. (1980); pyrolysis GC of the mycolate was performed according to Yassin et al. (1993a). Non-hydroxylated fatty acids were purified, identified and quantified by GC as described by Yassin (1988). Phospholipids were extracted, purified and identified as described previously (Yassin et al., 1993b). Menaquinones were extracted and purified according to

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB WWCC-22¹ is AM406674.
Collins et al. (1977). Mass spectral analyses of the menaquinones were recorded as described recently by Yassin & Hupfer (2006) in positive ion mode on a Q-TOF 2 mass spectrometer (Micromass) equipped with a nanospray source.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and the purification of PCR products were carried out using previously described procedures (Rainey et al., 1996). Purified PCR products were sequenced using a Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) according to the manufacturer’s protocol. A Genetic Analyzer (310 DNA; Applied Biosystems) was used for electrophoresis of the sequence reaction products. The 16S rRNA gene sequences of strain IMMIB WWCC-22T, as well as those of the other recognized species of the genus Gordonia retrieved from GenBank, were added to the ARB-database (Ludwig et al., 2004) and aligned using the appropriate tool from the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix was calculated using the correction of Jukes & Cantor (1969). The topologies of the resultant trees were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining data based on 1000 resamplings using the ARB package.

To establish the phylogenetic position of strain IMMIB WWCC-22T, its 16S rRNA gene sequence was determined in this study [1489 nucleotides; 96.5 % of the Escherichia coli sequence (Brosius et al., 1978)]. A tree depicting the phylogenetic relationship of the novel strain within the genus Gordonia is shown in Fig. 1. The novel strain formed a distinct subline within the genus Gordonia, branching proximal to the base of a subcluster of species, which includes Gordonia hirsuta, Gordonia amarae, Gordonia sihwensis and Gordonia hydrophobica. Bootstrap resampling, however, showed that the association of strain IMMIB WWCC-22T with this subcluster of species is not statistically significant and, from the tree construction analysis, it is evident that the novel strain does not exhibit a significant affinity with any recognized species. The novel strain shared closest 16S rRNA gene sequence similarity with the type strains of G. hydrophobica (97.6 %), Gordonia defluvii (97.5 %), Gordonia rubripertincta (97.4 %), Gordonia desulfuricans, (97.3 %), Gordonia namibiensis (97.3 %), Gordonia alkaniavorans (97.2 %), Gordonia westfalica (97.2 %), G. sihwensis (97.1 %) and Gordonia amicalis (97.1 %). Lower 16S rRNA gene sequence similarities were found with the type strains of the remaining Gordonia species. DNA–DNA relatedness studies were not carried out between strain IMMIB WWCC-22T and its phylogenetically closest relatives as it has already been established that representatives of other Gordonia species with similar 16S rRNA gene sequence similarities, for example Gordonia araii and Gordonia effusa and the type strains of G. amarae, G. hydrophobica and G. hirsuta (Kageyama et al., 2006), share DNA–DNA relatedness values well below the 70 % cut-off point recommended for the delineation of bacterial species (Wayne et al., 1987). The novel strain can be distinguished from its phylogenetically closest relatives by using a combination of phenotypic properties (Table 1).

Fig. 1. Maximum-likelihood tree showing the position of Gordonia malaquae sp. nov. IMMIB WWCC-22T in the genus Gordonia. The tree was based on comparison of 16S rRNA gene sequences that were at least 90 % complete (with regard to the E. coli sequence). Numbers at nodes are levels of bootstrap support (%) based on analyses of 1000 resampled datasets. Solid circles indicate that the corresponding nodes (groupings) are also recovered in neighbour-joining and maximum-parsimony trees. Bar, 5.0 % sequence divergence.
Strain IMMB WWCC-22\textsuperscript{T} has morphological properties consistent with its assignment to the genus *Gordonia*. The organism is aerobic and forms smooth, creamy colonies on Columbia and BHI agars. The cells are rod- and cocoid-like, stain Gram-positive and are non-acid–alcohol-fast. The novel strain grows at temperatures up to 37 °C, but not at 42 °C. The physiological properties of strain IMMB WWCC-22\textsuperscript{T} are given in detail in the species description below. The biochemical characteristics determined in this study that distinguish strain IMMB WWCC-22\textsuperscript{T} from *G. desulfuricans* DSM 44462\textsuperscript{T}, *G. hirsuta* DSM 44140\textsuperscript{T} (data from Linos et al., 2002); 4, *G. hydrophobica* DSM 44015\textsuperscript{T}; 5, *G. rubripertincta* DSM 43197\textsuperscript{T}; 6, *G. sihwensis* DSM 44576\textsuperscript{T} (Kim et al., 2003). All strains (not determined for strain numbers 3 and 4) were positive for acetate, 2,3-butanediol, citrate, glucose, paraaffin and trehalose and for hydrolysis of testosterone and urea. All strains (not determined for strain numbers 3 and 4) were negative for the utilization of adipic acid, adonitol, isoamyl alcohol, L-arabinose, cellobiose, gelatin, lactate, lactose, maltose, melezitose, raffinose, rhamnose and D-xyllose and for hydrolysis of testosterone and urea. All strains (not determined for strain numbers 3 and 4) were negative for nitrogen sources, but not acetamide, 2,3-butandiol, citrate, glucose, paraaffin, sucrose, trehalose and xylrose as carbon sources but not adonitol, adipate, iso-amyllumol, 1-arabinose, cellobiose, *meso* -erythritol, galactose, glucose, 1,2-propanediol, raffinose, rhamnose or sorbitol. Utilizes L-alanine, proline and serine as simultaneous carbon and nitrogen sources, but not acetamide, arginine, gelatin or ornithine.

### Table 1. Differential physiological characteristics of strain IMMB WWCC-22\textsuperscript{T} and other members of the genus *Gordonia*

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Forms smooth, cream coloured colonies on agar media. Cells are rod- and cocoid-like, Gram-positive and non-acid–alcohol-fast. Grows at temperatures between 22–37 °C, but not at 42 °C. Contains the salient chemotaxonomic characteristic of the genus *Gordonia*. Mycolic acids cleave on pyrolysis to release fatty acids of C\textsubscript{16:0} and C\textsubscript{18:0} as the major cleavage products. The fatty acid profile consists mainly of straight-chain saturated, unsaturated and 10-methyl branched fatty acids. Hydrolyses urea and testosterone, but not adenine, casein, elastin, ascinulin, gelatin, guanine, hyoxanthine, tyrosine or xanthine. Assimilates acetate, 2,3-butandiol, citrate, glucose, paraaffin, sucrose, trehalose and xylrose as carbon sources but not adonitol, adipate, iso-amyllumol, 1-arabinose, cellobiose, *meso* -erythritol, galactose, glucose, 1,2-propanediol, raffinose, rhamnose or sorbitol. Utilizes L-alanine, proline and serine as simultaneous carbon and nitrogen sources, but not acetamide, arginine, gelatin or ornithine.

**Description of *Gordonia malaquae* sp. nov.**

The type strain, IMMIB WWCC-22T (= DSM 45064T = CCUG 53555T), was isolated from sludge from a wastewater treatment plant, Taiwan.

Acknowledgements

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References


