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 C_{16:0} and C_{18:0} (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 subline 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-22^{T} (= DSM 45064^{T} = CCUG 53555^{T}).

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-22^{T} 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-22^{T} 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 growth characteristics. 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 diamino-pimelic 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 methanolation 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...
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-22\textsuperscript{T}, 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-22\textsuperscript{T}, 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-22\textsuperscript{T} 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 defluitii (97.5 %), Gordonia rubripertincta (97.4 %), Gordonia desulfuricans, (97.3 %), Gordonia namibiensis (97.3 %), Gordonia alkaniivorans (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-22\textsuperscript{T} 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-22\textsuperscript{T} 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.](image-url)
Strain IMMIB WWCC-22<sup>T</sup> 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 coccoid-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 IMMIB WWCC-22<sup>T</sup> are given in detail in the species description below. The biochemical characteristics determined in this study that distinguish strain IMMIB WWCC-22<sup>T</sup> from *G. desulfuricans* DSM 44462<sup>T</sup>, *G. hydrophobicum* DSM 44015<sup>T</sup> and *G. rubripertincta* DSM 43197<sup>T</sup> are presented in Table 1.

Chemotaxonomically, strain IMMIB WWCC-22<sup>T</sup> possesses chemical markers consistent with its phylogenetic assignment to the genus *Gordonia*. The cell wall contains meso-diaminopimelic acid as well as arabinose and galactose (i.e. wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). One-dimensional TLC of whole-cell acid methanolysates of the novel strain revealed the presence of two lipid spots on the chromatogram. The lower spot corresponded to mycolic acids, as identified from its *R<sub>f</sub>* value (0.55), and the higher spot corresponded to non-hydroxylated fatty acids. Pyrolysis GC of the purified mycolic acid methyl esters from strain IMMIB WWCC-22<sup>T</sup> released fatty acid methyl esters of C<sub>16:0</sub> (29% of total cleavage products) and C<sub>18:0</sub> (71.0%) as pyrolysis cleavage products. GC analyses of the non-hydroxylated fatty acid methyl esters revealed the presence of dodecanoate (0.9% of total fatty acids), tetradecanoate (5.0%), pentadecanoate (0.9%), cis-hexadecenoate (5.0%), hexadecanoate (40.0%), heptadecanoate (1.6%), octadecanoate (14.4%), octadecanoate (18.5%), tuberculostearic acid (10-methyl octadecanoate, 13.2%), eicosanoate (1.0%) and eicosanoate (0.15%) as the major cellular fatty acid methyl esters. Polar lipid analysis showed that the novel strain contains phosphatidylethanolamine, phosphatidyl-inositol, phosphatidylinositol dimannosides, phosphatidyl-glycerol and diphasatidylglycerol as the characteristic phospholipids (i.e. phospholipid type II *sensu* Lechevalier *et al.*, 1977). Mass spectral analysis of the main component from strain IMMIB WWCC-22<sup>T</sup> shows a strong peak at *m/z* 809.5 attributable to [M+Na]<sup>+</sup> in the high mass region. This corresponds to a dihydrogenated menaquinoine with nine isoprene units MK-9(9H2). The second band shows a strong peak at *m/z* 741.58 attributable to [M+Na]<sup>+</sup> in the high mass region. This corresponds to a dihydrogenated menaquinoine with eight isoprene units, MK-8(9H2).

It is apparent from the genotypic and phenotypic data that strain IMMIB WWCC-22<sup>T</sup> represents a novel species of the genus *Gordonia*, for which the name *Gordonia malaquae* is proposed.

**Table 1. Differential physiological characteristics of strain IMMIB WWCC-22<sup>T</sup> and other members of the genus *Gordonia***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Utilization as sole sources of energy and carbon:</td>
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<tr>
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<td>ND</td>
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<td>ND</td>
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<td>–</td>
<td>+</td>
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<tr>
<td>myo-Inositol</td>
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<tr>
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<tr>
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<tr>
<td>Utilization as sole sources of carbon and nitrogen:</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Serine</td>
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</tr>
</tbody>
</table>

It is apparent from the genotypic and phenotypic data that strain IMMIB WWCC-22<sup>T</sup> represents a novel species of the genus *Gordonia*, for which the name *Gordonia malaquae* is proposed.

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

*Gordonia malaquae* (mala’quae. L. adj. malus bad; L. n. aqua water; N.L. gen. n. malaquae of bad water, effluent).

Forms smooth, cream-coloured colonies on agar media. Cells are rod- and coccoid-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<sub>16:0</sub> and C<sub>18:0</sub> 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, ascorbic acid, gelatin, guanine, hypoxanthine, tyrosine or xanthine. Assimilates acetate, 2,3-butanediol, citrate, glucose, paraffin, sucrose, trehalose and xylose as carbon sources but not adonitol, adipect, iso-alamylcohol, 1-arabinose, cellobiose, meso-erythritol, galactose, gluconate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, myo-inositol, lactate, lactose, maltose, mannitol, melezitose, 1,2-propandiol, raffinose, rhamnose or sorbitol. Utilizes L-alanine, proline and serine as simultaneous carbon and nitrogen sources, but not acetamide, arginine, gelatin or ornithine.
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


