Sphingobium scionense sp. nov., an aromatic hydrocarbon-degrading bacterium isolated from contaminated sawmill soil

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This study characterized strain WP01\textsuperscript{T}, a Gram-staining-negative, rod-shaped, aerobic bacterium isolated from a polycyclic aromatic hydrocarbon-contaminated soil in New Zealand. Strain WP01\textsuperscript{T} shared many characteristics of the genus Sphingobium: the predominant respiratory quinone (89\%) was ubiquinone with ten isoprene units (Q-10); the major fatty acids were C_{18:1}ω7c, C_{16:1}ω7c, C_{16:0} and C_{14:0} 2-0H; spermidine was the major polyamine; the DNA G + C content was 63.8 mol\%; and the Sphingobium-specific 16S rRNA signatures were conserved. A point of difference from other species of the genus Sphingobium was that strain WP01\textsuperscript{T} reduced nitrate to nitrite. The polar lipid pattern consisted of the predominant compounds diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipids. 16S rRNA gene sequence analysis showed that, amongst the recognized species of the genus Sphingobium, strain WP01\textsuperscript{T} was most similar to Sphingobium yanoikuyae GIFU 9882\textsuperscript{T} and Sphingobium amienense YTT (\textgt;97\% 16S rRNA gene sequence similarities). The low DNA–DNA relatedness values between strain WP01\textsuperscript{T} and S. yanoikuyae GIFU 9882\textsuperscript{T} (46.6\%) and S. amienense DSM 16289\textsuperscript{T} (25.6\%) indicated no relatedness at the species level. On the basis of these characteristics, it is concluded that strain WP01\textsuperscript{T} should be considered as representing a novel species within the genus Sphingobium, for which the name Sphingobium scionense sp. nov. is proposed. The type strain is WP01\textsuperscript{T} (=DSM 19371\textsuperscript{T} = ICMP 13533\textsuperscript{T}).

Amongst the sphingomonads, many strains have been described that are able to degrade different aromatic hydrocarbons, including naphthalene and biphenyls (Lloyd-Jones & Lau, 1997; Pinyakong et al., 2003; Leys et al., 2004). Biphenyls and naphthalene represent two important classes of environmental pollutants, the polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Strain WP01\textsuperscript{T} was isolated previously from contaminated soil containing oil residues and Pinus radiata sap and sawdust and, prior to the division of the genus Sphingomonas by Takeuchi et al. (2001), had been tentatively identified as belonging to the genus Sphingomonas (Lloyd-Jones & Lau, 1997). In this study, a taxonomic reevaluation of strain WP01\textsuperscript{T} indicated that this strain should be regarded as a member of the genus Sphingobium. The genus Sphingomonas was described by Yabuuchi et al. (1990) as comprising strictly aerobic, chemoheterotrophic, yellow-pigmented, Gram-negative, rod-shaped bacteria that contain glycosphingolipids as cell-envelope components. Takeuchi et al. (1994) described four phylogenetic clusters in the genus, based on 16S rRNA gene sequence data, and subsequently combined phylogenetic, chemotaxonomic and physiological analyses to divide the genus into four genera: Sphingomonas, Sphingobium, Novosphingobium and Sphingopyxis (Takeuchi et al., 2001). Recently, the genus Sphingosinicella (Maruyama et al., 2006) has been added to the family Sphingomonadaceae. Here, we report on the classification of strain WP01\textsuperscript{T} as a novel member of the genus Sphingobium. The type species of this ever-expanding genus is Sphingobium yanoikuyae and species with validly published names include S. amienense (Ushiba et al., 2003), S. aromaticiconvertens (Wittich et al., 2007), S. chlorophenolicum (Takeuchi et al., 2001), S. chungbukense (Pal et al., 2005), S. cloacae (Prakash & Lal, 2006), S. francense (Pal et al., 2005), S. fuliginis (Prakash & Lal, 2006), S. herbicidovorans (Takeuchi et al., 2001), S. indicum (Pal et al., 2005), S. japonicum (Pal et al., 2005), S. olei (Young et al., 2007), S. xenophagum (Pal et al., 2006), S. yanoikuyae (Yabuuchi et al., 1990; Takeuchi et al., 2001), S. ummariense (Singh & Lal, 2009) and S. rhizovicinum (Young et al., 2008).

\textbf{Abbreviation:} PHA, polyhydroxyalkanoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WP01\textsuperscript{T} is EU009209.
The bacterium designated WP01\textsuperscript{T} is an aerobic, non-spore-forming, non-motile, Gram-staining-negative rod that was originally isolated for its ability to degrade phenanthrene. It is also capable of degrading naphthalene, biphenyl, m-xylene and benzoate (Lloyd-Jones & Lau, 1997) in mineral salts medium (Lloyd-Jones et al., 1999). On nutrient agar (per litre: 3 g beef extract, 5 g peptone; Difco), yellow, round, convex and shiny colonies are formed, appearing within 2–4 days at 30 °C, which is the optimal temperature for growth. Strain WP01\textsuperscript{T} is also able to grow in Luria–Bertani (LB) broth (per litre: 10 g tryptone, 5 g yeast extract, 10 g NaCl) and is able to tolerate 0.6 M NaCl in nutrient broth. Strain WP01\textsuperscript{T} is chemoheterotrophic and is able to assimilate the carboxylic acids malate, succinate, d-gluconate and benzoate, the carbohydrates l-arabinose, d-glucose, maltose, galactose, rhamnose and xylose, and the aromatic hydrocarbons m-xylene, biphenyl, naphthalene and phenanthrene. Biochemical characteristics (API 20 NE; bioMérieux) include a positive catalase reaction and the abilities to hydrolyse aesculin, to produce β-galactosidase and to reduce nitrate to nitrite. Compared with S. yanoikuyae JCM 7371\textsuperscript{T}, with which strain WP01\textsuperscript{T} shares a similar catabolic profile, the differences are the ability of strain WP01\textsuperscript{T} to reduce nitrate and its inability to utilize citrate (Table 1). The polyhydroxalkanoate (PHA) content and composition in biomass were determined according to the method of Riis & Mai (1988) and it was shown that strain WP01\textsuperscript{T} was able to accumulate the storage polymer PHA. The dominant ester formed by strain WP01\textsuperscript{T} to reduce nitrate and its inability to utilize citrate is 2-hydroxytetradecanoic acid (C14 : 02-OH; 9.0%). In addition, the following minor fatty acids were identified: C16 : 0 10:5c (1.7%), C17 : 0 10:7c (0.6%), C16 : 0 2-OH (0.7%) and C18 : 1 10:5c (1.3%). The fatty acid profile of strain WP01\textsuperscript{T} contains C16 : 0 10:5c (56.0%) versus 32%, C16 : 1 9.8% versus 8%, C16 : 0 11.9% versus 10% and C14 : 0 2-OH 9.0% versus 34%, for strain WP01\textsuperscript{T} and cluster II respectively (Takeuchi et al., 2001).

Polar lipid and respiratory quinone analyses were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall, DSMZ, Braunschweig, Germany. The dominant respiratory quinone was Q-10 (89%), with lesser amounts of Q-9 (11%). The polar lipid pattern predominately contained diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylylycerol and sphingoglycolipid 1, with lesser amounts of sphingoglycolipid 2. Significantly smaller amounts of phosphatidylcholine and trace amounts of an unidentified aminophospholipid, which migrated close to the origin, were also present. Strain WP01\textsuperscript{T} contained the same major polar lipids as those found in S. yanoikuyae IFO 15102\textsuperscript{T} (Busse et al., 1999); however, phosphatidyl-dimethylethanolamine and an unidentified glycolipid were absent.

Polyamines were extracted and analysed by HPLC as described by Takeuchi et al. (2001). The polyamine pattern of strain WP01\textsuperscript{T} had spermidine as the main polyamine (1.98 µmol g\textsuperscript{-1} wet weight), but homospermidine and putrescine were absent. DNA base compositions were determined by thermal denaturation (Marmur & Doty, 1962) using a spectrophotometer (DU800; Beckman) and the genomic DNA of Escherichia coli K-12 as the standard for the calibration of T\textsubscript{m} values. The DNA G+C content of strain WP01\textsuperscript{T} was 63.8 mol%, which was in accordance with the range described for the genus Sphingobium (61.7–64.9 mol%) (Yabuuchi et al., 1990; Takeuchi et al., 1995, 2001).

An almost full-length 16S rRNA gene sequence from strain WP01\textsuperscript{T} was obtained as described by Rochelle et al. (1995). Analysis of this and other published sequences revealed that strain WP01\textsuperscript{T} was most similar to bacteria previously described as belonging to S. yanoikuyae. Strain WP01\textsuperscript{T} shared 98% 16S rRNA gene sequence similarity with sequences from the S. yanoikuyae strains Q1 (GenBank accession number U37525) and B1 (U37524.1), with which strain WP01\textsuperscript{T} also exhibited sequence similarity in the genes encoding the enzymes required for the catabolism of

### Table 1. Biochemical characteristics of strain WP01\textsuperscript{T} and the type strains of S. amiense and S. yanoikuyae

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<th>Characteristic</th>
<th>1</th>
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<td>Assimilation of:</td>
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<tr>
<td>N-Acetyl-d-glucosamine</td>
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<td>Citrate</td>
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<td>Malate</td>
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<tr>
<td>d-Mannose</td>
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<td>+</td>
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<tr>
<td>β-Galactosidase activity</td>
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<td>Aesculin hydrolysis</td>
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<td>Nitrate reduction</td>
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Strains: 1, WP01\textsuperscript{T}; 2, S. amiense YT\textsuperscript{T}; 3, S. yanoikuyae JCM 7371\textsuperscript{T}. All strains were positive for assimilation of d-glucose and maltose. All strains were negative for urease activity. Data for taxa 2 and 3 were taken from Ushiba et al. (2003).
aromatic compounds (Lloyd-Jones & Lau, 1997). The 16S rRNA gene sequence of strain WP01T had high sequence similarity values with sequences from the type strains of the genus *Sphingobium*, *S. yanoikuyae* GIFU 9882T and *S. amiense* YT7 (97.31 % and 97.16 %, respectively). The 16S rRNA gene sequence from strain WP01T retained the nucleotide signatures that correspond to cluster II (genus *Sphingobium*) as defined by Takeuchi et al. (2001): 52 : 359 U : A, 134 G, 593 U, 987 : 1218 A : U and 990 : 1215 U : G. Phylogenetic analysis was performed by comparison with 16S rRNA gene sequences from other type strains belonging to the genus *Sphingobium*. The sequences were aligned using the CLUSTAL_X algorithm over 1335 aligned positions and a phylogenetic tree was constructed using the maximum-likelihood algorithm with 1000 bootstrap analyses using DNAMAN version 5.2.2 (Lynnon Biosoft). The results (Fig. 1) showed that strain WP01T is related to other species of the genus *Sphingobium* and is most closely related to *S. yanoikuyae* GIFU 9882T and *S. amiense* YT7.

DNA–DNA hybridization was used to compare the similarity of strain WP01T to its closest phylogenetic neighbours. Genomic DNA was extracted from strain WP01T and *S. yanoikuyae* GIFU 9882T and purified according to the method of Marmur (1961) with an additional proteinase K digestion and SDS treatment. DNA–DNA hybridization was carried out according to the methods of De Ley et al. (1970) and Huß et al. (1983) using a Perkin Elmer Lambda 35 UV/VIS spectrometer equipped with a PTP-6 Peltier system. The DNA–DNA relatedness value between strain WP01T and *S. yanoikuyae* GIFU 9882T was 46.6 %, which falls below the value of approximately 70 % that has been suggested as a threshold to delineate bacterial species (Grimont, 1999; Wayne et al., 1987). Additional DNA–DNA hybridization experiments between strain WP01T and *S. amiense* DSM 16289T were carried out by the identification service at DSMZ. The DNA–DNA relatedness value between strain WP01T and *S. amiense* DSM 16289T was 25.6 %.

Strain WP01T shares many characteristics of the genus *Sphingobium* (Takeuchi et al., 2001): it is strictly aerobic and catalase-positive, has Q10 as the predominant ubiquinone and C18:1ω7c as the dominant fatty acid. The DNA G+C content and 16S rRNA gene nucleotide signatures are also consistent with the genus. The ability to reduce nitrate and the low DNA–DNA relatedness values with *S. yanoikuyae* GIFU 9882T and *S. amiense* DSM 16289T suggest that strain WP01T should be considered as representing a novel species within the genus *Sphingobium*, for which the name *Sphingobium scionense* sp. nov. is proposed.

**Description of *Sphingobium scionense* sp. nov.**

*Sphingobium scionense* (sci.o.nen’se. N.L. neut. adj. scion-ense pertaining to Scion, a Crown Research Institute close to the isolation source of the type strain).

Cells are Gram-negative-staining, aerobic, non-spore-forming and non-motile rods measuring 0.5–0.6 × 1.6–2.0 µm that produce yellow, round, convex and shiny colonies within 2–4 days on nutrient agar at 30 °C. Growth is observed at 15–35 °C (optimum 30 °C) but not at 37 °C. Accumulates polyhydroxalkanoate granules in the form of poly-β-hydroxybutyrate from glucose and acetate. Positive for catalase and nitrate reductase. Negative for urease, oxidase and arginine dihydrogenase, indole production, acid production from glucose and assimilation of sorbitol, inositol, malonate, sucrose, lactose, adonitol and raffinose. The dominant fatty acids are C18:1ω7c, C16:1ω7c and C16:0 and the only hydroxy fatty acid is C14:0 2-OH. The dominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipid 1, with lesser amounts of sphingoglycolipid 2. Spermidine is the main polyamine.

The type strain, WP01T (≡DSM 19371T =ICMP 13533T), was isolated from hydrocarbon-contaminated sawmill soil, New Zealand. The DNA G+C content of the type strain is 63.8 mol%.

**Fig. 1.** Phylogenetic tree based on almost-complete 16S rRNA gene sequences (1335 aligned positions) showing the relationship between strain WP01T and type strains of species in the genus *Sphingobium*. The tree was constructed with the maximum-likelihood method. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. *Novosphingobium nitrogenifigens* Y88T was used as the outgroup. Bar, 0.005 substitutions per nucleotide position.
Acknowledgements

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References


