Sphingobium olei sp. nov., isolated from oil-contaminated soil

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The taxonomic status of a yellow-coloured bacterial isolate from an oil-contaminated soil sample was determined using a polyphasic taxonomic approach. Comparative analysis of 16S rRNA gene sequences showed that the novel isolate formed a distinct phyletic line within the genus *Sphingobium*. The generic assignment was confirmed by chemotaxonomic data, which revealed: a fatty acid profile that is characteristic of the genus *Sphingobium* consisting of straight-chain saturated and unsaturated as well as 2-OH fatty acids; a ubiquinone with ten isoprene units (Q-10) as the predominant respiratory quinone; a polar lipid pattern consisting of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylcholine and sphingoglycolipid, and spermidine as the major polyamine component. Genotypic and phenotypic data show that the new isolate merits classification as a representative of a novel species of the genus *Sphingobium*, for which the name *Sphingobium olei* sp. nov. is proposed. The type strain is IMMIB HF-1^T (=DSM 18999^T=CCUG 54329^T).

The genus *Sphingobium* Takeuchi *et al.* (2001) accommodates strictly aerobic, chemo-organotrophic, yellow- to whitish-brown-pigmented, Gram-negative, rod-shaped bacteria. Members of the genus can be characterized chemotaxonomically by having a fatty acid profile that contains C_{18:1}ω7c as the major fatty acid and C_{14:0} 2-OH as the main hydroxylated fatty acid. They contain ubiquinone Q-10 as the main respiratory quinone and spermidine as the major polyamine. The polar lipid pattern consists of diphosphatidylglycerol, phosphatidylglycerol, sphingoglycolipid, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and phosphatidylcholine (Busse *et al.*, 1999; Stolz *et al.*, 2000; Pal *et al.*, 2005; Prakash & Lal, 2006; Wittich *et al.*, 2007). Members of the genus *Sphingobium* represent environmental isolates that play an important role in the bioremediation and biodegradation of pollutants. At the time of writing, the genus *Sphingobium* comprised 12 recognized species. In this paper, the taxonomic characterization of a yellow-pigmented isolate, IMMIB HF-1^T, preliminarily identified as a member of the genus *Sphingobium*, is presented.

Strain IMMIB HF-1^T was isolated on nutrient agar (NA) from oil-contaminated soil near the oil refinery located in Kaohsiung County, Taiwan. The isolate was subsequently cultivated on brain-heart infusion (BHI) agar (Becton Dickinson no. 237100) and tryptone soy agar (TSA; Oxoid CM 131) to determine its morphological characteristics. Fermentation and enzymic tests were performed using the API Coryne, API 20 Strep and API 20E systems and assimilation tests were performed using the API 20NE and API 50CH systems according to the manufacturer's instructions (bioMérieux), except for the time of incubation. All strips were read after 7 days incubation at 37 °C. Salt tolerance was determined by cultivating the organism in tryptone soy broth supplemented with NaCl at final concentrations of 0.0–12.0 %.

The chemotaxonomic characteristics of strain IMMIB HF-1^T were determined by cultivating the organism at 37 °C in shake flasks containing BHI broth for 1 week. At maximum growth, the purity of the culture was checked and the organism was killed with formaldehyde (1 %, v/v), harvested by centrifugation, washed with distilled water and freeze-dried. Lipids were extracted by acid methanolysis as described by Minnikin *et al.* (1980). Fatty acids were analysed as described recently by Yassin *et al.* (2007).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB HF-1^T is AM489507.

Polar lipids were determined using TLC, as described by Yassin *et al.* (1993). Respiratory quinones were extracted and purified according to Collins *et al.* (1977). Mass spectral analyses of the quinones were recorded in positive ion mode on a Q-TOF 2 mass spectrometer (Micromass) equipped with a nanospray source as described by Yassin & Hupfer (2006). For the compounds under study, the major ions observed with electrospray were protonated pseudo-molecular ions, $[M + Na]^+$. The identity of the ubiquinone was verified by observing the diagnostic ion at m/z 197, which represents the benzylium ion. For polyamine analysis, strain IMMIB HF-1^T was grown on PYE medium (0.3% peptone from casein, 0.3% yeast extract, pH 7.2), harvested at approximately 70% of the maximum optical density and, after lyophilization, polyamines were extracted and analysed as described by Busse & Auling (1988) and Stolz *et al.* (2007).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA and the purification of PCR products were carried out according to the methods of Rainey *et al.* (1996). Purified PCR products were sequenced using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) using the manufacturer's protocol. A genetic analyzer (310; Applied Biosystems) was used for electrophoresis of reaction products. The 16S rRNA gene sequences of species of the genus *Sphingobium* with validly described names retrieved from GenBank were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the respective tools of 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 tree were evaluated in bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings.

The almost complete 16S rRNA gene sequence (1471 nt) of strain IMMIB HF-1^T was determined in this study. A tree depicting the phylogenetic affinity of this isolate within the genus *Sphingobium* is shown in Fig. 1. It is evident from the tree that strain IMMIB HF-1^T formed a distinct subline within the genus *Sphingobium*, branching proximal to the base of a subcluster of species that included *Sphingobium cloacae*, *Sphingobium yanoikuyae* and *Sphingobium amiense*. However, bootstrap resampling showed that the association of strain IMMIB HF-1^T with this subcluster of species was not statistically significant and, from the tree construction analysis, it is evident that strain IMMIB HF-1^T does not exhibit a significant affinity with any recognized species. Highest sequence similarities were shown with the type strains of *S. amiense* (97.0%), *Sphingobium xenophagum* (96.7%) and *S. cloacae* (96.5%); other species showed lower levels of similarity. However, sequence divergence values of 3% or greater are considered to be strong evidence that organisms are not related at the species level (Stackebrandt & Goebel, 1994). Support for the distinctiveness of strain IMMIB HF-1^T was also evident from phenotypic analyses (Table 1).

Chemotaxonomically, strain IMMIB HF-1^T possessed chemical markers that support its assignment to the genus *Sphingobium*. Cellular fatty acid analysis revealed the presence of C_{14:0} 2-OH (2.86%) and C_{18:1}ω7c (46.23%) as major hydroxylated and non-hydroxylated fatty acids, respectively. The detailed fatty acid profiles of strain

Fig. 1. Maximum-likelihood tree showing the position of *Sphingobium olei* sp. nov. IMMIB HF-1^T among species of the genus *Sphingobium*. The tree was based on a comparison of 16S rRNA gene sequences that were at least 90% complete (with regard to the *Escherichia coli* sequence). Bar, 5.0% sequence divergence.

Table 1. Differential biochemical characteristics of strain IMMIB HF-1^T and *Sphingobium* species

Strains: 1, *Sphingobium olei* sp. nov. IMMIB HF-1^T; 2, *S. amiense* YT^T; 3, *S. cloacae* JCM 10874^T; 4, *S. yanoikuyae* JCM 7371^T; 5, *S. chlorophenolicum* DSM 8671; 6, *S. herbicidovorans* DSM 11019^T; 7, *S. chungbukense* KCTC 2955^T; 8, *S. indicum* B90A^T; 9, *S. japonicum* UT26^T; 10, *S. francense* Sp + ^T; 11, *S. xenophagum* BN6^T; 12, *S. aromaticum* DSM 12677^T. Data are from the following sources: strain 1, this study; strains 2–6, Ushiba *et al.* (2003); strain 7, Kim *et al.* (2000); strains 8–10, Pal *et al.* (2005); strain 11, Stolz *et al.* (2000) and strain 12, Wittich *et al.* (2007). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Assimilation of:												
D-Glucose	+	+	–	+	+	+	+	+	+	+	+	–
L-Arabinose	+	–	–	+	–	+	+	+	+	+	+	–
Fructose	–	ND	–	–	–	–	+	+	+	–	–	+
D-Mannose	–	–	–	–	–	–	+	–	–	–	–	–
N-Acetylglucosamine	–	–	–	+	–	–	–	ND	ND	ND	–	–
Malate	+	–	–	+	–	+	–	ND	ND	ND	+	–
Gluconate	+	–	–	+	–	+	+	ND	ND	ND	ND	–
Maltose	+	+	–	+	–	+	+	ND	ND	ND	+	–
Aesculin hydrolysis	+	–	–	+	+	+	–	–	+	+	+	–
Nitrate reduction to nitrite	–	–	–	–	–	–	–	ND	ND	ND	ND	–
β-Galactosidase	–	–	–	+	–	–	–	ND	ND	ND	–	+

IMMIB HF-1^T and other *Sphingobium* species are shown in Table 2. Polar lipid analysis showed that the isolate contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylcholine and sphingoglycolipid as characteristic polar lipids (data not shown). Mass spectral analysis of the main isoprenoid components isolated from strain IMMIB HF-1^T showed a strong peak at *m/z* 885.6, attributable to [M+Na]⁺ in the high mass region. This corresponds to ubiquinone with ten isoprene units (Q-10). The polyamine pattern of strain IMMIB HF-1^T contained spermidine as the predominant compound [23.0 µmol (g dry weight)^{–1}] and only trace amounts of 1,3-diaminopropane, putrescine and spermine [≤0.1 µmol (g dry weight)^{–1}]. This polyamine pattern is in agreement with those observed for members of the genus *Sphingobium* (Busse *et al.*, 1999; Takeuchi *et al.*, 2001).

Strain IMMIB HF-1^T was examined for a range of phenotypic characteristics. The organism consisted of Gram-negative, rod-shaped cells. On NA, BHI agar and TSA, colonies were yellow, slightly elevated and smooth with entire margins. The isolate was aerobic and positive in tests for catalase and oxidase. It hydrolysed gelatin, hippurate and aesculin. It had alkaline phosphatase, α-glucosidase, pyrrolidonyl arylamidase, leucine aminopeptidase and pyrazinamidase activities. It had no urease activity and was unable to reduce nitrate to nitrite. The detailed biochemical properties of strain IMMIB HF-1^T are given in the species description below. Differential biochemical characteristics of strain IMMIB HF-1^T and recognized species of the genus *Sphingobium* are given in Table 1.

From the above, based on both phenotypic and phylogenetic criteria, it is suggested that strain IMMIB HF-1^T

represents a novel species within the genus *Sphingobium*, for which the name *Sphingobium olei* sp. nov. is proposed.

Description of *Sphingobium olei* sp. nov.

Sphingobium olei (o'le.i. L. gen. neut. n. *olei* off/from oil, as the organism was isolated from oil-contaminated soil).

Cells are Gram-negative and rod-shaped. On NA, TSA and BHI agar, colonies are yellow and slightly elevated with entire margins. Aerobic, catalase- and oxidase-positive, grows at temperatures of 22–37 °C and tolerates 1.0 % NaCl. Possesses the salient chemotaxonomic characteristics of the genus *Sphingobium*. The detailed fatty acid profile is presented in Table 2. The respiratory quinone consists of ubiquinone with ten isoprene units (Q-10). Contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylcholine, sphingoglycolipid and an unknown phospholipid. Major cellular polyamine is spermidine [23.0 µmol (g dry weight)^{–1}] and trace amounts of 1,3-diaminopropane, putrescine and spermine [≤0.1 µmol (g dry weight)^{–1}] are also present. Hydrolyses gelatin, hippurate and aesculin. Assimilates D-galactose, gentiobiose, D-glucose, glycerol, L-arabinose, arbutin, maltose, potassium gluconate, malic acid, starch, sucrose, trehalose and D-xylose, but not D-adonitol, adipic acid, amygdalin, D-arabinose, D- or L-arabitol, phenylacetic acid, capric acid, D-cellobiose, trisodium citrate, dulcitol, erythritol, fructose, D- or L-fucose, N-acetylglucosamine, glycogen, inositol, inulin, D-lactose, D-lyxose, D-mannose, D-mannitol, D-melezitose, D-melibiose, D-raffinose, ribose, salicin, L-sorbose, D-tagatose, turanose, L-xylose or xylitol. Produces acid from starch, arbutin, D-fucose, gentiobiose, D-glucose, maltose, sucrose, trehalose and D-xylose, but not

Table 2. Cellular fatty acid content of strain IMMIB HF-1^T and species of the genus *Sphingobium*

Strains: 1, *Sphingobium olei* sp. nov. IMMIB HF-1^T; 2, *S. amiense* JCM 11777^T; 3, *S. chlorophenolicum* ATCC 33790^T; 4, *S. chungbukense* DJ77^T; 5, *S. cloacae* JCM 10874^T; 6, *S. francense* CCM 7288^T; 7, *S. herbicidovorans* DSM 11019^T; 8, *S. indicum* CCM 7286^T; 9, *S. japonicum* CCM 7287^T; 10, *S. aromaticiconvertens* RW16^T; 11, *S. xenophagum* DSM 6383^T; 12, *S. yanoikuyae* IFO 15102^T; 13, *S. fuliginis* TKP^T. Data are from the following sources: strain 1, this study; strain 2, Ushiba *et al.* (2003); strains 3, 7, 10–12, Wittich *et al.* (2007); strain 4, Kim *et al.* (2000); strain 5, Fujii *et al.* (2001); strains 6, 8 and 9, Pal *et al.* (2005); strain 13, Prakash & Lal (2006). Values shown are percentages of total fatty acids; –, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
C _{14:0}	7.59	—	5	—	0.1	0.2	—	—	0.2	1.2	—	—	0.45
C _{15:0}	4.93	—	—	—	—	—	—	—	—	—	—	—	0.24
C _{16:0}	8.56	8.0	9.5	12.0	7.2	16.5	7.4	19.0	10.0	7.8	8.0	11.2	8.33
C _{17:0}	1.37	—	—	—	0.3	—	—	—	—	—	—	—	—
C _{18:0}	0.81	—	—	—	0.7	0.4	—	0.9	—	—	—	—	0.30
C _{16:1} ω5 <i>c</i>	—	—	2.4	1.0	3.3	1.2	1.6	0.9	2.2	—	2.0	2.7	1.29
C _{16:1} ω9 <i>c</i>	—	—	—	—	0.7	—	—	—	—	—	—	—	—
C _{17:1} ω6 <i>c</i>	10.85	2.0	6.4	2.0	—	—	1.7	—	—	—	2.5	3.2	1.70
C _{17:1} ω8 <i>c</i>	—	—	—	—	—	—	—	—	—	—	—	—	0.28
C _{17:0} cyclo 7-8	—	—	—	—	—	—	—	—	—	2.7	—	—	—
C _{18:1} ω5 <i>c</i>	—	—	2.5	3.0	1.3	1.1	2.0	1.6	1.4	1.6	1.6	2.1	0.97
C _{12:0} 2-OH	—	—	—	—	—	—	1.0	—	—	—	—	—	—
C _{13:0} 2-OH	—	—	—	—	—	—	2.6	—	—	—	—	—	—
C _{14:0} 2-OH	2.86	7.0	9.4	9.0	2.8	5.1	5.5	6.8	4.3	11.8	6.7	7.1	10.51
C _{15:0} 2-OH	—	—	—	—	—	—	—	—	—	—	—	—	0.44
C _{16:0} 2-OH	—	—	—	—	0.2	0.2	—	0.2	—	—	—	0.9	0.52
Summed features*													
4	16.70	13.0	9.6	6.0	—	—	13.4	0.2	—	—	23.9	16.3	—
7	46.23	62.0	60.1	67.0	80.0	62.5	68.3	55.7	66.7	74.8	55.2	56.0	65.80

*According to GC-MS analysis of fatty acids extracted from strain IMMIB HF-1^T, summed feature 4 corresponds to C_{16:1}ω7*c* and summed feature 7 corresponds to C_{18:1}ω7*c*. According to data received from the Microbial Identification System, summed feature 4 consists of C_{16:1}ω7*c* and/or iso-C_{15:0} 2-OH. Summed feature 7 consists of one or more of C_{18:1}ω7*c*, C_{18:1}ω9*t* and C_{18:1}ω12*t*.

from amygdalin, D- or L-arabinose, D- or L-arabitol, D-cellobiose, dulcitol, erythritol, D-fructose, L-fucose, N-acetylglucosamine, glycerol, glycogen, inositol, inulin, D-lactose, D-lyxose, D-mannitol, D-mannose, D-melezitose, D-melibiose, D-raffinose, L-rhamnose, D-ribose, salicin, L-sorbose, D-sorbitol, D-tagatose, turanose or xylitol. Negative for oxidative fermentation of amygdalin, L-arabinose, D-glucose, inositol, D-mannitol, D-melibiose, L-rhamnose, D-sorbitol and sucrose. Positive for alkaline phosphatase, α-glucosidase, pyrrolidonyl arylamidase, leucine aminopeptidase and pyrazinamidase activities, but negative for arginine dihydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, 2-nitrophenyl β-D-galactopyranoside, 4-nitrophenyl β-D-galactopyranoside, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, nitrate reductase and urease activities. Produces acetoin (Voges-Proskauer reaction), but not indole or H₂S.

The type strain, IMMIB HF-1^T (=DSM 18999^T=CCUG 54329^T), was isolated from oil-contaminated soil near the oil refinery located in Kaohsiung County, Taiwan.

Acknowledgements

We are grateful to Hans-Jürgen Busse for analysis of the polyamine pattern and to Professor Dr Hans-Georg Trüper for nomenclatural advice. This research work was kindly supported by a grant from the National Science Council and the Council of Agriculture, Executive Yuan, Taiwan, Republic of China.

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