Sphingobium vulgare sp. nov., isolated from freshwater sediment

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A Gram-negative, motile, non-spore-forming bacterial strain, designated HU1-GD12^T, was isolated from freshwater sediment. The strain was characterized by using a polyphasic approach in order to determine its taxonomic position. Comparative analysis of the 16S rRNA gene sequence showed that the isolate constituted a distinct branch within the genus *Sphingobium*, showing the highest level of sequence similarity with respect to *Sphingobium ummariense* RL-3^T (96.2%). Strain HU1-GD12^T had a genomic DNA G + C content of 66.8 mol% and Q-10 as the predominant respiratory quinone. Furthermore, the major polyamine component (spermidine) in the cytoplasm and the presence of sphingoglycolipids suggested that strain HU1-GD12^T belonged to the family *Sphingomonadaceae*. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain HU1-GD12^T represents a novel species of the genus *Sphingobium*, for which the name *Sphingobium vulgare* sp. nov. is proposed. The type strain is HU1-GD12^T (=LMG 24321^T=KCTC 22289^T).

The genus *Sphingobium* was proposed by Takeuchi *et al.* (2001) to accommodate three species of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990) with two other new genera, *Novosphingobium* and *Sphingopyxis*, because the genus *Sphingomonas* represented a broad range of heterogeneous species with respect to physiology, phylogenetics and ecology. The nomenclatural system proposed by Takeuchi *et al.* (2001) has been generally accepted and used (Ushiba *et al.*, 2003; Pal *et al.*, 2005, 2006; Prakash & Lal, 2006; Wittich *et al.*, 2007; Young *et al.*, 2007, 2008; Singh & Lal, 2009). At the time of writing, the genus *Sphingobium* comprised 15 species with validly published names.

Sediment from a freshwater lake was investigated for bacterial culturability and diversity by the authors (Lim *et al.*, 2008). One isolate, HU1-GD12^T, was found to be phylogenetically related to members of the genus *Sphingobium*. Sediment samples were collected from a littoral zone of Lake Hakha (36° 16' 60" N, 127° 18' 32" E), a typical freshwater lake in South Korea, and subjected to dilution-to-extinction cultivation (Button *et al.*, 1993) in 96-well plates. Cells in the plates were enriched with unsupplemented lake water media of low nutritional status for

3 months, followed by the detection of extinction wells, as previously described by Lim *et al.* (2008). Single colonies were isolated on 1/10 R2A agar plates and then transferred to R2A agar and cultivated at 30 °C. Cells were maintained in a glycerol suspension (20 %, w/v) at –70 °C.

Colonies were used directly for 16S rRNA gene PCR and amplified fragments were sequenced using the primers 27F and 1100R (Lane, 1991), 341F and 534R (Muyzer *et al.*, 1993) and 907F (Lane *et al.*, 1985). The 16S rRNA gene sequence of strain HU1-GD12^T was assembled with Seqman II 5.0 (DNASTAR) (Swindell & Plasterer, 1997) and compared with all 16S rRNA gene sequences available in the GenBank database. Alignment of 23 sequences was carried out with CLUSTAL_X (Thompson *et al.*, 1997). Gaps at the 5' and 3' ends and ambiguous bases were removed from the alignment using BIOEDIT (Hall, 1999). Phylogenetic trees based on comparison of 1372 bases were constructed by the neighbour-joining (Saitou & Nei, 1987) and the maximum-parsimony (Fitch, 1971) algorithms using MEGA version 3.1 (Kumar *et al.*, 2004). The maximum-likelihood method, employing the DNAML program in the PHYLIP software package (Felsenstein, 1989), was also used for phylogenetic analysis. Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983) and bootstrap values were based on 1000 replications (Felsenstein, 1985). Chromosomal DNA was extracted and purified using Qiagen Genomic-tip system 100/G for determination of G + C content. DNA G + C content was measured as described by Mesbah *et al.* (1989) using a reverse-phase HPLC (Younglin Instrument Co.).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HU1-GD12^T is FJ177535.

An additional phylogenetic tree based on maximum-likelihood analysis of 16S rRNA gene sequences and the polar lipid profiles of strains HU1-GD12^T and *Sphingobium ummariense* RL-3^T are available with the online version of this paper.

The Gram reaction was performed by the non-staining method as described by Buck (1982). Cell morphology was observed under a Nikon light microscope ($\times 1000$) and a transmission electron microscope (model CM-20; Philips) using cells grown for 2 days at 30 °C on R2A agar. For the latter, cells were negatively stained with 1% (w/v) phosphotungstic acid. After air-drying, grids were examined by TEM. Catalase activity was determined by means of bubble production in 3% (v/v) H_2O_2 . Oxidase activity was determined using 1% (w/v) tetramethyl *p*-phenylenediamine. Growth at different temperatures (4, 10, 18, 24, 30, 37 and 45 °C) and at various pH values (pH 4.0–10.0, with intervals of 0.5 pH unit) was assessed after 5 days incubation. Salt tolerance was tested on R2A medium supplemented with 1–10% (w/v) NaCl after 5 days incubation. Growth on nutrient agar (NA) and trypticase soy agar (TSA) was also evaluated at 30 °C.

Physiological characteristics were determined using ID 32GN, API 20NE, API 20E and API ZYM strips according to the manufacturer's instructions (bioMérieux). The results of the API tests were read after 2 days incubation, except for those of the API ZYM kits (4 h). Anaerobic growth was tested on R2A broth containing thioglycolate (1 g l^{-1}) in a serum bottle with the headspace substituted with nitrogen gas. DNase activity was detected on DNase test agar (Scharlau) by flooding culture plates with 1 M HCl. Hydrolysis of starch, chitin, aesculin and skimmed milk were examined according to Atlas (2004). Activities for the degradation of cellulose and xylan were studied according to Ten *et al.* (2004). Nitrate reduction tests were carried out both aerobically and anaerobically in a serum bottle supplemented with 10 mM KNO_3 as an electron donor, and were monitored by an ion chromatograph (Metrohm) equipped with a conductivity detector and an anion exchange column. Isoprenoid quinones were determined as described previously (Komagata & Suzuki, 1987) using Sep-Pak Vac silica cartridges (Waters). Cellular fatty

acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI) after 2 days incubation at 30 °C on R2A. The fatty acids analysed by GC (6890; Hewlett Packard) were identified by the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987). Polyamines were extracted and analysed as described by Busse & Auling (1988) and Schenkel *et al.* (1995).

The 16S rRNA gene sequence of strain HU1-GD12^T determined in this study was 1394 bp in length. Sequence similarity calculations after a simple alignment analysis indicated that strain HU1-GD12^T was closely related to *Sphingobium ummariense* RL-3^T (96.2%), *S. herbicidovorans* MBIC 3166^T (96.1%), *S. japonicum* UT26^T (96.1%) and *S. fuliginis* TKP^T (96.0%). Lower levels of sequence similarities (<96.0%) were found with the other recognized species of the genus *Sphingobium*. The relationship between strain HU1-GD12^T and other members of the genus *Sphingobium* was confirmed in the neighbour-joining phylogenetic tree (Fig. 1) and in the maximum-likelihood phylogenetic tree (see Supplementary Fig. S1 in IJSEM Online). Strain HU1-GD12^T formed a distinct subline within the genus *Sphingobium*.

Strain HU1-GD12^T was Gram-negative staining, strictly aerobic, motile and rod-shaped. Colonies grown on R2A agar plates (Difco) for 3 days were circular, concentric, entire, convex, yellow and 0.5–0.7 mm in diameter. Catalase and oxidase reactions were positive. Strain HU1-GD12^T showed growth on R2A, NA and TSA media. On R2A agar, strain HU1-GD12^T was able to grow at between 18 and 37 °C with an optimal growth temperature of 30 °C. Cells were also cultivated in R2A broth media with

Fig. 1. Neighbour-joining phylogenetic tree, constructed from a comparative analysis of 16S rRNA gene sequences, showing the relationships between strain HU1-GD12^T and related species. Filled circles indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bootstrap values (expressed as percentages of 1000 replications) >65% are shown at the branch points. Bar, 0.01 substitutions per 1 nt position.

the addition of 0–1 % NaCl and showed growth over the pH range 5.5–9.0 (optimum, pH 7.5). Strain HU1-GD12^T was able to grow with only a few carbon source components. The physiological characteristics of strain HU1-GD12^T are summarized in the species description and comparisons of characteristics with closely related type strains are shown in Table 1.

The G+C DNA content of strain HU1-GD12^T was 66.8 mol%. The fatty acids of strain HU1-GD12^T (Table 2) mainly comprised one or more of C_{18:1}ω7c, C_{18:1}ω9c, C_{18:1}ω9t and C_{18:1}ω12t (included within summed feature 7) and C_{16:0}. The major polyamine component was spermidine. The major isoprenoid quinone was ubiquinone Q-10. The polar lipid profiles of strain HU1-GD12^T and the closely related strain *S. ummariense* RL-3^T were obtained (see Supplementary Fig. S2). Differences in the presence of unknown glycolipids (GL1–3) and phosphatidylcholine, together with differences in the amounts of diphosphatidylglycerol, clearly distinguished strains HU1-GD12^T and RL-3^T from each other. These chemotaxonomical data supported the affiliation of strain HU1-GD12^T to the genus *Sphingobium*.

All of the characteristics determined for strain HU1-GD12^T were in accordance with those expected for a member of the genus *Sphingobium*. However, the phylogenetic distances from recognized species of the genus *Sphingobium* and the combination of unique phenotypic characteristics indicated that strain HU1-GD12^T was not affiliated to any recognized species of the genus. Therefore, it is concluded that strain HU1-GD12^T represents a novel species of the genus *Sphingobium*, for which the name *Sphingobium vulgare* sp. nov. is proposed.

Table 1. Physiological characteristics of strain HU1-GD12^T and related type strains of species of the genus *Sphingobium*

Strains: 1, HU1-GD12^T; 2, *S. ummariense* RL-3^T; 3, *S. fuliginis* TKP^T (data for all strains are from this study). +, Positive; –, negative. All strains showed positive results for assimilation of D-glucose and negative results for arginine dihydrolase, urease, gelatin hydrolysis, acid production from glucose, nitrate reduction and assimilation of N-acetylglucosamine, caprate, trisodium citrate, L-fucose, malate and D-mannose.

Characteristic	1	2	3
Indole production	–	–	+
Enzyme activity:			
β-Glucosidase	–	+	+
β-Galactosidase	–	+	–
Assimilation of:			
Maltose	–	+	+
L-Arabinose	–	+	+
Potassium gluconate	–	+	+
Sucrose	+	+	–
Salicin	–	+	–
Melibiose	–	+	–

Table 2. Cellular fatty acid profiles of strain HU1-GD12^T and related type strains of the genus *Sphingobium*

Strains: 1, HU1-GD12^T; 2, *S. ummariense* RL-3^T; 3, *S. fuliginis* TKP^T (data for all strains are from this study). Values shown are percentages of total fatty acids. –, Not detected.

Fatty acid	1	2	3
C _{16:0}	15.08	14.00	16.57
C _{16:1} ω5c	–	1.42	–
C _{17:1} ω6c	–	2.10	–
C _{14:0} 2-OH	5.51	9.61	8.36
Summed features*			
4	1.71	4.10	5.89
7	77.70	66.60	69.18

*Data for all strains are based on the Microbial Identification System. Summed feature 4 consists of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 7 consists of one or more of C_{18:1}ω7c, C_{18:1}ω9c, C_{18:1}ω9t and C_{18:1}ω12t.

Description of *Sphingobium vulgare* sp. nov.

Sphingobium vulgare (vul.ga're. L. neut. adj. *vulgare* common, referring to the lack of specific characteristics).

Cells are Gram-negative staining, strictly aerobic, motile, rod-shaped, 0.4–0.6 μm in diameter and 1.0–2.5 μm in length. Colonies grown on R2A agar for 3 days are circular, concentric, entire, convex and yellow. Catalase- and oxidase-positive. Grows at 18–37 °C, pH 5.5–9.0 and with 0–1 % (w/v) NaCl. Growth occurs on NA or TSA media. Nitrate is not reduced. Sphingoglycolipid is present. Spermidine is the major polyamine component. The major isoprenoid quinone is ubiquinone Q-10. Casein, cellulose, chitin, DNA, starch and xylan are not degraded. H₂S production and acetoin production from pyruvate are not observed. No acid is produced from amygdalin, L-arabinose, inositol, D-mannitol, D-melibiose, L-rhamnose, D-sorbitol or D-sucrose. Activities for lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are not present. 2-Naphthyl phosphate (pH 8.5), naphthol-AS-BI-β-D-glucuronide and naphthol-AS-BI-phosphate are hydrolysed, but the following substances are not hydrolysed: 1-nitrophenyl-N-acetyl-β-D-glucosaminide, 2-naphthyl butyrate, 2-naphthyl caprylate, 2-naphthyl myristate, 2-naphthyl phosphate (pH 5.4), 2-naphthyl α-L-fucopyranoside, 2-naphthyl α-D-glucopyranoside, 2-naphthyl β-D-galactopyranoside, 6-bromo-2-naphthyl α-D-galactopyranoside, 6-bromo-2-naphthyl β-D-glucopyranoside, 6-bromo-2-naphthyl α-D-mannopyranoside, L-cystyl 2-naphthylamide, L-leucyl 2-naphthylamide, L-valyl 2-naphthylamide, N-benzoyl-DL-arginine 2-naphthylamide and N-glutaryl-phenylalanine 2-naphthylamide. D-Glucose and D-sucrose are the only substances that can be utilized as sole carbon source. The following compounds tested in this study are not assimilated: L-alanine, β-acetylglucosamine, glycogen, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, inositol, itaconate,

2-ketogluconate, lactate, malonate, D-mannitol, L-proline, phenylacetate, propionate, D-ribose, L-rhamnose, L-serine, D-sorbitol, suberate and valerate. Additional physiological characteristics and detailed fatty acid profiles are presented in Tables 1 and 2, respectively.

The type strain, HU1-GD12^T (=LMG 24321^T=KCTC 22289^T), was isolated from freshwater sediment of Lake Hakha, South Korea. The G+C content of genomic DNA is 66.8 mol% (as determined by HPLC).

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