Olivibacter soli sp. nov., *Olivibacter ginsengisoli* sp. nov. and *Olivibacter terrae* sp. nov., from soil of a ginseng field and compost in South Korea

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Three novel strains, designated Gsoil 034^T, Gsoil 060^T and Jip13^T, isolated from soil from a ginseng field and compost in South Korea, were characterized using a polyphasic approach to clarify their taxonomic positions. These isolates were found to be Gram-negative, aerobic and heterotrophic, non-spore-forming, non-motile and rod-shaped. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these three isolates formed a cluster with the monospecific genus *Olivibacter* within the family *Sphingobacteriaceae* but were clearly separated from *Olivibacter sitiensis*. The 16S rRNA gene sequence similarities between these isolates and the type strain of *O. sitiensis* were in the range 88.5–90.1 %. Phenotypic and chemotaxonomic data (MK-7 as the major isoprenoid quinone and iso-C_{15:0} 2-OH and/or C_{16:1ω7c}, iso-C_{15:0} and iso-C_{17:0} 3-OH as the major fatty acids) supported the affiliation of these strains to the genus *Olivibacter*. However, the results of physiological and biochemical tests allowed phenotypic differentiation of the isolates from *Olivibacter* species with validly published names. Therefore strains Gsoil 034^T, Gsoil 060^T and Jip13^T represent three novel species of the genus *Olivibacter*, for which the names *Olivibacter soli* sp. nov. (type strain Gsoil 034^T = KCTC 12645^T = LMG 23492^T), *Olivibacter ginsengisoli* sp. nov. (type strain Gsoil 060^T = KCTC 12646^T = LMG 23491^T) and *Olivibacter terrae* sp. nov. (type strain Jip13^T = KCTC 12644^T = LMG 23494^T) are proposed.

The genus *Olivibacter* of the family *Sphingobacteriaceae* was proposed by Ntougias *et al.* (2007), and, at the time of writing, comprises just one species, *Olivibacter sitiensis*. In this study, we report on the taxonomic characterization of three *Olivibacter*-like bacterial strains, Gsoil 034^T, Gsoil 060^T and Jip13^T.

Strains Gsoil 034^T and Gsoil 060^T were isolated from soil from a ginseng field in Pocheon Province, South Korea. The soil sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0), serially diluted in the same buffer and then spread on one-fifth-strength modified R2A plates containing the following (l⁻¹): 0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g Casamino acids, 0.25 g soytone,

0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K₂HPO₄, 0.05 g MgSO₄, 0.05 g CaCl₂ and 15 g agar. The plates were incubated at 30 °C for 1 month. Single colonies on the plates were purified by transferring them onto new plates for incubation once again on full-strength modified R2A or on one-half-strength modified R2A. Strain Jip13^T was originally isolated from compost, composed of cow dung and rice straw, collected in Chungwon Province, South Korea. The compost sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0), serially diluted and then spread onto R2A agar (Difco) plates. The plates were incubated at 30 °C for 3 weeks. Single colonies on the plates were purified by transferring them onto new plates. The purified colonies were tentatively identified using partial sequences of the 16S rRNA genes (Im *et al.*, 2003, 2005). As strains Gsoil 034^T, Gsoil 060^T and Jip13^T could grow well both on full-strength modified R2A agar and on commercial R2A

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Jip13^T, Gsoil 034^T and Gsoil 060^T are AB267714, AB267715 and AB267716, respectively.

agar (Difco), they were routinely cultured on R2A agar at 30 °C and maintained as glycerol suspensions (20 %, w/v) at -70 °C.

Extraction of genomic DNA was done using a commercial genomic DNA extraction kit (Core Biosystem). The 16S rRNA genes were amplified from the chromosomal DNA using the universal bacterial primer set 9F and 1512R; the purified PCR products were sequenced by Solgent Co. Ltd (Daejeon, Republic of Korea) (Kim *et al.*, 2005). Full sequences of the 16S rRNA genes were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using the CLUSTAL_X program (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using a neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) with the MEGA3 program (Kumar *et al.*, 2004); bootstrap percentages were calculated from 1000 replications (Felsenstein, 1985).

Almost-complete 16S rRNA gene sequences (1469, 1483 and 1482 bp) were determined for strains Gsoil 034^T, Gsoil 060^T and Jip13^T and subjected to comparative analyses. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these isolates are clustered with *O. sitiensis* AW-6^T within the family *Sphingobacteriaceae* (Fig. 1). The 16S rRNA gene sequence similarity between these isolates and *O. sitiensis* AW-6^T ranged from 88.5 to 90.1 %. The three strains showed 93.9–94.7 % sequence similarity with respect to each other, suggesting that they differ at the species level (Stackebrandt & Goebel, 1994).

Gram-reaction testing was performed by using the non-staining method, as described by Buck (1982). Cell morphology was observed at ×1000 magnification with a light microscope (E600; Nikon) using cells grown for 3 days at 30 °C on R2A agar. Catalase activity was determined from bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Carbon-source utilization and enzyme activities were tested by using API 20NE, API ID 32GN and API ZYM test kits (bioMérieux). Growth at different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and pH (pH 5.0–10.0, in increments of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar and MacConkey agar was also evaluated, at 30 °C.

Morphological observation of strains Gsoil 034^T, Gsoil 060^T and Jip13^T grown on R2A agar plates for 5 days showed that the cells were similar to each other, i.e. all were non-motile rods, 0.6–0.9 µm wide and 0.9–1.8 µm long. Colonies of the three strains grown on R2A agar were also similar to each other, i.e. smooth, circular, convex, opaque, yellowish and 1.0–2.0 mm in diameter. All three strains grew on nutrient agar and trypticase soy agar but none grew on MacConkey agar. Strains Gsoil 034^T and Gsoil 060^T reduced nitrate to nitrite, unlike strain Jip13^T. Other physiological characteristics of strains Gsoil 034^T, Gsoil 060^T and Jip13^T are summarized in the species descriptions. Phenotypic characteristics that serve to differentiate these strains from their closest phylogenetic relatives are listed in Table 1.

To measure the G+C content of the chromosomal DNA, genomic DNA from the novel strains was extracted and

Fig. 1. Rooted phylogenetic tree, based on 16S rRNA gene sequences, for strains Gsoil 034^T, Gsoil 060^T and Jip13^T and related taxa in the family *Sphingobacteriaceae*. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with a Kimura two-parameter distance matrix (Kimura, 1983) and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 60 % are shown at branch points. Bar, 2 substitutions per 100 nucleotide positions.

Table 1. Comparison of the phenotypic characteristics of strains Gsoil 034^T, Gsoil 060^T and Jip13^T and *O. sitiensis* AW-6^T

Data for *O. sitiensis* AW-6^T were taken from Ntougias *et al.* (2007). All strains were positive for catalase and for assimilation of sucrose and glucose. All were negative for Gram stain, sporulation and assimilation of acetate, citrate, serine and tryptophan.

Characteristic	Gsoil 034 ^T	Gsoil 060 ^T	Jip13 ^T	<i>O. sitiensis</i> AW-6 ^T
Growth at 42 °C	—	—	—	+
Growth with 4 % NaCl	+	+	+	—
Oxidase activity	+	—	+	+
Assimilation of:				
Mannitol	—	+	+	—
Inositol	—	+	—	—
L-Histidine	—	—	—	+
Maltose	+	+	+	—
Sorbitol	—	—	—	+
DNA G + C content (mol%)	39.2	43.6	40.6	45.6

purified, as described by Moore & Dowhan (1995), enzymically degraded into nucleosides and then determined as described by Mesbah *et al.* (1989), using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and then re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac cartridges silica (Waters) and subsequently analysed by HPLC as described previously (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for strains grown on one-tenth-strength trypticase soy agar (Difco) for 3 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by gas chromatography (6890; Hewlett Packard) were identified using the Microbial Identification software package (Sasser, 1990).

The DNA G + C contents of strains Gsoil 034^T, Gsoil 060^T and Jip13^T were 39.2, 43.6 and 40.6 mol%, respectively. The major respiratory quinone was MK-7. As shown in Table 2, the major fatty acids in all three strains were summed feature 4 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), iso-C_{15:0} and iso-C_{17:0} 3-OH, which is consistent with the pattern for *O. sitiensis*. However, some qualitative and quantitative differences in the fatty acid composition served to distinguish these three strains from each other and also from *O. sitiensis* (Table 2).

As the 16S rRNA gene sequence difference used as the threshold for defining a novel genomic species (Stackebrandt & Goebel, 1994) is 3 %, the sequence similarities (88.5–90.1 %) for the three strains with respect to *O. sitiensis* seem somewhat low for clustering together within the same genus. However, few genus-specific characteristics, which are mainly defined by chemotaxonomic data (predominant isoprenoid quinone, fatty acid profile and DNA G + C content), were found in this study. Likewise, sequence similarity values for *Sphingobacterium*

species and *Pedobacter* species are also relatively low: the lowest sequence similarity value, between the type strains of *Sphingobacterium spiritivorum* and *Sphingobacterium thalpophilum*, is only 90.8 %, while the level of similarity between the type strains of *Pedobacter himalayensis* and *Pedobacter sandarakinus* is 92.3 %. Therefore, on the basis of the data and observations described above, it is appropriate to conclude that strains Gsoil 034^T, Gsoil 060^T and Jip13^T should be assigned to the genus *Olivibacter* as the type strains of three novel species, for which the names *Olivibacter soli* sp. nov., *Olivibacter ginsengisoli* sp. nov. and *Olivibacter terrae* sp. nov., respectively, are proposed.

Description of *Olivibacter soli* sp. nov.

Olivibacter soli (so'li. L. gen. n. *soli* of the soil).

Cells are 0.6–0.8 × 1.5–1.8 µm in size. Colonies grown on R2A agar plates are smooth, circular, convex, non-luminescent, opaque and yellowish in colour within 5 days at 30 °C. Growth is observed at temperatures of 15–37 °C, at pH 6.0–8.0 and with 0–4 % (w/v) NaCl. The optimal growth temperature and pH are 30 °C and pH 6.5–7.5. *N*-Acetyl-D-glucosamine, L-alanine, arabinose, L-fucose, glycogen, gluconate, D-mannose, melibiose, rhamnose and D-ribose are assimilated. Positive for hydrolysis of aesculin and gelatin and for *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, arginine dihydrolase, cystine arylamidase, α-fucosidase, α-glucosidase, β-glucosidase, leucine arylamidase, urease and valine arylamidase and glucose fermentation. Negative reactions are observed for esterase (C4), esterase lipase (C8), lipase (C14), naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase and α-mannosidase and for assimilation of adipate, caprate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, 2-keto-gluconate, 5-ketogluconate, lactate, malate, malonate, phenylacetate, L-proline, propionate, salicin, suberate and

Table 2. Fatty acid profiles of strains Gsoil 034^T, Gsoil 060^T and Jip13^T and *O. sitiensis* AW-6^T

Data for *O. sitiensis* AW-6^T were taken from Ntougias *et al.* (2007). Values are percentages of total fatty acids; —, not detected.

Fatty acid	Gsoil 034 ^T	Gsoil 060 ^T	Jip13 ^T	<i>O. sitiensis</i> AW-6 ^T
Saturated				
C _{14:0}	—	—	2.0	0.5
C _{16:0}	5.7	10.4	7.7	5.5
C _{18:0}	1.3	1.5	—	—
Unsaturated				
C _{16:1} ω5c	1.3	—	—	1.3
Branched-chain				
iso-C _{15:0}	32.5	33.6	35.9	29.3
iso-C _{15:0} 3-OH	2.2	3.3	2.6	1.9
anteiso-C _{15:1}	1.1	—	—	—
iso-C _{15:1} F	—	—	—	0.3
iso-C _{17:0}	—	—	0.8	0.4
iso-C _{17:0} 3-OH	12.1	12.4	11.5	11.2
iso-C _{17:1} ω9c	1.5	0.9	1.2	2.0
anteiso-C _{17:1} B and/or iso-C _{17:1} I	—	—	—	0.5
Hydroxy				
C _{16:0} 2-OH	1.8	2.4	2.6	0.9
C _{16:0} 3-OH	1.4	2.0	1.1	0.7
C _{16:1} 2-OH	—	—	—	0.6
Unknown*				
ECL 13.565	—	—	—	0.9
ECL 13.961	1.2	2.1	—	—
ECL 16.582	—	—	—	1.1
Summed feature 4†	37.3	31.6	34.7	43.2

*Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified. ECL, Equivalent chain-length.

†Summed feature 4 comprises iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

valerate. The DNA G+C content of the type strain is 39.2 mol%.

The type strain, Gsoil 034^T (=KCTC 12645^T =LMG 23492^T), was isolated from soil from a ginseng field in Pocheon Province, South Korea.

Description of *Olivibacter ginsengisoli* sp. nov.

Olivibacter ginsengisoli (gin.seng.i.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field).

Cells are 0.7–0.9 × 1.2–1.6 µm. Colonies grown on R2A agar plates are smooth, circular, convex, non-luminescent, opaque and yellowish in colour within 5 days at 30 °C. Growth is observed at temperatures of 15–30 °C, at pH 6.0–8.0 and with 0–4% (w/v) NaCl. The optimal growth temperature and pH are 30 °C and pH 6.8–7.5. *N*-Acetyl-D-glucosamine, arabinose, gluconate, D-mannose, melibiose, rhamnose and salicin are assimilated. Positive for hydrolysis of aesculin and gelatin and *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, arginine dihydrolase, esterase (C4), α-galactosidase,

α-glucosidase, β-glucosidase, leucine arylamidase, urease and valine arylamidase and for glucose fermentation. Negative reactions are observed for cystine arylamidase, esterase lipase (C8), α-fucosidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase and α-mannosidase and for assimilation of adipate, L-alanine, caprate, L-fucose, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, 2-keto-gluconate, 5-ketogluconate, lactate, malate, malonate, phenylacetate, L-proline, propionate, D-ribose, suberate and valerate. The DNA G+C content of the type strain is 43.6 mol%.

The type strain, Gsoil 060^T (=KCTC 12646^T =LMG 23491^T), was isolated from soil from a ginseng field in Pocheon Province, South Korea.

Description of *Olivibacter terrae* sp. nov.

Olivibacter terrae (ter'rae. L. gen. n. *terrae* of the earth).

Cells are 0.6–0.8 × 0.9–1.3 µm. Colonies grown on R2A agar plates are smooth, circular, convex, non-luminescent, opaque and yellowish in colour within 5 days at 30 °C.

Growth is observed at temperatures of 15–30 °C, at pH 6.0–8.0 and with 0–4 % (w/v) NaCl. The optimal growth temperature and pH are 30 °C and pH 7.0. *N*-Acetyl-D-glucosamine, arabinose, glycogen, gluconate, D-mannose, rhamnose and D-ribose are assimilated. Positive for hydrolysis of aesculin and gelatin and for *N*-acetyl- β -glucosaminidase, acid phosphatase, alkaline phosphatase, arginine dihydrolase, cystine arylamidase, esterase (C4), esterase lipase (C8), α -galactosidase, α -glucosidase, β -glucuronidase, β -glucuronidase, leucine arylamidase, urease and valine arylamidase and glucose fermentation. Negative reactions are observed for α -fucosidase, β -galactosidase, lipase (C14), naphthol-AS-BI-phosphohydrolase and α -mannosidase and for assimilation of adipate, L-alanine, caprate, L-fucose, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, 2-ketogluconate, 5-ketogluconate, lactate, malate, malonate, melibiose, phenylacetate, L-proline, propionate, salicin, suberate and valerate. The DNA G + C content of the type strain is 40.6 mol%.

The type strain, Jip13^T (=KCTC 12644^T =LMG 23494^T), was isolated from compost, composed of cow dung and rice straw, collected in Chungwon Province, South Korea.

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