Rhodanobacter ginsenosidimutans sp. nov., isolated from soil of a ginseng field in South Korea

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A novel gammaproteobacterium, designated Gsoil 3054^T, was isolated from soil of a ginseng field in Pocheon province, South Korea, and was characterized using a polyphasic approach to determine its taxonomic position. The strain was Gram-negative, aerobic, non-motile, non-spore-forming and rod-shaped. Phylogenetic analysis based on 16S rRNA gene sequences showed that the strain belonged to the genus *Rhodanobacter*; it was most closely related to *Rhodanobacter fulvus* Jip2^T and *Rhodanobacter thiooxydans* LCS2^T (97.9 and 97.2 % 16S rRNA gene sequence similarity, respectively). Chemotaxonomic data, i.e. Q-8 as the predominant ubiquinone and iso-C_{15:0}, 10-methyl C_{16:0} and iso-C_{17:0} as major fatty acids, also supported classification of strain Gsoil 3054^T in the genus *Rhodanobacter*. However, DNA–DNA hybridization values of Gsoil 3054^T with *R. fulvus* Jip2^T and *R. thiooxydans* LCS2^T were 45 and 31 %, respectively. Moreover, physiological and biochemical tests enabled strain Gsoil 3054^T to be differentiated phenotypically from other established species of *Rhodanobacter*. Therefore, the isolate represents a novel species, for which the name *Rhodanobacter ginsenosidimutans* sp. nov. is proposed; the type strain is Gsoil 3054^T (=KACC 12822^T =DSM 21013^T =KCTC 22231^T =LMG 24457^T).

The genus *Rhodanobacter* was originally described by Nalin *et al.* (1999) on the basis of a lindane-degrading bacterium isolated from soil of a wood-treatment site. Subsequently, however, the only known deposit of the type strain of the type species of the genus, *Rhodanobacter lindaniclasticus* LMG 18385^T, could not be recovered, and the strain no longer exists (Mergaert *et al.*, 2002). In recent years, several novel species have been classified as members of the genus *Rhodanobacter*, which at the time of writing comprises *R. lindaniclasticus* and five further species with validly published names: *Rhodanobacter fulvus* (Im *et al.*, 2004), *R. spathiphylli* (De Clercq *et al.*, 2006), *R. thiooxydans* (Lee *et al.*, 2007) and *R. ginsengisoli* and *R. terrae* (Weon *et al.*, 2007). In this paper, a polyphasic taxonomic approach was used to characterize a novel *Rhodanobacter*-like strain (Gsoil 3054^T) from soil of a ginseng field in Pocheon province, South Korea.

The soil sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0) and the suspension was spread on one-fifth-strength modified R2A agar plates [full-strength modified R2A agar contained (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 (agar amount is constant irrespective of medium strength)] after serial dilution with 50 mM phosphate buffer (pH 7.0). Plates were incubated at 30 °C for 1 month. Single colonies on the plates were purified by transferring them onto new plates of full- or half-strength modified R2A followed by incubation at 30 °C. Purified colonies were tentatively identified from partial 16S rRNA gene sequences (Im *et al.*, 2005). They were routinely cultured on R2A agar (Difco) at 30 °C and maintained as glycerol suspensions (20 %, w/v) at –70 °C.

The Gram reaction was performed by the non-staining method as described by Buck (1982). Morphology of cells grown for 3 days at 30 °C on R2A agar was observed under a Nikon light microscope at ×1000. Motility was determined with an optical microscope using the hanging drop technique (Skerman, 1967). Catalase activity was determined by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetra-methyl *p*-phenylenediamine. Carbon source utilization and enzyme activities were tested by using the API 20NE, API ID32 GN and API ZYM test kits (bioMérieux). Anaerobic growth was assessed in serum bottles containing R2A broth and sodium thioglycollate (1 g l⁻¹) with the upper air layer

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 3054^T is EU332826.

replaced with nitrogen gas. Growth at different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and pH (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 10 days incubation in R2A broth. For the pH experiments, three different buffers were used (final concentration, 50 mM): acetate buffer for pH 5.0–5.5, phosphate buffer for pH 6.0–8.0 and Tris buffer for pH 8.5–10.0. Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl and incubated for 5 days at 30 °C. Growth on nutrient agar and trypticase soy agar (TSA) was also evaluated at 30 °C.

Cells of strain Gsoil 3054^T were aerobic, Gram-negative, non-motile, non-spore-forming rods. Colonies grown on R2A agar plates for 3 days were smooth, circular, yellowish and 1.5–4.0 mm in diameter. The isolate grew well on nutrient agar and TSA. Other physiological characteristics of strain Gsoil 3054^T are summarized in the species description and a comparison of selective characteristics of strain Gsoil 3054^T with those of closely related type strains is shown in Table 1.

For measurement of the chromosomal DNA G+C content, genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995) and enzymically degraded into nucleosides; G+C content was 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 re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane/quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed by HPLC as described previously (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for strains grown on TSA (Difco) for 5 days. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acids were analysed by GC (Hewlett Packard 6890) and identified by the Microbial Identification software package (Sasser, 1990).

The genomic DNA G+C content of strain Gsoil 3054^T was 65.5 mol%. Q-8 was the major ubiquinone. As shown in Table 2, the major fatty acids of strain Gsoil 3054^T were iso-C_{15:0} (22.6 %), 10-methyl C_{16:0} (20.7 %) and iso-C_{17:0} (12.2 %), which is consistent with fatty acid data for *R. fulvus* and *R. thiooxydans*; however, qualitative and quantitative differences in fatty acid compositions distinguished strain Gsoil 3054^T from *R. fulvus* and *R. thiooxydans* (Table 2).

Extraction of genomic DNA was performed with a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Ten *et al.* (2008). Full 16S rRNA gene sequences were compiled using SEQMAN software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with CLUSTAL_X (Thompson *et al.*, 1997) and

Table 1. Phenotypic characteristics of strain Gsoil 3054^T and related taxa

Strains: 1, strain Gsoil 3054^T; 2, *R. fulvus* Jip2^T; 3, *R. thiooxydans* LCS2^T; 4, *R. spathiphylli* B39^T; 5, *R. lindaniclasticus* RP5557^T; 6, *R. terrae* GP18-1^T; 7, *R. ginsengisoli* GR17-7^T. All strains are positive for catalase and oxidase. All strains are negative for glucose fermentation, indole production, arginine dihydrolase and urease. All strains assimilate D-glucose, but not L-arabinose, D-mannitol, potassium gluconate, phenylacetic acid, L-rhamnose, inositol, sodium malonate, potassium 5-ketogluconate or D-sorbitol. Data for taxa 2–7 are from Lee *et al.* (2007) and Weon *et al.* (2007). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Motility	–	+	–	–	–	–	+
Nitrate reduction	–	–	+	–	–	–	–
Protease (gelatin)	–	–	+	–	ND	–	+
Esterase (C4)	+	–	+	–	ND	+	+
Esterase lipase (C8)	+	+	–	+	ND	+	+
α-Galactosidase	+	+	–	–	ND	–	–
β-Galactosidase	+	+	+	–	–	+	–
β-Glucuronidase	+	+	–	–	ND	–	–
N-Acetyl-β-glucosaminidase	+	+	–	+	ND	–	+
Utilization of:							
D-Mannose	+	+	–	–	–	+	–
N-Acetylglucosamine	+	+	+	+	–	+	+
Inositol	+	–	–	–	–	–	–
Maltose	+	+	+	+	–	+	+
Sodium acetate	–	–	–	+	ND	–	+
Melibiose	+	+	–	–	–	–	–
L-Fucose	–	–	–	–	–	+	–
D-Ribose	–	–	+	–	–	–	–
Sucrose	+	+	–	–	–	–	–
Glycogen	–	–	+	–	–	–	–
Salicin	+	+	–	–	–	–	–
Valerate	+	–	+	+	ND	–	+
DNA G+C content (mol%)	65.5	65.3	64.6	67.6	63.0	62.5	61.0

gaps were edited with BIOEDIT (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using MEGA3 (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985). DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and means of the remaining three values are quoted as relatedness values.

The nearly complete 16S rRNA gene sequence (1504 bp) of strain Gsoil 3054^T was determined and subjected to

Table 2. Cellular fatty acid profiles of strain Gsoil 3054^T and related taxa

All data are from this study. Strains were grown for 5 days on TSA. —, <1 % or not detected.

Fatty acid	Strain Gsoil 3054 ^T	<i>R. fulvus</i> Jip2 ^T	<i>R. thiooxydans</i> LCS2 ^T
iso-C _{11:0}	4.5	4.1	4.3
Unknown (ECL 11.798)*	1.6	2.0	1.9
iso-C _{11:0} 3-OH	3.8	3.7	3.5
iso-C _{14:0}	—	—	1.3
iso-C _{13:0} 3-OH	3.8	3.7	2.8
iso-C _{15:0}	22.6	19.5	20.8
anteiso-C _{15:0}	3.1	3.2	5.9
iso-C _{16:0}	1.6	2.4	7.0
Summed feature 4†	4.8	3.2	4.2
C _{16:0}	11.8	12.4	3.5
10-Methyl C _{16:0}	20.7	9.4	24.5
C _{16:0} 3-OH	6.2	14.9	1.1
iso-C _{17:0}	12.2	12.0	14.0
C _{17:0} cyclo	1.4	4.5	—
C _{19:0}	—	—	1.0

*ECL, Equivalent chain-length (i.e. the identity of the fatty acid is unknown).

†Summed feature 4 contains iso-C_{15:0} 2-OH and/or C_{16:1}ω7clt.

comparative analysis. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain Gsoil 3054^T belonged to the genus *Rhodanobacter* (Fig. 1). The closest phylogenetic neighbours of strain Gsoil 3054^T were *R. fulvus* Jip2^T (97.9 % sequence similarity) and *R. thiooxydans* LCS2^T (97.2 %), followed by *R. spathiphylli* B39^T (96.7 %), *R. lindaniclasticus* RP5557^T (96.2 %), *R. terrae* GP18-1^T (95.5 %) and *R. ginsengisoli* GR17-7^T (95.0 %). DNA–DNA relatedness values of strain Gsoil 3054^T with *R. fulvus* Jip2^T and *R. thiooxydans* LCS2^T were 45 and 31 %, respectively.

These low values, together with 16S rRNA gene sequence similarities of less than 97 % with other strains, suggest that strain Gsoil 3054^T represents a novel species in the genus *Rhodanobacter* according to Wayne *et al.* (1987) and Stackebrandt & Goebel (1994).

In conclusion, phylogenetic analysis based on 16S rRNA gene sequences indicated that strain Gsoil 3054^T belongs to the genus *Rhodanobacter* (Fig. 1) and chemotaxonomic data, i.e. Q-8 as the predominant ubiquinone and iso-C_{15:0}, 10-methyl C_{16:0} and iso-C_{17:0} as major fatty acids, support the classification of strain Gsoil 3054^T in the genus *Rhodanobacter*. However, the DNA–DNA relatedness values and biochemical tests (Table 1) enabled strain Gsoil 3054^T to be differentiated from established species of *Rhodanobacter*. Therefore, strain Gsoil 3054^T should be assigned to the genus *Rhodanobacter* as the type strain of a novel species, for which the name *Rhodanobacter ginsenosidimutans* sp. nov. is proposed.

Description of *Rhodanobacter ginsenosidimutans* sp. nov.

Rhodanobacter ginsenosidimutans (gin.se.no.si.di.mu'tans. N.L. n. *ginsenosidum* ginsenoside; L. part. adj. *mutans* transforming, converting; N.L. part. adj. *ginsenosidimutans* ginsenoside-converting).

Cells are Gram-negative, aerobic, non-motile and rod-shaped, 0.4–0.6 µm wide and 1.1–1.3 µm long after 3 days on R2A agar (Difco). Colonies grown on R2A agar for 3 days are smooth, circular, convex, transparent and yellowish. On R2A agar, grows at 15–37 °C, but not at 4 or 42 °C. Growth occurs at pH 4–10 and in up to 5 % NaCl; optimal growth is at pH 7.0 and without NaCl. Substrate utilization, enzyme production and other physiological characteristics are indicated in Table 1. In addition to the carbon sources listed in Table 1, utilizes histidine and L-alanine as sole carbon sources. Does not utilize adipate, caprate, citrate, 3-hydroxybenzoate,

Fig. 1. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationship between strain Gsoil 3054^T and related strains. The tree was made using the neighbour-joining method (Saitou & Nei, 1987) with a two-parameter distance matrix (Kimura, 1983) and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 0.02 substitutions per nucleotide position.

4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, 2-keto-gluconate, DL-lactate, malate, propionate, L-proline, L-serine or suberate as sole carbon sources. According to the API ZYM gallery, produces acid phosphatase, α -glucosidase, β -glucosidase, leucine arylamidase, α -mannosidase and valine arylamidase, but not α -chymotrypsin, cystine arylamidase, α -fucosidase, lipase (C14), naphthol-AS-BI-phosphohydrolase or trypsin. Q-8 is the predominant ubiquinone and iso-C_{15:0}, 10-methyl C_{16:0} and iso-C_{17:0} are the predominant cellular fatty acids.

The type strain is Gsoil 3054^T (=KACC 12822^T =DSM 21013^T =KCTC 22231^T =LMG 24457^T), isolated from soil of a ginseng field of Pocheon province, South Korea. The genomic DNA G+C content of the type strain is 65.6 mol%.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Applications Center Program, Ministry of Education, Science and Technology (grant MG08-0101-2-0), Republic of Korea. We thank Jean Euzéby for his help with the etymology of the species epithet.

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