Rhodanobacter ginsengisoli sp. nov. and *Rhodanobacter terrae* sp. nov., isolated from soil cultivated with Korean ginseng

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Two bacterial isolates from ginseng fields in Korea, strains GR17-7^T and GP18-1^T, were characterized using a polyphasic approach. Phylogenetic analysis of their 16S rRNA gene sequences revealed a clear affiliation with the *Gammaproteobacteria*, and showed that the closest phylogenetic relationships were with members of the genus *Rhodanobacter*. The 16S rRNA gene sequence similarity between strains GR17-7^T and GP18-1^T was 97.2%. Both strains showed 16S rRNA gene sequence similarities of 95.2–96.9% to type strains of recognized *Rhodanobacter* species. The G+C contents of the DNA of strains GR17-7^T and GP18-1^T were 61.0 and 62.5 mol%, respectively. According to the DNA–DNA hybridization tests, the hybridization value between strains GR17-7^T and GP18-1^T was 34%. Strains GR17-7^T and GP18-1^T showed less than 32% DNA–DNA relatedness with *Rhodanobacter fulvus* KCTC 12098^T and *Rhodanobacter spathiphylli* LMG 23181^T. Strains GR17-7^T and GP18-1^T were aerobic, Gram-negative, rod-shaped, and catalase- and oxidase-positive. Major fatty acids of both strains were iso-C_{17:1}ω₉c and iso-C_{16:0}. Based on the data presented, two novel *Rhodanobacter* species are proposed, with the names *Rhodanobacter ginsengisoli* sp. nov. (type strain GR17-7^T=KACC 11762^T=DSM 18993^T) and *Rhodanobacter terrae* sp. nov. (type strain GP18-1^T=KACC 11761^T=DSM 19241^T).

The genus *Rhodanobacter* was proposed for Gram-negative, aerobic, yellow-coloured bacteria isolated from soil. The major fatty acids are iso-C_{17:1}ω₉c, iso-C_{15:0} and iso-C_{17:0}, and the DNA G+C contents are in the range 63–67.8 mol%. Phylogenetically, these micro-organisms are related to the genera *Dyella*, *Frateuria* and *Fulvimonas* within the family *Xanthomonadaceae*. At the time of writing the genus comprised *Rhodanobacter lindaniclasticus*, *Rhodanobacter fulvus* and *Rhodanobacter spathiphylli* (Nalin *et al.*, 1999; Im *et al.*, 2004; De Clercq *et al.*, 2006).

In the course of a study of bacterial diversity in fields cultivated with Korean ginseng (*Panax ginseng* C. A. Meyer), two bacterial strains, GR17-7^T and GP18-1^T, were isolated in Yeongju and Ansung provinces, respectively, South Korea. Soil samples were suspended in sterilized water and diluted solutions were spread on R2A agar (Difco) and incubated at 28 °C. Purified colonies were obtained from subcultures.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains GR17-7^T and GP18-1^T are EF166075 and EF166076, respectively.

The isolates were tested for a number of key characteristics, such as Gram staining, catalase, oxidase and hydrolysis of casein, DNA, gelatin, starch and Tween 80, by using standard procedures (Smibert & Krieg, 1994). Growth was assessed at 5, 10, 20, 25, 30, 35 and 40 °C, at pH 4, 5, 6, 7, 8, 9 and 10, and at 0, 1, 3, 5 and 7% (w/v) NaCl. Hydrolysis of carboxymethylcellulose (0.1%, w/v; Sigma), chitin from crab shells (1%, w/v), pectin (0.5%, w/v) and tyrosine (0.5%, w/v) was also determined. Cell morphology and motility were observed using light microscopy (AXIO; Zeiss) and transmission electron microscopy (model 912AB; LEO). In addition, the strains were characterized by using the whole test-spectrum of the API 20NE, API ID 32GN and API ZYM systems (bioMérieux), according to the manufacturer's instructions. The API ZYM test strip was read after 4 h incubation at 37 °C and the other API test strips were examined after 5 days at 28 °C. The two strains were aerobic, Gram-negative, rod-shaped and catalase- and oxidase-positive. Strain GR17-7^T grew well on R2A medium and trypticase soy agar (TSA; Difco), grew weakly on nutrient agar (NA; Difco) and did not grow on MacConkey agar (Difco).

Strain GP18-1^T grew well on R2A medium and weakly on NA, but did not grow on TSA or MacConkey agar. The phenotypic characteristics of strains GR17-7^T and GP18-1^T are summarized and compared with those of type strains of closely related taxa in Table 1.

Fatty acid methyl esters were extracted and prepared from cells grown on R2A agar for 48 h at 28 °C by using the standard protocol of the Microbial Identification System (MIDI; Microbial ID). The DNA G+C content was determined as described by Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18 S; Supelco). Strains GR17-7^T, GP18-1^T, *R. spathiphylli* LMG 23181^T and *R. fulvus* KCTC 12098^T contained a variety of fatty acids, including iso-C_{16:0}, iso-C_{17:1}ω9c, iso-C_{15:0}, iso-C_{11:0} 3-OH, iso-C_{11:0}, anteiso-C_{15:0}, summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) and unknown 11.799 (Table 2). Strain GR17-7^T contained iso-C_{17:1}ω9c (16.2 %), iso-C_{16:0} (14.8 %) and iso-C_{11:0} 3-OH (10.2 %), and GP18-1^T contained iso-C_{17:1}ω9c (19.3 %), iso-C_{16:0} (19.1 %) and iso-C_{15:0} (10.4 %) as the major fatty acids. The DNA G+C contents of strains GR17-7^T and GP18-1^T were 61.0 and 62.5 mol%, respectively, which were slightly lower than those of other members of the genus *Rhodanobacter*.

The 16S rRNA gene was amplified by PCR with primers fD1 and rP2 (Weisburg *et al.*, 1991), and the entire PCR fragment was sequenced directly (Hiraishi, 1992). Multiple sequence alignments were performed using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic trees were constructed by using the neighbour-joining method with the MEGA3 program package (Kumar *et al.*, 2004). DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG High Prime DNA labelling and detection starter kit II (Roche Molecular Biochemicals). The hybridized DNA was visualized using the DIG luminescent detection kit (Roche). Phylogenetically, strains GR17-7^T and GP18-1^T were found to be closely related to members of the genus *Rhodanobacter* (Fig. 1). The 16S rRNA gene sequence similarity between strains GR17-7^T and GP18-1^T was 97.2 %. Both strains showed 95.2–96.9 % sequence similarities with the type strains of the three recognized species of the genus *Rhodanobacter*. According to the DNA–DNA hybridization tests, the relatedness between strains GR17-7^T and GP18-1^T was 34 %. Strains GR17-7^T and GP18-1^T exhibited less than 32 % DNA–DNA relatedness with *R. fulvus* KCTC 12098^T and *R. spathiphylli* LMG 23181^T.

For *R. spathiphylli* LMG 23181^T, we obtained phenotypic results (for example, gelatin hydrolysis, assimilation patterns and enzymic reactions) that were slightly different from those reported by De Clercq *et al.* (2006). Also, the fatty acid compositions of *R. fulvus* KCTC 12098^T and *R. spathiphylli* LMG 23181^T determined in this study were also slightly different from those given by De Clercq *et al.* (2006). These discrepancies were supposed to be due to differences in the cultivation conditions used, such as media and incubation periods. Phenotypically, strains

Table 1. Differential phenotypic characteristics of strains GR17-7^T and GP18-1^T and type strains of *Rhodanobacter* species

Strains: 1, GR17-7^T (*Rhodanobacter ginsengisoli* sp. nov.; data from this study); 2, GP18-1^T (*Rhodanobacter terrae* sp. nov.; this study); 3, *R. lindaniclasticus* LMG 18385^T (Nalin *et al.*, 1999); 4, *R. fulvus* KCTC 12098^T (Im *et al.*, 2004 and this study); 5, *R. spathiphylli* LMG 23181^T (De Clercq *et al.*, 2006 and this study). All strains are positive for catalase and oxidase. All strains are negative for nitrate reduction, 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, D-ribose, inositol, sucrose, sodium malonate, potassium 5-ketogluconate, glycogen, D-sorbitol or potassium 2-ketogluconate. With the exception of *R. lindaniclasticus* LMG 18385^T, all strains assimilate 3-hydroxybutyric acid, but not adipic acid, itaconic acid, suberic acid, L-alanine, 3-hydroxybenzoic acid, L-serine, propionic acid, L-histidine or 4-hydroxybenzoic acid (data not reported for *R. lindaniclasticus* LMG 18385^T). All strains except *R. lindaniclasticus* LMG 18385^T show positive activities for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but are negative for lipase (C14), trypsin, α-chymotrypsin, α-mannosidase and α-fucosidase (data not reported for *R. lindaniclasticus* LMG 18385^T). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5
Motility	+	–	–	+	–
Aesculin hydrolysis	+	+	+	+	–
Gelatin hydrolysis	+	–	ND	–	+
Assimilation of:					
D-Mannose	–	+	–	+	–
N-Acetylglucosamine	+	+	–	+	+
Maltose	+	+	–	+	+
Capric acid	–	–	+	–	–
Malic acid	–	+	+	–	–
Trisodium citrate	–	–	+	–	–
Sodium acetate	+	–	ND	–	+
Lactic acid	–	–	+	–	–
Salicin	–	–	–	+	–
D-Melibiose	–	–	–	+	–
L-Fucose	–	+	–	–	–
Valeric acid	+	–	ND	–	+
L-Proline	+	+	ND	–	–
Enzymic activities					
Valine arylamidase	+	–	ND	+	+
Cystine arylamidase	–	–	ND	–	+
α-Galactosidase	–	–	–	+	–
β-Galactosidase	–	+	–	+	–
β-Glucuronidase	–	–	ND	+	–
α-Glucosidase	+	–	ND	+	+
β-Glucosidase	+	–	ND	+	–
N-acetyl-β-glucosaminidase	+	–	ND	+	+
DNA G+C content (mol%)	61.0	62.5	63.0	65.3	67.6

GR17-7^T and GP18-1^T differed from each other in properties such as motility, gelatin hydrolysis, assimilation patterns of several substrates and enzymic reactions. In

Table 2. Cellular fatty acid compositions of strains GR17-7^T and GP18-1^T and type strains of *Rhodanobacter* species

Strains: 1, GR17-7^T (*Rhodanobacter ginsengisoli* sp. nov.); 2, GP18-1^T (*Rhodanobacter terrae* sp. nov.); 3, *R. fulvus* KCTC 12098^T; 4, *R. spathiphylli* LMG 23181^T. All data are from this study. All strains were grown for 2 days on R2A medium. Values are percentages of total fatty acids. —, <0.5% or not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4
iso-C _{11:0}	8.3	3.7	3.6	4.2
iso-C _{11:0} 3-OH	10.2	5.6	5.3	6.0
iso-C _{12:0} 3-OH	3.1	1.7	1.2	3.9
iso-C _{13:0} 3-OH	5.5	2.6	2.6	2.1
C _{14:0}	—	—	0.9	—
iso-C _{14:0}	2.2	1.9	2.6	4.9
iso-C _{15:0}	7.3	10.4	16.2	8.3
iso-C _{15:1} F	—	—	0.8	—
anteiso-C _{15:0}	5.0	7.3	6.3	4.8
C _{16:0}	1.3	4.1	5.1	1.5
iso-C _{16:0}	14.8	19.1	13.2	28.4
iso-C _{16:1} H	0.6	—	—	1.1
C _{17:0} cyclo	—	—	3.2	—
C _{17:1ω6c}	7.2	2.7	—	5.3
iso-C _{17:0}	4.7	8.8	5.2	2.8
iso-C _{17:1ω9c}	16.2	19.3	16.8	15.3
anteiso-C _{17:0}	—	0.7	—	—
C _{18:0}	1.0	1.0	1.1	—
C _{18:1ω7c}	—	—	0.7	—
iso-C _{18:0}	0.6	0.5	—	0.5
Summed feature 3*	3.8	6.5	8.8	5.7
Unknown ECL 11.799	6.4	2.8	2.8	2.7

*Summed feature 3 contains iso-C_{15:0} 2-OH and/or C_{16:1ω7c}.

addition, the two strains could be differentiated from other members of the genus *Rhodanobacter* on the basis of phenotypic properties, fatty acid compositions and DNA G+C contents (Tables 1 and 2).

On the basis of the data presented here, strains GR17-7^T and GP18-1^T represent two novel species of *Rhodanobacter*, for which we propose the names *Rhodanobacter ginsengisoli* sp. nov. and *Rhodanobacter terrae* sp. nov.

Description of *Rhodanobacter ginsengisoli* sp. nov.

Rhodanobacter ginsengisoli (gin.sen'gi.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil from a ginseng field, the source of the type strain).

Cells are Gram-negative, motile, aerobic rods (0.5–0.6 × 2.0–3.5 µm). Temperature range for growth is 5–35 °C; no growth occurs at 40 °C. Optimum temperature for growth is 28 °C. Grows at pH 5.0–9.0. Tolerates up to 3% NaCl, but not 5% NaCl. Colonies on R2A medium are yellow, circular and slightly convex with clear margins. Positive for catalase and oxidase. Negative for nitrate reduction, glucose fermentation, indole production, arginine dihydrolase, urease and β-galactosidase. Hydrolyses aesculin, casein, gelatin, Tween 80 and tyrosine, but not chitin, carboxymethylcellulose, DNA, pectin or starch. Major fatty acids are iso-C_{17:1ω9c}, iso-C_{16:0} and iso-C_{11:0} 3-OH. The DNA G+C content of the type strain is 61.0 mol%.

The type strain, GR17-7^T (=KACC 11762^T=DSM 18993^T), was isolated from a soil sample from a ginseng field in Yeongju province, South Korea.

Description of *Rhodanobacter terrae* sp. nov.

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

Cells are Gram-negative, non-motile, aerobic rods (0.5 × 2.0–5.0 µm). Temperature range for growth is 5–30 °C; no growth occurs at 35 °C. Optimum temperature for growth is 25–28 °C. Grows at pH 4.0–8.0. Tolerates up to 2% NaCl, but not 3% NaCl. Colonies on R2A medium are yellow, circular and slightly convex with clear margins. Positive for catalase, oxidase and β-galactosidase. Negative

Fig. 1. Phylogenetic dendrogram constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships between strains GR17-7^T, GP18-1^T and related species. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 2 substitutions per 100 nucleotide positions.

for nitrate reduction, glucose fermentation, indole production, arginine dihydrolase and urease. Hydrolyses aesculin, Tween 80 and tyrosine, but not casein, chitin, carboxymethylcellulose, DNA, gelatin, pectin or starch. Major fatty acids are iso-C_{17:1}ω9c, iso-C_{16:0} and iso-C_{15:0}. The DNA G + C content of the type strain is 62.5 mol%.

The type strain, GP18-1^T (=KACC 11761^T=DSM 19241^T), was isolated from a soil sample from a ginseng field in Ansung province, South Korea.

Acknowledgements

This study was supported by the National Institute of Agricultural Biotechnology (NIAB grant no. 06-4-11-19-1), Rural Development Administration, Republic of Korea.

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