NOTE

Rhodanobacter umsongensis sp. nov., Isolated from a Korean Ginseng Field[§]

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A bacterial isolate designated GR24-2^T was isolated from Korean soil used for cultivating ginseng (Panax ginseng C. A. Meyer). The strain was aerobic, Gram-negative, motile, and rod-shaped. It grew optimally at 28-30°C, pH 7.0, and in a range of 0-1% NaCl. Phylogenetically, the strain clustered with members of the genus Rhodanobacter. The strain exhibited the highest sequence similarities (>98%) with R. panaciterrae LnR5-47^T (98.4%), R. soli DCY45^T (98.2%), and *R. ginsengisoli* GR17-7^T (98.0%). However, it also showed high sequence similarities (>97%) with some other Rhodanobacter and Dyella species. The strain contained Q-8 as the predominant respiratory quinone. The major fatty acids (greater than 10% of the total fatty acids) were iso- $C_{17:1} \omega_9 c$ (24.5%), iso-C_{16:0} (22.8%), anteiso-C_{15:0} (10.5%), and iso-C_{15:0} (10.1%). Its major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and an unknown aminophospholipid. The DNA G+C content of strain GR24-2^T was 65.6 mol%. The strain showed less than 70% DNA relatedness values between the closely related Rhodanobacter and Dyella species. The phylogeny, phenotype, DNA-DNA hybridization, and chemotaxonomic data generated in this study reveal that the isolate is a novel species of the genus Rhodanobacter. The name proposed for this strain is Rhodanobacter umsongensis sp. nov. (type strain $GR24-2^{T} = KACC \ 12917^{T} = DSM \ 21300^{T}$).

Keywords: Rhodanobacter umsongensis, 16S rRNA gene sequence, taxonomy

The genus Rhodanobacter was proposed by Nalin et al. (1999). Currently, the genus comprises 11 species: R. lindaniclasticus (Nalin *et al.*, 1999), which was isolated from soil samples from a wood-treatment site and degrades lindane (y-hexachlorocvclohexane); R. fulvus (Im et al., 2004), which was isolated from soil mixed with rotten rice straw and produces β -galactosidase; R. spathiphylli (De Clercq et al., 2006), which was isolated from the rhizospheres of Spathiphyllum plants grown in a compost-amended potting mix; R. thiooxydans (Lee et al., 2007), which was isolated from a biofilm on sulfur particles used in an autotrophic denitrification process; R. ginsengisoli, R. terrae (Weon et al., 2007), R. ginsenosidimutans (An et al., 2009), and R. soli (Bui et al., 2010), which were isolated from the soil of ginseng fields in South Korea; R. panaciterrae (Wang et al., 2011), which was isolated from the soil of ginseng in China and has ginsenoside-converting activity; Rhodanobacter caeni (Woo et al., in press), which was isolated from a sewage sludge sample; and Rhodanobacter denitrificans (Prakash et al., 2011), which was isolated from nitrate-rich zones of a contaminated aquifer. Unfortunately, the type strain RP5557^{T} for *R. lindaniclasticus* was no longer available (Mergaert et al., 2002). Members of this genus are Gram-negative, catalase- and oxidase-positive, non-sporeforming rods, with ubiquinone 8 as the major quinone, and iso- $C_{16:0}$, iso- $C_{17:1} \omega 9c$, and iso- $C_{15:0}$ as the major fatty acids. G+C contents of the genus are in the range of 61.0-67.6 mol%. Phylogenetic analyses have revealed that the genus is closely related with the genera *Dyella* and *Frateuria* within the family Xanthomonadaceae (Nalin et al., 1999; Im et al., 2004; De Clercq et al., 2006; Lee et al., 2007; Weon et al., 2007; An et al., 2009; Bui et al., 2010; Wang et al., 2011).

Ginseng (*Panax ginseng* C. A. Meyer) has been cultivated in Korea since ancient times. We isolated bacteria from the soil of a ginseng field in Umsong Province (36° 56′ N 127° 44′ E), South Korea, to investigate the phylogenetic diversity. A novel bacterial strain, GR24-2^T, was found. We determined its taxonomic position by performing phylogenetic analyses based on 16S rRNA gene sequences, chemotaxonomic characteristics, and phenotypic characteristics. Our results provide evidence that strain GR24-2^T represents a new bacterial species.

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. DNA from strain GR24-2^T was isolated and purified according to the method described by Ausubel *et al.* (1987). The 16S rRNA gene was amplified using the universal primers

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Fig. 1. Neighbor-joining tree constructed from comparative analyses of 16S rRNA gene sequences showing the relationships between GR24-2^T and related taxa. Bootstrap values (expressed as percentages of 1,000 replications) greater than 70% are shown at branch points. Dots indicate that the corresponding nodes were also recovered from trees generated with the maximum parsimony and maximum likelihood algorithms. Bar, 0.01 expected changes.

fD1 and rP2 (Weisburg *et al.*, 1991) and sequenced as described by Weon *et al.* (2005). We obtained 1,477 bp fragments. This sequence was aligned with type strains of related genera and species using SILVA Aligner (Pruesse *et al.*, 2007). Phylogenetic trees were reconstructed using the neighboroining (Saitou and Nei, 1987), maximum-parsimony (Fitch, 1971), and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA 5 software (Kumar *et al.*, 2008) with bootstrap values based on 1,000 replications.

According to the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007), strain GR24-2^T exhibited high sequence similarities with *R. panaciterrae* LnR5-47^T (98.4%), *R. soli* DCY45^T (98.3%), and *R. ginsengisoli* GR17-7^T (98.0%). In addition, it showed high sequence similarities (>97%) with some other species of *Rhodanobacter* and *Dyella*. In the neighbor-joining analysis (Fig. 1), the strain was clustered with other *Rhodanobacter* species with 79% bootstrap support. The maximum parsimony and maximum likelihood trees also supported this grouping.

To assess the DNA relatedness among the closely related species, DNA–DNA hybridization was performed using the filter hybridization method described by Seldin and Dubnau (1985). Probe labeling was conducted using the non-radioactive DIG-High prime system (Roche) and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA-DNA relatedness was quantified using a densitometer (Bio-Rad); the new strain showed less than 70% DNA relatedness between the closely related *Rhodanobacter* and *Dyella* species with *R. caeni* MJ01^T (48±5%), *R. denitri-ficans* 2APBS1^T (45±3%), *R. ginsengisoli* GR17-7^T (47±2%), *R. panaciterrae* LnR5-47^T (55±2%; reciprocal value of 49± 3%), *R. soli* DCY45^T (46±4%), *R. spathiphylli* B39^T (51±3%), *R. terrae* GP18-1^T (56±3%), *R. thiooxydans* LCS2^T (57±4%), *D. ginsengisoli* Gsoil 3046^T (51±2%), *D. japonica* XD53^T $(29\pm4\%)$, and *D. soli* JS12-10^T (49\pm5\%).

Analyses of phenotypic characteristics (e.g., tests for Gram staining, catalase, oxidase, hydrolyses of carboxymethylcellulose, casein, chitin, and so forth) were performed using the methods described by Smibert and Krieg (1994). The pH range (pH 4.0-10.0 at intervals of 1.0 pH units) for growth was determined in R2A broth adjusted with citratephosphate buffer or tris-hydrochloride buffer (Breznak and Costilow, 1994). Growth at 0, 1, 2, 3, and 5% (w/v) NaCl was investigated in R2A broth. Growth at various temperatures (5-45°C at intervals of 5°C) was measured on R2A agar medium. Growth under anaerobic conditions was tested in GasPak (BBL) jars at 28°C for 15 days. H₂S production was determined on Kligler iron agar (BBL). Cell morphology was examined using light microscopy (AXIO; Zeiss) and transmission electron microscopy (model 912AB; LEO) after incubation for 24 h at 28°C. Physiological and biochemical properties were further determined with API 20NE, API ID 32GN, and API ZYM (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 strain was strictly aerobic, Gram-negative, rod-shaped, and catalase- and oxidase-positive. It grew on R2A, nutrient agar (NA; Difco), and trypticase soy agar (TSA; Difco), but not on MacConkey agar (Difco). Strain GR24-2^T was found to be motile and to hydrolyze gelatin. In addition, the strain could be differentiated from other members of the genus Rhodanobacter based on phenotypic properties (Table 1).

For quinone and polar lipid analyses, cells were grown on R2A medium at 28°C. The presence of isoprenoid quinones was investigated using HPLC, as described previously (Groth *et al.*, 1996). Fatty acid methyl esters were extracted after cells were grown on R2A for 48 h at 28°C and prepared using the

Table 1. Differential phenotypic characteristics of GR24-2^T and closely related members of the genus *Rhodanobacter*

Strains: 1, Rhodanobacter umseongensis GR24-2^T; 2, Rhodanobacter ginsengisoli KACC 11762^T; 3, Rhodanobacter panaciterrae KACC 12826^T; 4, Rhodanobacter soli KCTC 22620^T. All data were obtained from this study unless indicated. All strains are Gram-negative and positive for catalase and oxidase. All strains are negative for arginine dihydrolase, indole production, nitrate reduction, and urease. All strains assimilate D-glucose, but not adipic acid, L-alanine, L-arabinose, capric acid, L-fucose, glycogen, L-histidine, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, inositol, itaconic acid, lactic acid, malic acid, D-mannitol, phenylacetic acid, potassium gluconate, D-melibiose, potassium 5-ketogluconate, propionic acid, L-rhamnose, D-saccharose, salicin, L-serine, sodium malonate, suberic acid, or trisodium citrate (based on API 20NE and API ID 32GN test strips). All strains show positive activities for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase, but are negative for α -fucosidase, α -galactosidase, β -galactosidase, lipase (C14), α mannosidase, and trypsin (based on API ZYM test strips). +, Positive; -, negative; W, weakly positive.

Characteristics	1	2	3	4
Motility	+	+	_a	+ ^b
Gelatin hydrolysis	+	+	_ ^a	+ ^b
Assimilation of :				
D-Mannose	-	-	W	-
N-Acetylglucosamine	+	+	+	-
D-Maltose	+	+	+	W
D-Ribose	-	-	+	-
Sodium acetate	+	+	-	W
Valeric acid	-	+	-	-
3-Hydroxybutyric acid	+	+	-	+
L-Proline (PRO)	+	+	+	-
Enzymatic activity:				
Cystine arylamidase	+	-	+	+
α-Glucosidase	-	+	-	-
β-Glucosidase	-	+	+	-
N-Acetyl-β-glucosaminidase	-	+	+	-
^a Data from Wang <i>et al.</i> (2011).				

^b Data from Bui *et al.* (2010).

standard protocol of the Microbial Identification System (MIDI; Microbial ID). Polar lipids were analyzed according to Minnikin et al. (1984). DNA G+C content was determined as described by Mesbah et al. (1989) using a reverse-phase column (Supelcosil LC-18-S; Supelco). The strain GR24-2¹ exhibited typical gamma-proteobacterial quinone systems, with the predominant compound ubiquinone Q-8. The major fatty acids (>10% of the total fatty acids) of $GR24-2^{1}$ were iso-C_{17:1} w9c (24.5%), iso-C_{16:0} (22.8%), anteiso-C_{15:0} (10.5%), and iso- $C_{15:0}$ (10.1%). The overall fatty acid pattern of the strain was consistent with other members of the genus Rhodanobacter, however the qualitative and quantitative differences among strains were found (Table 2). The major polar lipids of the strain were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and an unknown aminophospholipid, which was also recovered from R. ginsengisoli KACC 11762¹ (see Supplementary data Fig. S1). The DNA G+C content of strain $GR24-2^1$ was 65.6 mol%.

In conclusion, strain GR24-2¹ was classified into the genus *Rhodanobacter* based on phylogenetic analyses, polar lipid pattern, and fatty acid composition. Upon comparing GR24-2^T with the closely related *Rhodanobacter* species, strain GR24-2^T can be differentiated from these species based on

motility, gelatin hydrolysis, assimilation patterns of some substrates, enzymatic activities, quantitative differences in fatty acids, and DNA-DNA relatedness. Therefore, strain GR24-2^T should be considered a novel species of the genus *Rhodanobacter*, for which the name *Rhodanobacter umseongensis* sp. nov. is proposed.

Description of Rhodanobacter umsongensis sp. nov.

Rhodanobacter umsongensis (um.song.en'sis. N.L. masc. adj. *umsongensis* referring to the Umsong Region in Korea, where the bacteria were first found).

Cells are strictly aerobic, Gram-negative, motile, rod-shaped $(0.5-0.6\times2.0-5.0 \ \mu\text{m})$ and are catalase- and oxidase-positive. They grow on R2A, NA, and TSA, but not on MacConkey agar. Colonies are yellow and circular, convex with clear margins on R2A medium. Growth occurs at 5-30°C (optimum, 28°C), at pH 5-9 (optimum, pH 7), and up to 2% (w/v) NaCl. Cells hydrolyze Tween 80, but not carboxymethylcellulose, casein, chitin, DNA, pectin, starch, or tyrosine. They are negative in methyl red and Voges-Proskauer tests. H₂S is not produced. Cells are positive for aesculin and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease (API 20NE test strips). Cells assimilate D-glucose, *N*-acetylglucosamine, D-maltose, sodium acetate, 3-hydroxybutyric acid, and L-proline, but not L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, Lrhamnose, D-ribose, inositol, D-saccharose, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, Lserine, salicin, D-melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, or 4-hydroxybenzoic acid (API 20NE and API ID 32GN

Table 2. Cellular fatty acids of strain GR24-2^T and *Rhodanobacter species* Strains: 1, strain GR24-2^T (data from this study); 2, *Rhodanobacter ginsengisoli* GR17-7^T (Weon *et al.*, 2007); 3, *Rhodanobacter panaciterrae* LnR5-47^T (Wang *et al.*, 2011); 4, *Rhodanobacter soli* DCY45^T (Bui *et al.*, 2010). All strains were grown in R2A medium at 28°C for 2 days. Values given are percentages of total fatty acids. -, <1.0% or not detected.

Fatty acids	1	2	3	4			
iso-C _{11:0}	3.6	8.3	3.5	7.1			
iso-C _{11:0} 3-OH	3.4	10.2	4.4	4.0			
iso-C _{12:0} 3-OH	1.4	3.1	1.6	1.2			
iso-C _{13:0} 3-OH	1.7	5.5	3.0	2.2			
iso-C _{14:0}	3.1	2.2	2.6	4.5			
anteiso-C _{15:0}	10.5	5.0	4.3	5.0			
iso-C _{15:0}	10.1	7.3	12.5	15.9			
C _{16:0}	2.5	1.3	2.3	1.6			
iso-C _{16:0}	22.8	14.8	20.6	15.9			
iso-C _{16:1} H	1.0	-	-	-			
iso-C _{17:0}	4.3	4.7	5.7	3.7			
iso- $C_{17:1} \omega 9c$	24.5	16.2	20	23.8			
$C_{17:1}\omega 6c$	-	7.2	5.3	1.6			
C _{18:0}	-	1.0	-	-			
Unknown 11.799	1.2	6.4	-	2.8			
Summed feature 3 ^a	6.1	3.8	6.3	5.5			
^a Summed feature 3 contains iso-C ₁₅₀ , 2-OH and/or C ₁₆₁ ω 7c							

test strips). They have positive alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, and naph-thol-AS-BI-phosphohydrolase activities, but are negative for lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-alactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosi-dase activities (API ZYM test strips). The quinone system consists of Q-8 (88.8%) and Q-7 (10.3%). The major fatty acids are iso-C_{17:1} ω9c (24.5%), iso-C_{16:0} (22.8%), anteiso-C_{15:0} (10.5%), and iso-C_{15:0} (10.1%). The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, an unknown aminophospholipid, and diphosphatidylglycerol. The G+C content of genomic DNA is 65.6 mol%.

The type strain, $GR24-2^{T}$ (KACC 12917^T =DSM 21300^T), was isolated from a soil sample from a ginseng field in Umsong Province, South Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of $GR24-2^{T}$ is FJ821731.

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