Rhizobium soli sp. nov., isolated from soil

Jung-Hoon Yoon, So-Jung Kang, Hwe-Su Yi, Tae-Kwang Oh and Choong-Min Ryu

Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Taejon, Republic of Korea

Correspondence
Jung-Hoon Yoon
jhyoon@kribb.re.kr
Choong-Min Ryu
cmryu@kribb.re.kr

A Gram-negative, non-motile, pale-yellow, rod-shaped bacterial strain, DS-42^T, was isolated from a soil in Korea and its taxonomic position was investigated by a polyphasic study. Strain DS-42^T grew optimally at 25 °C and pH 7.0–8.0. Strain DS-42^T did not form nodules on three different legumes, and the *nodD* and *nifH* genes were also not detected by PCR. Strain DS-42^T contained Q-10 as the predominant ubiquinone. The major cellular fatty acid was $C_{18:1}\omega7c$. The DNA G+C content was 60.8 mol%. Phylogenetic analyses based on 16S rRNA, *atpD* and *recA* gene sequences showed that strain DS-42^T belonged to the genus *Rhizobium*. Strain DS-42^T showed 16S rRNA gene sequence similarity of 94.1–97.7 % to the type strains of recognized *Rhizobium* species. DNA–DNA relatedness between strain DS-42^T and the type strains of *Rhizobium huautlense*, *R. galegae*, *R. loessense* and *R. cellulosilyticum* was 13–19 %, indicating that strain DS-42^T was distinct from them genetically. Strain DS-42^T can also be differentiated from these four phylogenetically related *Rhizobium* species by various phenotypic properties. On the basis of phenotypic properties, phylogenetic distinctiveness and genetic data, strain DS-42^T is considered to represent a novel species of the genus *Rhizobium*, for which the name *Rhizobium soli* sp. nov. is proposed. The type strain is DS-42^T (=KCTC 12873^T =JCM 14591^T).

The genus *Rhizobium* was first proposed by Frank (1889) and its description was emended by Young *et al.* (2001). Phylogenetically, the genus forms an evolutionary lineage within the family *Rhizobiaceae* of the *Alphaproteobacteria* (Lee *et al.*, 2005). Recent descriptions of novel species, e.g. eight species in 2008 and 2009 (Berge *et al.*, 2009; Gu *et al.*, 2008; Han *et al.*, 2008; Hunter *et al.*, 2007, 2008; Peng *et al.*, 2008; Ramírez-Bahena *et al.*, 2008; Tian *et al.*, 2008), have increased considerably the number of species belonging to the genus *Rhizobium*. At the time of writing, the genus *Rhizobium* comprises 31 species with validly published names (Euzéby, 1997). Members of the genus *Rhizobium* have generally been isolated from nodules on leguminous plants (Peng *et al.*, 2008; Wei *et al.*, 2003). Recently, some *Rhizobium* species have been isolated from other sources (García-Fraile *et al.*, 2007; Hunter *et al.*, 2007; Quan *et al.*, 2005). In this study, we report on the taxonomic characterization of a *Rhizobium*-like bacterial strain, DS-42^T, which was isolated from a soil from Korea. The aim of the present work was to determine the exact taxonomic position of strain DS-42^T by using a polyphasic characterization that included determination of phenotypic properties, phylogenetic investigations based on 16S rRNA, *atpD* and *recA* gene sequences and genetic analysis.

Strain DS-42^T was isolated by means of standard dilution plating technique at 25 °C on 10-fold-diluted nutrient agar (Difco). The type strains of four *Rhizobium* species were used as reference strains for DNA–DNA hybridization, phenotypic characterization and fatty acid analysis: *Rhizobium huautlense* LMG 18254^T, *R. galegae* LMG 6214^T, *R. loessense* CIP 108030^T and *R. cellulosilyticum* DSM 18291^T. These reference strains were cultivated under the culture conditions recommended by the culture collections. The morphological, physiological and biochemical characteristics of strain DS-42^T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 25 °C. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. Flagellation was determined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at 4, 10, 15 and 20–32 °C (at intervals of 1 °C) was measured on TSA. Growth in the absence of NaCl and at 0.5 % and 1.0–10.0 % (in

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *atpD* and *recA* gene sequences of strain DS-42^T are respectively EF363715, GQ260191 and GQ260192.

Neighbour-joining trees based on *atpD* and *recA* gene sequences are available as supplementary material with the online version of this paper.

Table 1. Phenotypic characteristics of *Rhizobium soli* sp. nov. DS-42^T and type strains of phylogenetically related *Rhizobium* species

Strains: 1, *R. soli* DS-42^T; 2, *R. huautlense* LMG 18254^T; 3, *R. galegae* LMG 6214^T; 4, *R. loessense* CIP 108030^T; 5, *R. cellulosilyticum* DSM 18291^T. Data are from this study unless indicated. +, Positive; –, negative; W, weakly positive; ND, no data available. All strains are Gram-negative, aerobic, rod-shaped and non-spore-forming. All strains are positive for growth at 1 % (w/v) NaCl (not determined for *R. galegae* LMG 6214^T), activity of catalase, oxidase, alkaline phosphatase, leucine arylamidase and α-glucosidase, hydrolysis of aesculin, utilization of D- and L-arabinose, ribose, D-xylose, galactose, glucose, fructose, mannose, mannitol, sorbitol, *N*-acetylglucosamine, aesculin, cellobiose, D-arabitol and 2-ketogluconate and susceptibility to polymyxin B, streptomycin, gentamicin, tetracycline and neomycin. All strains were negative for production of H_2S and indole, activity of urease [reported as positive by García-Fraile *et al.* (2007) for *R. huautlense* LMG 18254^T, *R. galegae* LMG 6214^T and *R. cellulosilyticum* DSM 18291^T], arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase and α-mannosidase, hydrolysis of casein, starch, tyrosine, xanthine and Tweens 20, 40, 60 and 80, utilization of methyl α-D-mannoside, methyl α-glucoside, amygdalin, inulin, melezitose, starch and glycogen and susceptibility to lincomycin.

Characteristic	1	2	3	4	5
Origin	Soil (Korea)	*Sesbania herbacea* (Mexico)	*Galega orientalis* (Finland)	*Astragalus campanatus* (China)	*Populus alba* (Spain)
Cell size (μm)	0.4–0.7 × 1.0–4.5	ND	0.9–1.0 × 1.5–1.8[a*]	0.5–0.7 × 1.8–2.1[a]	ND
Flagella	None	ND	One or two[a]	ND	ND
pH range for growth	5.5–8.5	5.0–9.0[a]	5.0–9.5[a]	7.0–10.0[a]	6–8[b]
Growth at/in:					
40 °C	–	+[a]	–[a]	–[a]	–[b]
2 % (w/v) NaCl	+	–[a]	–[a]	+[a]	+[b]
Nitrate reduction	–	+	–	–	–
Hydrolysis of hypoxanthine	–	+	–	–	–
Enzyme activity (API ZYM, API 20E)					
Esterase (C4)	W	+	+	–	W
Esterase lipase (C8)	W	+	+	–	W
Acid phosphatase	W	–	+	+	W
β-Galactosidase	–	–	–	+	–
β-Glucosidase	–	+	+	W	W
N-Acetyl-β-glucosaminidase	–	–	–	+	–
α-Fucosidase	–	–	–	W	–
Tryptophan deaminase	–	–	+	–	–
Utilization of:					
Glycerol	+	+	+	+	–
Erythritol	+	–	–	+	–
L-Xylose	–	+	–	–	–
Adonitol	–	+	–	+	–
Methyl β-D-xyloside	+	+	+	+	–
Sorbose	–	+	–	–	–
Rhamnose	–	+	+	+	+
Dulcitol	–	+	–	+	–
Inositol	–	+	+	+	+
Salicin	–	+	–	–	W
Maltose	W	+	+	–	+
Lactose	–	+	–	+	–
Melibiose	+	+	+	+	–
Sucrose	–	+	+	+	+
Trehalose	W	+	–	–	+
Raffinose	–	+	+	+	–
Xylitol	+	+	–	+	+
Gentiobiose	W	+	–	+	+†
Turanose	–	+	+	+	+
D-Lyxose	W	+	–	+	+

Table 1. cont.

Characteristic	1	2	3	4	5
D-Tagatose	−	+	−	+	−
D-Fucose	−	+	−	−	+
L-Fucose	+	+	+	−	+
L-Arabitol	−	+	−	+	−
Gluconate	+	−	+	−	+
5-Ketogluconate	−	−	−	−	+
Susceptibility to:					
Penicillin G	−	−	+	−	−
Chloramphenicol	+	+	+	−	+
Ampicillin	−	+	+	−	−
Cephalothin	+	+	+	−	+
Novobiocin	+	+	+	−	+
Carbenicillin	+	+	+	−	+
Kanamycin	+	−	+	+	+
Oleandomycin	+	−	−	−	+
DNA G+C content (mol%)	60.8	57.0[a]	63.0[a]	59.5[a]	57[b]

*Data taken from: *a*, Quan *et al.* (2005); *b*, García-Fraile *et al.* (2007).
†Reported as negative by Wei *et al.* (2003) and García-Fraile *et al.* (2007).

increments of 1.0 %) (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was omitted. The pH range for growth was determined in nutrient broth (Difco) that was adjusted to pH 4.5–10.5 (at intervals of 0.5 pH units) by using sodium acetate/acetic acid and Na_2CO_3 buffers. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Aesculin hydrolysis and nitrate reduction were studied as described previously (Lányí, 1987). Antibiotic susceptibility was tested on TSA plates using discs containing the following amounts of antibiotic: polymyxin B (100 U), streptomycin (50 μg), penicillin G (20 U), chloramphenicol (100 μg), ampicillin (10 μg), cephalothin (30 μg), gentamicin (30 μg), novobiocin (5 μg), tetracycline (30 μg), kanamycin (30 μg), lincomycin (15 μg), oleandomycin (15 μg), neomycin (30 μg) and carbenicillin (100 μg). Utilization of various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50CH and API ZYM systems (bioMérieux); utilization of various substrates was determined by inoculating the API 50CH strip with cells suspended in AUX medium (bioMérieux).

A nodulation test was performed by using the common leguminous species *Medicago truncatula* (barrel medick, a relative of alfalfa), *Phaseolus lunatus* (lima bean) and *Glycine max* (soybean). Seeds of *M. truncatula* and *P. lunatus* were surface-sterilized by soaking for 1 min in 100 % ethanol and seeds of *G. max* were surface-sterilized by soaking for 10 min in 1 % (w/v) sodium hypochlorite solution. The seeds were then washed five times with sterile distilled water. After germination, 2-day-old seedlings were transferred to round plastic Petri dishes (150 mm in diameter) containing solid nitrogen-free Murashige and Skoog (MS) salt medium (Gibco-BRL), which includes 0.8 % (w/v) plant agar and 1.5 % (w/v) sucrose. After incubation for 2 days, primary roots were inoculated by dripping 200 μl bacterial suspension at 10^8 c.f.u. ml^{-1} onto the root from the tip to the base. The positions of the root tips and the smallest emergent root hairs were marked on the plastic pouches immediately after inoculation with the aid of a dissecting microscope at ×12 magnification. The plants were cultured vertically for 3–4 weeks in a growth chamber at 25 °C with a 12 h photoperiod. *Sinorhizobium meliloti* 1021 was used as a positive control for nodulation on *M. truncatula*, and water treatment was used as a negative control.

Cell biomass for DNA extraction and for analysis of isoprenoid quinones was obtained from cultures grown with shaking at 150 r.p.m. for 3 days in trypticase soy broth (Difco, pH 7.3) at 25 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). PCR amplifications of *atpD* and *recA* genes were performed

under the conditions described by Yoon *et al.* (1998) using primers described by Tian *et al.* (2008). The PCR products were purified with the QIAquick PCR purification kit (Qiagen). The amplified *atpD* and *recA* genes were cloned into the pGEM T-easy vector (Promega) according to the manufacturer's instructions. Sequences of the *atpD* and *recA* genes were determined for both strands by extension from vector-specific priming sites (primers T7 and SP-6 from the pGEM T-easy vector). PCR amplifications of *nodD* and *nifH* genes were performed by using primers and conditions described by Poly *et al.* (2001) or Rivas *et al.* (2002). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain DS-42^T, *R. huautlense* LMG 18254^T, *R. galegae* LMG 6214^T, *R. loessense* CIP 108030^T and *R. cellulosilyticum* DSM 18291^T was harvested after incubation for 3 days at 25 °C on solid medium (CIP medium no. 57) that contained (l^{-1}) 4 g glucose, 4 g yeast extract, 10 g malt extract, 2 g $CaCO_3$ and 15 g agar (pH 7.2). Fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). 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 in each sample were excluded, and the means of the remaining three values are quoted as DNA–DNA relatedness values.

Cells of strain DS-42^T were Gram-negative, non-motile, aerobic rods, 0.4–0.7 μm wide and 1.0–4.5 μm long. The strain grew at 4 and 31 °C, with optimum growth at 25 °C. Strain DS-42^T grew optimally at pH 7.0–8.0 and in the presence of 0.5–1.0 % (w/v) NaCl. Morphological, cultural, physiological and biochemical characteristics of strain DS-42^T are given in the species description and in Table 1. Nodulation tests were performed by investigating the ability of strain DS-42^T to form nodules on three different leguminous species, *M. truncatula*, *P. lunatus* and *G. max*. No nodule formation was observed in three repeated experiments (not shown). Even when high-dosage inoculation of strain DS-42^T was used on the root hairs, no swollen tissues appeared. The *nodD* and *nifH* genes were also not detected by PCR in strain DS-42^T.

The almost-complete 16S rRNA gene sequence of strain DS-42^T determined in this study comprised 1436 nt (approx. 96 % of the *Escherichia coli* 16S rRNA sequence). Sequence analyses of the 16S rRNA, *atpD* and *recA* genes showed that strain DS-42^T was phylogenetically most closely related to the genus *Rhizobium*. In the neighbour-joining tree based on 16S rRNA gene sequences, strain DS-42^T fell within the clade comprising *Rhizobium* species, particularly forming a cluster with *R. huautlense*, *R. galegae*, *R. cellulosilyticum* and *R. loessense* (Fig. 1). In phylogenetic trees constructed using the maximum-likelihood and maximum-parsimony algorithms, strain DS-42^T fell within the clade encompassed by the genus *Rhizobium*. Strain DS-42^T exhibited 16S rRNA gene sequence similarity of 97.7, 97.1, 96.9 and 96.7 % to the type strains of *R. huautlense*, *R. galegae*, *R. cellulosilyticum* and *R. loessense*, respectively, and of 94.1–96.9 % to the type strains of the other *Rhizobium* species. In neighbour-joining trees based on *atpD* and *recA* gene sequences, strain DS-42^T formed distinct phylogenetic lineages within the clade comprising *Rhizobium* species (Supplementary Figs S1 and S2, available in IJSEM Online). Strain DS-42^T exhibited 85.3–91.3 % *atpD* gene sequence

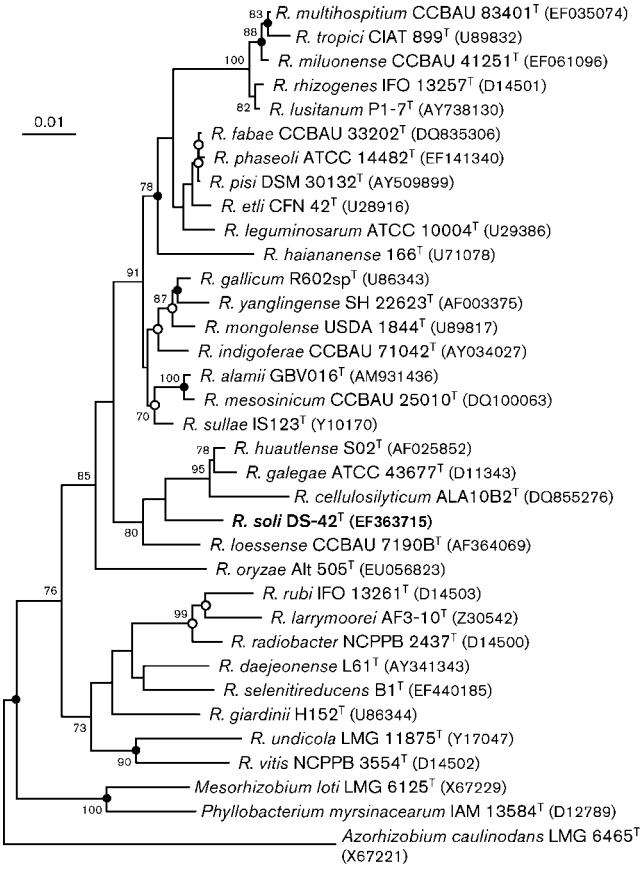

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of *Rhizobium soli* sp. nov. DS-42^T, *Rhizobium* species and other related taxa. Bootstrap values (expressed as percentages of 1000 replications) ⩾70 % are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood or maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

similarity and 84.2–88.0 % *recA* gene sequence similarity to the *Rhizobium* type strains used in this study.

Strain DS-42^T contained ubiquinone-10 (Q-10), at a peak area ratio of approximately 95 %, as the predominant isoprenoid quinone. The cellular fatty acid profile of strain DS-42^T is shown in Table 2, together with those of *R. huautlense* LMG 18254^T, *R. galegae* LMG 6214^T, *R. loessense* CIP 108030^T and *R. cellulosilyticum* DSM 18291^T, also analysed in this study. The fatty acid profiles of the five strains were essentially similar in that $C_{18:1}\omega7c$ is the major fatty acid, although there were differences in the proportions of some fatty acids (Table 2). The DNA G+C content of strain DS-42^T was 60.8 mol%, which is in the range reported for known *Rhizobium* species (Table 1).

Strain DS-42^T exhibited mean DNA–DNA relatedness of 13–19 % to the type strains of phylogenetically related *Rhizobium* species [*R. huautlense* LMG 18254^T (15 %), *R. galegae* LMG 6214^T (19 %), *R. loessense* CIP 108030^T (19 %) and *R. cellulosilyticum* DSM 18291^T (13 %)]. These values indicate that strain DS-42^T represents a genomic species distinct from these four *Rhizobium* species (Wayne *et al.*, 1987). Strain DS-42^T is clearly distinguishable from these four *Rhizobium* species by differences in some phenotypic characteristics, including enzyme activities, utilization of substrates and susceptibility to antibiotics (Table 1). The phylogenetic distinctiveness, together with the DNA–DNA relatedness data and differential phenotypic properties, is sufficient to allocate strain DS-42^T to a species that is separate from recognized *Rhizobium* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-42^T is considered to represent a novel species within the genus *Rhizobium*, for which the name *Rhizobium soli* sp. nov. is proposed.

Description of *Rhizobium soli* sp. nov.

Rhizobium soli (so′li. L. gen. n. *soli* of soil).

Cells are Gram-negative, non-spore-forming, aerobic rods, 0.4–0.7 × 1.0–4.5 μm. Non-motile. Colonies on TSA are circular, convex, smooth, glistening, pale yellow and 1.2–2.0 mm in diameter after incubation for 3 days at 25 °C. Growth occurs at 4 and 31 °C, with optimum growth at 25 °C, but not at 32 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.5 and 8.5, but not at pH 5.0 or 9.0. Growth occurs in the presence of 0.5–3.0 % (w/v) NaCl, with optimum growth in the presence of 0.5–1.0 % (w/v) NaCl. Nodulation is not observed on three different legumes and the *nodD* and *nifH* genes are not detected by PCR. Catalase- and oxidase-positive. Urease-negative. Nitrate reduction is negative. H_2S and indole are not produced. Aesculin is hydrolysed, but casein, starch, tyrosine, hypoxanthine, xanthine and Tweens 20, 40, 60 and 80 are not. Glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, methyl β-D-xyloside, galactose, glucose, fructose, mannose, mannitol, sorbitol, *N*-acetyl-glucosamine, aesculin, cellobiose, melibiose, xylitol, L-fucose, D-arabitol, gluconate and 2-ketogluconate are utilized and maltose, trehalose, gentiobiose and D-lyxose are utilized weakly, but L-xylose, adonitol, sorbose, rhamnose, dulcitol, inositol, methyl α-D-mannoside, methyl α-glucoside, amygdalin, salicin, lactose, sucrose,

Table 2. Cellular fatty acid compositions of *R. soli* sp. nov. DS-42^T and type strains of phylogenetically related *Rhizobium* species

Strains: 1, *R. soli* DS-42^T; 2, *R. huautlense* LMG 18254^T; 3, *R. galegae* LMG 6214^T; 4, *R. loessense* CIP 108030^T; 5, *R. cellulosilyticum* DSM 18291^T. All data are from this study. Values are percentages of total fatty acids; fatty acids that represented <0.5 % in all strains were omitted. −, Not detected.

Fatty acid	1	2	3	4	5
Straight-chain					
$C_{16:0}$	10.2	9.1	7.7	2.3	12.5
$C_{18:0}$	1.2	1.3	0.9	6.0	0.5
Unsaturated					
$C_{15:1}\omega8c$	−	0.7	0.8	−	−
$C_{18:1}\omega7c$	70.5	76.2	76.2	81.9	70.3
Hydroxy					
$C_{16:0}$ 3-OH	1.8	1.5	2.0	1.7	2.4
$C_{18:1}$ 2-OH	−	1.3	−	−	−
$C_{18:0}$ 3-OH	−	−	0.7	0.6	−
11-Methyl $C_{18:1}\omega7c$	1.0	−	−	−	−
10-Methyl $C_{19:0}$	0.8	1.2	2.1	−	0.7
$C_{19:0}$ cyclo $\omega8c$	1.0	2.4	3.9	0.6	2.9
Summed features*					
2	4.9	5.0	4.6	6.0	5.8
3	8.0	1.1	0.5	0.5	4.1

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 contained iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH. Summed feature 3 contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

inulin, melezitose, raffinose, starch, glycogen, turanose, D-tagatose, D-fucose, L-arabitol and 5-ketogluconate are not utilized. Acid is produced from D-glucose, L-rhamnose, melibiose and L-arabinose, but not from D-mannitol, inositol, D-sorbitol, sucrose or amygdalin. Alkaline phosphatase, leucine arylamidase and α-glucosidase activities are present and esterase (C4), esterase lipase (C8) and acid phosphatase activities are weak, but arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Susceptible to carbenicillin, cephalothin, chloramphenicol, kanamycin, novobiocin, oleandomycin, polymyxin B, streptomycin, gentamicin, tetracycline and neomycin, but not to ampicillin, lincomycin or penicillin G. The predominant ubiquinone is Q-10. The major fatty acid is $C_{18:1}\omega7c$. The DNA G+C content of the type strain is 60.8 mol%.

The type strain, DS-42^T (=KCTC 12873^T =JCM 14591^T), was isolated from a soil from Dokdo, an island of Korea.

Acknowledgements

This work was supported by the 21C Frontier Program of Microbial Genomics and Applications (grant MG05-0401-2-0) from the Ministry of Education, Science and Technology (MEST) of the Republic of Korea.

References

Berge, O., Lodhi, A., Brandelet, G., Santaella, C., Roncato, M.-A., Christen, R., Heulin, T. & Achouak, W. (2009). *Rhizobium alamii* sp. nov., an exopolysaccharide-producing species isolated from legume and non-legume rhizospheres. *Int J Syst Evol Microbiol* **59**, 367–372.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

Euzéby, J. P. (1997). List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* **47**, 590–592. http://www.bacterio.cict.fr

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Frank, B. (1889). Über die Pilzsymbiose der Leguminosen. *Ber Dtsch Bot Ges* **7**, 332–346 (in German).

García-Fraile, P., Rivas, R., Willems, A., Peix, A., Martens, M., Martínez-Molina, E., Mateos, P. F. & Velázquez, E. (2007). *Rhizobium cellulosilyticum* sp. nov., isolated from sawdust of *Populus alba*. *Int J Syst Evol Microbiol* **57**, 844–848.

Gu, C. T., Wang, E. T., Tian, C. F., Han, T. X., Chen, W. F., Sui, X. H. & Chen, W. X. (2008). *Rhizobium miluonense* sp. nov., a symbiotic bacterium isolated from *Lespedeza* root nodules. *Int J Syst Evol Microbiol* **58**, 1364–1368.

Han, T. X., Wang, E. T., Wu, L. J., Chen, W. F., Gu, J. G., Gu, C. T., Tian, C. F. & Chen, W. X. (2008). *Rhizobium multihospitium* sp. nov., isolated from multiple legume species native of Xinjiang, China. *Int J Syst Evol Microbiol* **58**, 1693–1699.

Hunter, W. J., Kuykendall, L. D. & Manter, D. K. (2007). *Rhizobium selenireducens* sp. nov. a selenite-reducing α-*Proteobacteria* isolated from a bioreactor. *Curr Microbiol* **55**, 455–460.

Hunter, W. J., Kuykendall, L. D. & Manter, D. K. (2008). *Rhizobium selenireducens* sp. nov. In *List of New Names and New Combinations Previously Effectively, but not Validly, Published*, Validation List no. 121. *Int J Syst Evol Microbiol* **58**, 1057.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Lee, K.-B., Liu, C.-T., Anzai, Y., Kim, H., Aono, T. & Oyaizu, H. (2005). The hierarchical system of the '*Alphaproteobacteria*': description of *Hyphomonadaceae* fam. nov., *Xanthobacteraceae* fam. nov. and *Erythrobacteraceae* fam. nov. *Int J Syst Evol Microbiol* **55**, 1907–1919.

Peng, G., Yuan, Q., Li, H., Zhang, W. & Tan, Z. (2008). *Rhizobium oryzae* sp. nov., isolated from the wild rice *Oryza alta*. *Int J Syst Evol Microbiol* **58**, 2158–2163.

Poly, F., Monrozier, L. J. & Bally, R. (2001). Improvement in the RFLP procedure for studying the diversity of *nifH* genes in communities of nitrogen fixers in soil. *Res Microbiol* **152**, 95–103.

Quan, Z.-X., Bae, H.-S., Baek, J.-H., Chen, W.-F., Im, W.-T. & Lee, S.-T. (2005). *Rhizobium daejeonense* sp. nov., isolated from a cyanide treatment bioreactor. *Int J Syst Evol Microbiol* **55**, 2543–2549.

Ramírez-Bahena, M. H., García-Fraile, P., Peix, A., Valverde, A., Rivas, R., Igual, J. M., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2008). Revision of the taxonomic status of the species *Rhizobium leguminosarum* (Frank 1879) Frank 1889^{AL}, *Rhizobium phaseoli* Dangeard 1926^{AL} and *Rhizobium trifolii* Dangeard 1926^{AL}. *R. trifolii* is a later synonym of *R. leguminosarum*. Reclassification of the strain *R. leguminosarum* DSM 30132 (=NCIMB 11478) as *Rhizobium pisi* sp. nov. *Int J Syst Evol Microbiol* **58**, 2484–2490.

Rivas, R., Velázquez, E., Willems, A., Vizcaíno, N., Subba-Rao, N. S., Mateos, P. F., Gillis, M., Dazzo, F. B. & Martínez-Molina, E. (2002). A new species of *Devosia* that forms a unique nitrogen-fixing root-nodule symbiosis with the aquatic legume *Neptunia natans* (L.f.) Druce. *Appl Environ Microbiol* **68**, 5217–5222.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, Technical Note no. 101. Newark, DE: MIDI.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Tian, C. F., Wang, E. T., Wu, L. J., Han, T. X., Chen, W. F., Gu, C. T., Gu, J. G. & Chen, W. X. (2008). *Rhizobium fabae* sp. nov., a bacterium that nodulates *Vicia faba*. *Int J Syst Evol Microbiol* **58**, 2871–2875.

Valverde, A., Igual, J. M., Peix, A., Cervantes, E. & Velázquez, E. (2006). *Rhizobium lusitanum* sp. nov. a bacterium that nodulates *Phaseolus vulgaris*. *Int J Syst Evol Microbiol* **56**, 2631–2637.

Wang, E. T., van Berkum, P., Beyene, D., Sui, X. H., Dorado, O., Chen, W. X. & Martínez-Romero, E. (1998). *Rhizobium huautlense* sp. nov., a symbiont of *Sesbania herbacea* that has a close phylogenetic relationship with *Rhizobium galegae*. *Int J Syst Bacteriol* **48**, 687–699.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic

Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Wei, G. H., Tan, Z. Y., Zhu, M. E., Wang, E. T., Han, S. Z. & Chen, W. X. (2003). Characterization of rhizobia isolated from legume species within the genera *Astragalus* and *Lespedeza* grown in the Loess Plateau of China and description of *Rhizobium loessense* sp. nov. *Int J Syst Evol Microbiol* **53**, 1575–1583.

Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.

Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.

Yoon, J.-H., Kim, I.-G., Shin, D.-Y., Kang, K. H. & Park, Y.-H. (2003). *Microbulbifer salipaludis* sp. nov., a moderate halophile isolated from a Korean salt marsh. *Int J Syst Evol Microbiol* **53**, 53–57.

Young, J. M., Kuykendall, L. D., Martínez-Romero, E., Kerr, A. & Sawada, H. (2001). A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie *et al.* 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*. *Int J Syst Evol Microbiol* **51**, 89–103.