Journal of Microbiology (2013) Vol. 51, No. 2, pp. 262–267
DOI 10.1007/s12275-013-2563-5

NOTE

Paraherbaspirillum soli gen. nov., sp. nov. Isolated from Soil

Rangasamy Anandham[1], Soo-Jin Kim[2], Ji Young Moon[2], Hang-Yeon Weon[2], and Soon-Wo Kwon[2*]

[1]*Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai, India*
[2]*Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea*

(Received October 16, 2012 / Accepted November 30, 2012)

A bacterial strain, designated JS5-2^T, was isolated from soil collected from Jeju Island, Republic of Korea. The cells of the strain were Gram-negative, nonspore forming, catalase- and oxidase-positive, aerobic, nonmotile and rod-shaped. Strain JS5-2^T exhibited 96.2–97.2, 95.1–96.3, and 95.4–95.8% 16S rRNA gene sequence similarities to the genera *Herbaspirillum*, *Oxalicibacterium*, and *Herminiimonas*, respectively. The highest sequence similarities were with *Herbaspirillum autotrophicum* IAM 14942^T (97.2%) and *Herbaspirillum frisingense* GSF30^T (97.1%). The major fatty acids of strain JS5-2^T were $C_{16:0}$ (35.0%), $C_{17:0}$ cyclo (19.9%), $C_{18:1}$ *ω7c* (11.4%), and summed feature 3 ($C_{16:1}$ *ω7c*/$C_{15:0}$ iso 2-OH) (15.2%), and the major polar lipids of strain JS5-2^T were diphosphatidylglycerol and an unknown aminophospholipid. The strain contained Q-8 as the predominant ubiquinone. DNA-DNA relatedness values between strain JS5-2^T and *H. autotrophicum* IAM 14942^T, and *H. frisingense* GSF30^T were 32 and 35%, respectively. The DNA G+C content of strain JS5-2^T was 59.0 mol%. On the basis of phenotypic, genotypic, and physiological evidence, strain JS5-2^T represents a novel species of a new genus, for which the name *Paraherbaspirillum soli* gen. nov., sp. nov. is proposed. The type strain JS5-2^T (=KACC 12633^T =NBRC 106496^T) is proposed.

Keywords: DNA-DNA hybridization, *Paraherbaspirillum soli*, taxonomy, 16S rRNA gene sequence

During a course of study on the microbial diversity in soil collected in Jeju Island, Republic of Korea, we isolated many novel strains. In this study, we describe the taxonomic position of strain JS5-2^T isolated from Jeju soil. The results suggest that this strain represents a novel species belonging to a new genus in the family *Oxalobacteriaceae* of *Betaproteobacteria*; hence, *Paraherbaspirillum soli* sp. nov. is proposed for strain JS5-2^T. The family *Oxalobacteriaceae* is metabolically diverse, and includes some strict anaerobes and nitrogen-fixing organisms. Currently, the family *Oxalobacteriaceae* contains 13 genera including *Collimonas*, *Duganella*, *Glaciimonas*, *Herbaspirillum*, *Herminimonas*, *Janthinobacterium*, *Massilia*, *Naxibacter*, *Oxalicibacterium*, *Oxalobacter*, *Pseudoduganella*, *Telluria*, and *Undibacterium* (http://www.bacterio.cict.fr/).

In order to isolate a culturable bacteria from the soil of Jeju Island, a soil sample was serially diluted with 0.85% (w/v) NaCl and the appropriate 10-fold dilutions were plated on R2A agar (Difco, USA). The plates were incubated at 28°C for 4 days. JS5-2^T was one of the isolates that grew on the R2A medium, and was distinguishable due to the light yellow color of the colony.

Genomic DNA was isolated by the method of Ausubel *et al.* (1987), except that the lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer pair 9F and 1512R (Weisburg *et al.*, 1991), and the purified PCR products were sequenced by Solgent (Korea). Identification of phylogenetic neighbors and calculation of pairwise levels of 16S rRNA gene sequence similarity were achieved using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007). Sequence alignment and analysis of the data were performed using the arb software package (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 100 database (release August 2009; Pruesse *et al.*, 2007). Phylogenetic trees were constructed using MEGA version 5.0 (Tamura *et al.*, 2011) on the basis of the neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Kluge and Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms. A nearly full-length 16S rRNA gene sequence (1,473 bp) of the strain JS5-2^T was obtained. Strain JS5-2^T exhibited 96.2–97.2, 95.1–96.3, and 95.4–95.8% 16S rRNA gene sequence similarities to the genus *Herbaspirillum*, *Oxalicibacterium*, and *Herminiimonas* species, respectively. The highest sequence similarities were shown with *Herbaspirillum autotrophicum* IAM 14942^T (97.2%) and *Herbaspirillum frisingense* GSF30^T (97.1%). In the neighbor-joining phylogenetic tree (Fig. 1), strain JS5-2^T formed a group with two species of the genus *Oxalobacter*, despite a low bootstrap value, that was separate from the genera *Herbaspirillum*, *Collimonas*, *Oxalicibacterium*, and *Herminiimonas*. Interestingly, members of the

*For correspondence. E-mail: swkwon1203@korea.kr; Tel.: +82-31-299-1860; Fax: +82-31-299-1869
The GenBank accession number for the 16S rRNA gene sequences of strainJS5-2^T is FJ812351.

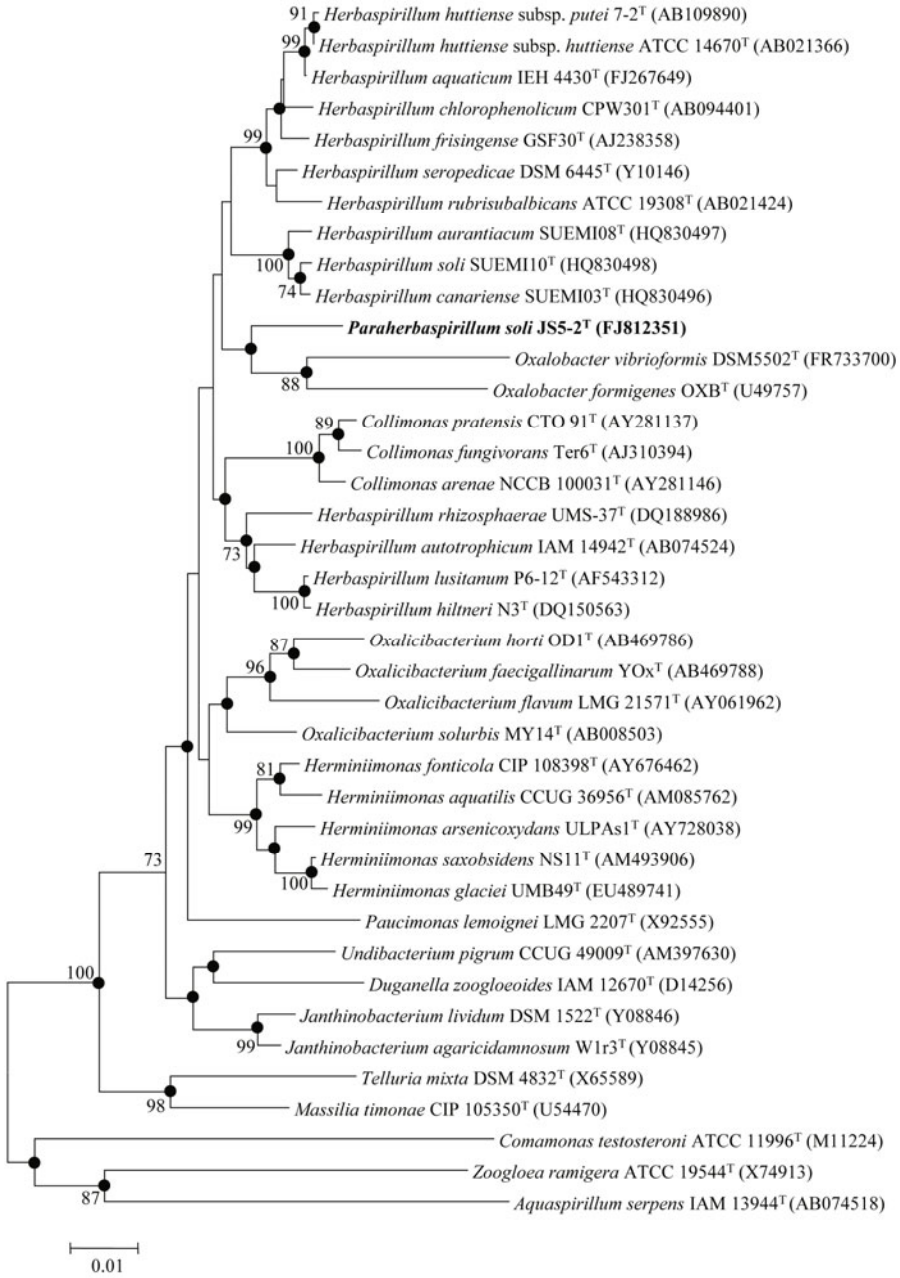

Fig. 1. Neighbor-joining tree based on almost full length 16S rRNA coding gene sequences showing the phylogenetic position of strain JS5-2^T. Numbers at nodes indicate percentages of 1,000 bootstrap re-samplings; only values >70% are given. Bar, 0.01 substitutions per nucleotide position. Dots indicate that the corresponding branches were also recovered both in the maximum-parsimony and maximum-likelihood trees.

genus *Herbaspirillum* were divided into two groups. These topologies were also supported by maximum-likelihood and maximum-parsimony tree (Fig. 1).

Cell morphology and motility were observed under a phase-contrast microscope (AXIO; Zeiss, Germany) and transmission electron microscope (912AB; LEO, Germany) after cultivation of the strain on R2A at 28°C. Gram reaction, presence of catalase and oxidase activities, and hydrolysis of casein, DNA and starch were determined as described previously (Smibert and Krieg, 1994). Hydrolysis of carboxymethylcellulose (CM-cellulose) (0.1%, w/v), chitin from crab shells (1%, w/v), pectin (0.5%, w/v) and tyrosine (0.5%, w/v) were also examined. Anaerobic growth was investigated using incubation in the BBL GasPak Anaerobic System (Difco) for 14 days at 28°C on R2A agar containing 0.5% Na_2SO_4, 0.5% $NaNO_3$, 0.5% $NaHCO_3$ or 0.02% $FeCl_3$. Growth at different temperatures (5, 10, 15, 20, 25, 30, 35, 37, 40, and 45°C) was assessed after 7 days incubation in R2A broth. Various pH values (4.0–10.0 at intervals of 0.5 pH units) were tested on R2A broth buffered with citrate/phosphate (pH 4.0–4.5), 10 mM MES (pH 5.0–6.0), 10 mM PIPES (pH 6.5–7.0) or 10 mM Tris/HCl (7.5–10) and adjusted with NaOH or HCl. Salt tolerance was tested on liquid R2A medium supplemented with 0–7% (w/v) NaCl (at intervals of 1% NaCl) up to 7 days of incubation at 28°C. Carbon source utilization and some enzyme activities were tested using the API 20 NE, API ZYM, and API ID 32 GN systems (bioMérieux, France) according to the manufacturer's instruc-

Table 1. Differential characteristics of strain JS5-2^T and related taxa within the family *Oxalobacteriaceae*
Taxa: 1, JS5-2^T; 2, *Collimonas* (De Boer *et al*., 2004; Höppener-Ogawa *et al*., 2008); 3, *Duganella* (Hiraishi *et al*., 1997; Kämpfer *et al*., 2012); 4, *Herbaspirillum* (Data from this study; Aragno and Schlegel, 1978; Baldani *et al*., 1986, 1996; Kirchhof *et al*., 2001; Valverde *et al*., 2003; Ding and Yokota, 2004; Im *et al*., 2004; Rothballer *et al*., 2006; Jung *et al*., 2007; Dobritsa *et al*., 2010; Carro *et al*., 2012); 5, *Herminiimonas* (this study; Fernandes *et al*., 2005; Kämpfer *et al*., 2006; Muller *et al*., 2006; Loveland-Curtez *et al*., 2009; Lang *et al*., 2007); 6, *Oxalicibacterium* (Tamer *et al*., 2002; Sahin *et al*., 2009, 2010) ; 7, *Oxalobacter* (Allison *et al*., 1985; Dehning and Schink, 1989); 8, *Paucimonas* (Jendrossek, 2001); 9, *Pseudoduganella* (Kämpfer *et al*., 2012). +, Positive; -, negative; ND, not determined.

Characteristics	1	2	3	4	5	6	7	8	9
Source	Soil	Soil	Soil, wastewater	Water, soil, plant	Water, sludge, glacier, rock	Plant litter, chicken dung, soil	Colon, rumen, sediment	Soil	Soil
O2 requirement	Aerobe	Aerobe	Aerobe	Aerobe	Aerobe	Aerobe	Strictly anaerobe	Aerobe	Aerobe
Morphology	Rod	ND	Rod	Rod/spirillum	Rod	Rod	Rod	Rod	Rod
Motility	-	+	+	+	+	+	+/-	+	+
Major polar lipids[a]	APL, DPG	ND	PG, PE	PE, PG	PE, PG, DPG	PE, PG	ND	ND	DPG, PG, PE
G+C contents (mol%)	59	57–62	63–64	60–67	51–59	56–64	48–52	59	63

[a]APL, Aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol

tions. Nitrogen fixing ability of strain JS5-2^T was evaluated by (i) polymerase chain reaction (PCR) specific for the *nifH* gene (Ueda *et al*., 1995) using the 16F (5′-GCIWTYTAYGG IAARGGIGG-3′) and 407R (5′-AAICCRCCRCAIACIACR TC-3′) primers; (ii) the ability to grow in semi-solid nitrogen-free JNFb medium, in which the formation of a characteristic pellicle within the medium indicated nitrogen fixation; and (iii) acetylene reduction assay (ARA) using gas chromatography (DS 6200, Donam Instruments Inc., Korea) fitted with a flame ionization detector and a Porapak-Q column (Döbereiner, 1995).

Cellular fatty acid analysis was conducted for strain JS5-2^T along with the reference strains *Collimonas fungivorans* KACC 12192^T, *Duganella violaceinigra* KACC 11669^T, *Duganella zoogloeoides* KACC 11690^T, *Herbaspirillum autotrophicum* DSM 732^T, *Herbaspirillum chlorophenolicum* KACC 11649^T, *Herbaspirillum frisingense* KACC 12182^T, *Herbaspirillum hiltneri* KACC 12211^T, *Herbaspirillum huttiense* KACC 12180^T, *Herbaspirillum lusitanum* KACC 11687^T, *Herbaspirillum putei* KACC 11685^T, *Herbaspirillum rubri-*

Table 2. Fatty acid compositions of JS5-2^T and related taxa within the family *Oxalobacteriaceae*
Taxa: 1, JS5-2^T; 2, *Collimonas* (this study; Jendrossek, 2001); 3, *Duganella* (this study; Kämpfer *et al*., 2012); 4, *Herbaspirillum* (this study; Dobritsa *et al*., 2010; Carro *et al*., 2012); 5, *Herminiimonas* (5) (this study; Kämpfer *et al*., 2006; Jung *et al*., 2007; Lang *et al*., 2007; Loveland-Curtez *et al*., 2009); 6, *Oxalicibacterium* (Sahin *et al*., 2009, 2010); 7, *Oxalobacter* (Allison *et al*., 1985); 8, *Paucimonas* (Mergaert *et al*., 1996); 9, *Pseudoduganella* (this study). -, not detected or <0.5%.

Fatty acids		1	2	3	4	5	6	7	8	9
$C_{10:0}$		-	-	0–0.9	0.3–0.5	-	-	-	-	0.5
$C_{10:0}$ 3-OH		0.9	2.0	5.6–10.3	1.4–5.7	6.5–8.6	0–8.4	-	3.6	5.1
$C_{12:0}$		3.8	6.5	3.7–13.5	0.6–5.2	-	-	1	5.3	7.0
$C_{12:0}$ 2-OH		2.7	3.8	-	0–3.1	-	-	-	-	3.0
$C_{12:0}$ 3-OH		3.6	5.8	-	3.5–5.3	-	-	6	5.7	4.9
$C_{14:0}$		-	1.4	0.5–0.8	0–4.0	0–5.0	0–5.4	1	1.3	0.8
$C_{14:0}$ 2-OH		-	-	-	0–3.7	-	-	-	4.7	-
$C_{14:0}$ 3-OH		-	-			-	-	10	-	-
$C_{15:1}$ *ω6c*		-	-	-	-	0–0.6	0–1.9	-	-	-
$C_{16:0}$		35.0	30.3	24.7–34.2	19.0–36.0	20.4–36.4	14.0–37.2	33	12.8	32.1
$C_{16:1}$ 2-OH		4.9	-	-	-	-	-	-	-	-
$C_{16:1}$ *ω5c*		-	-	-	-	-	0–0.9	-	-	
$C_{17:0}$		-	-	-	-	0–1.6	1.3–3.8	-	-	-
$C_{17:0}$ cyclo		19.9	4.1	-	0–29.2	3.3–28.4	2.2–41.6	34	1.9	-
$C_{18:0}$		1.1	3.7	-	0–2.3	0–0.9	0–3.6	-	-	-
$C_{18:1}$ *ω5c*		-	-	-	-	-	0–2.7	-	-	-
$C_{18:1}$ *ω6c*		-	-	-	-	-	0–11.1	-	-	-
$C_{18:1}$ *ω7c*		11.4	11.8	5.7–8.6	6.0– 24.2	5.7–9.0	0–11.9	-	25.5	4.3
$C_{18:1}$ *ω7c* 11-methyl		-	-	-	0–1.5	0–0.6	-	-	-	-
$C_{18:1}$ *ω9c*		-	-	-	-	-	0–1.7	-	-	-
$C_{19:0}$ *ω8c* cyclo		0.5	-	-	0–2.9	0–8.4	1.1–14.9	14	-	-
Summed feature[a]	3	15.2	30.8	41.2–49.7	10.1–45.5	11.6–52.7	2.8–37.3	-	39.3	41.5
	5	-	-	-	0–1.0	0–1.5	-	-	-	-
	7	-	-	-	0–0.7	-	-	-	-	-

[a] Summed feature 3 included $C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH. Summed feature 5 included $C_{18:2}$ *ω6,9c* and/or anteiso-$C_{18:0}$. Summed feature 7 included unknown 18.846 and/or $C_{19:1}$ *ω6c*.

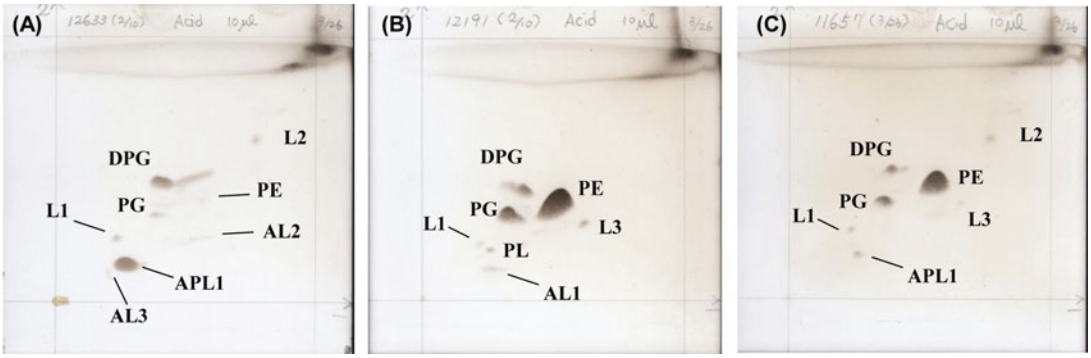

Fig. 2. Polar lipid profile of strain JS5-2^T (A), *Herbaspirillum seropedicae* KACC 12191^T (B), *Herminiimonas fonticola* CIP 108398^T (C) after separation by two-dimensional TLC. DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL, unknown aminolipids; APL, unknown aminophospholipid; L, unknown lipid.

subalbicans KACC 12181^T, *Herbaspirillum seropedicae* KACC 12191^T, *Herminiimonas aquatilis* KACC 11671^T, and *Herminiimonas fonticola* KACC 11657^T. For the fatty acid analysis, all strains were grown on R2A medium for 2 days at 28°C. The fatty acid methyl esters were identified and quantified by using the TSBA 6 database (version 6.10) of the Sherlock Microbial Identification System (MIDI). Isoprenoid quinones were purified and analyzed by HPLC as described by Minnikin *et al.* (1984). The G+C contents were determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), using a reverse-phased column (Supelcosil LC-18-S; Supelco). The polar lipid profiles of strain JS5-2^T, *Herbaspirillum seropedicae* KACC 12191^T and *Herminiimonas fonticola* CIP 108398^T were determined according to the method of Minnikin *et al.* (1984). Ethanolic molybdatophosphoric acid, α-naphthol-sulphuric acid reagent, Dragendorff reagent, ninhydrin and molybdenum blue reagent were used to detect total lipids, glycolipids, quaternary nitrogen and aminophospholipids, respectively. To determine genomic relatedness, the filter hybridization method was performed according to Seldin and Dubnau (1985). Probe labeling was conducted using the non-radioactive DIG-High prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA-DNA relatedness was quantified by using a densitometer (Bio-Rad, USA).

Cells of strain JS5-2 were Gram-negative, non-spore forming, aerobic, non-motile rods (1.1–3.0 μm long and 0.6–0.8 μm wide). Growth occurred on R2A and NA, but not on TSA or MacConkey agar. After 2 days of growth on R2A, an irregular light yellow-colored colony formed. The strain did not require salt for growth; however, growth occurred in medium containing 2% NaCl. The temperature and pH ranges for growth were 5–30°C (optimum, 28–30°C) and pH 5.0–9.0 (optimum, pH 7.0), respectively. Phenotypically, strain JS5-2^T could be differentiated from *Oxalobacter* based on its aerobic growth and higher G+C contents, and from the genera *Herbaspirillum*, *Herminiimonas*, and *Collimonas* on the basis of motility. The other differentiating characteristics of strain JS5-2^T from closely related genera within the family are given in Table 1. JS5-2^T did not show the characteristic white pellicle formation in semi-solid JNFb medium and did not exhibit acetylene reduction activity. However, strain JS5-2^T exhibited a positive amplicon of the expected size for *nifH* gene (data not shown).

Strain JS5-2^T contained $C_{16:0}$ (35.0%), $C_{17:0}$ cyclo (19.9%), summed feature ($C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2-OH) (15.2%) and $C_{18:1}$ $\omega7c$ (11.4%) as the major fatty acid components, and moderate amounts of $C_{16:1}$ 2-OH (4.9%), $C_{12:0}$ (3.8%) and $C_{12:0}$ 2-OH (3.6%). It is noteworthy that strain JS5-2^T had a unique fatty acid component, $C_{16:1}$ 2-OH, which was absent from other members of the family *Oxalobacteraceae*. Additionally, the fatty acid composition of strain JS5-2^T can be differentiated from that of the closely related genus *Oxalobacter* on the basis of the high abundance of summed feature 3 and $C_{18:1}$ $\omega7c$ (Table 2). The polar lipids of strain of JS5-2^T were composed of a large amount of diphosphatidylglycerol (DPG) and one unknown aminophospholipid (APL1), and small amounts of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), two unknown amino lipids (AL2 and AL3) and two unknown lipids (L1 and L2) (Fig. 2). In contrast, *Herbaspirillum seropedicae* KACC 12191^T and *Herminiimonas fonticola* CIP 108398^T contained a large amount of PE and PG, and no or trace amounts of one unknown APL1 (Fig. 2). The major isoprenoid quinone of strain JS5-2^T was ubiquinone (Q-8). The genomic DNA G+C content of the strain JS5-2^T was 59.0. DNA–DNA relatedness values between strain JS5-2^T and *Herbaspirillum autotrophicum* DSM 732^T and *Herbaspirillum frisingense* KACC 12182^T were 32 and 35%, respectively.

On the basis of phylogenetic chemotaxonomic analysis, strain JS5-2^T represents a novel genus and species, for which the name *Paraherbaspirillum soli* gen. nov., sp. nov. is proposed.

Description of *Paraherbaspirillum* gen. nov.

Paraherbaspirillum (Pa·ra·her.ba.spi'ril.lum. Gr. Prep. *Para* like, beside; N.L.mas.n. *Herbaspirillum* a bacterial genus; N.L.masc.n. *Paraherbaspirillum* like *Herbaspirillum*, referring to the close relationship to the genus).

Cells were Gram-negative, non-spore forming, catalase- and oxidase-positive, aerobic, nonmotile and rod shaped. The major fatty acids were $C_{16:0}$, $C_{17:0}$ cyclo, summed feature 3 ($C_{16:1}$ $\omega7c$/ iso$C_{15:0}$ 2-OH) and $C_{18:1}$ $\omega7c$. The polar lipids were composed of a large amount of diphosphatidylglycerol

and one unknown aminophospholipid, and a small amount of phosphatidylglycerol, phosphatidylethanolamine, two unknown amino lipids and two unknown lipids. The major isoprenoid quinone was ubiquinone (Q-8). The G+C content of the type strain of the type species was 59.0 mol%. Phylogenetically, the genus belongs to the family *Oxalobacteriaceae*. The type species is *Paraherbaspirillum soli* sp. nov.

Description of *Paraherbaspirillum soli* sp. nov.

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

It displayed the following characteristics in addition to those given in the genus description. After 2 days of growth on R2A, an irregular light yellow-colored colony was formed. Cells were rod-shaped (1.1–3.0 μm long and 0.6–0.8 μm wide). Growth occurred on R2A and NA, but not on TSA or MacConkey agar. Salt was not required for growth; however growth occurred in medium containing 2% NaCl (optimum 0–1%). The temperature and pH ranges for growth were 5–30°C (optimum 28–30°C) and pH 5.0–9.0 (optimum pH 7.0), respectively. The strain hydrolyzed tyrosine, pectin, xanthine, hypoxanthine and Tween 80, but not casein, starch, DNA, chitin and CM-cellulose. The strain was negative for nitrogen fixation, nitrate reduction, indole production, Voges-Proskauer test, glucose fermentation, urease, arginine dihydrolase, and aesculin hydrolysis. L-arabinose, *N*-acetylglucsoamine, potassium gluconate, D-ribose, inositol, sodium acetate, lactic acid, L-alanine, glycogen, L-serine, propionic acid, L-histidine, 3-hydroxybutyric acid, and L-proline were assimilated. D-glucose, D-mannose, D-mannitol, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-saccharose, itaconic acid, suberic acid, sodium malonate, potassium 5-ketogluconate, salicin, D-melibiose, L-fucose, D-sorbitol, valeric acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, and potassium 2-ketogluconate were not assimilated. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and napthol-AS-BI-phosphohydrolase activities were present. Trypsin, α-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase, and *α*-fucosidase activities were absent.

The type strain, JS5-2^T (=KACC 12633^T =NBRC 106496^T), was isolated from soil samples collected from Jeju Island, Republic of Korea.

This study was supported by the "Research Program for Agricultural Science & Technology Development" (Project No. PJ008666), National Academy of Agricultural Science, Rural Development Administration, Republic of Korea.

References

Allison, M.J., Dawson, K.A., Mayberry, W.R., and Foss, J.G. 1985. *Oxalobacter formigenes* gen. nov., sp. nov.: Oxalate-degrading anaerobes that inhabit the gastrointestinal tract. *Arch. Microbiol.* **141**, 1–7.

Aragno, M. and Schlegel, H.G. 1978. *Aquaspirillum autotrophicum*, a new species of hydrogen-oxidizing, facultatively autotrophic bacteria. *Int. J. Syst. Bacteriol.* **28**, 112–116.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (eds.). 1987. Current protocols in molecular biology. Greene/Wiley Interscience, New York, N.Y., USA.

Baldani, J.I., Baldani, V.L.D., Seldin, L., and Döbereiner, J. 1986. Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. *Int. J. Syst. Bacteriol.* **36**, 86–93.

Baldani, J.I., Pot, B., Kirchhof, G., Falsen, E., Baldani, V.L.D., Olivares, F.L., Hoste, B., Kersters, K., Hartmann, A., Gillis, M., and Döbereiner, J. 1996. Emended description of *Herbaspirillum*; inclusion of [*Pseudomonas*] *rubrisubalbicans*, a mild plant pathogen, as *Herbaspirillum rubrisubalbicans* comb. nov.; and classification of a group of clinical isolates (EF group 1) as *Herbaspirillum* species 3. *Int. J. Syst. Bacteriol.* **46**, 802–810.

Carro, L., Rivas, R., León-Barrios, M., González-Tirante, M., Velázquez, E., and Valverde, A. 2012. *Herbaspirillum canariense* sp. nov., *Herbaspirillum aurantiacum* sp. nov. and *Herbaspirillum soli* sp. nov., isolated from volcanic mountain soil, and emended description of the genus *Herbaspirillum*. *Int. J. Syst. Evol. Microbiol.* **62**, 1300–1306.

Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., and Lim, Y.W. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **57**, 2259–2261.

De Boer, W., Leveau, J.H.J., Kowalchuk, G.A., Klein Gunnewiek, P.J.A., Abeln, E.C.A., Figge, M.J., Sjollema, K., Janse, J.D., and Van Veen, J.A. 2004. *Collimonas fungivorans* gen. nov., sp. nov., a chitinolytic soil bacterium with the ability to grow on living fungal hyphae. *Int. J. Syst. Evol. Microbiol.* **54**, 857–864.

Dehning, I. and Schink, B. 1989. Two new species of anaerobic oxalate-fermenting bacteria, *Oxalobacter vibrioformis* sp. nov. and *Clostridium oxalicum* sp. nov., from sediment samples. *Arch. Microbiol.* **153**, 79–84.

Ding, L. and Yokota, A. 2004. Proposals of *Curvibacter gracilis* gen. nov., sp. nov. and *Herbaspirillum putei* sp. nov. for bacterial strains isolated from well water and reclassification of [*Pseudomonas*] *huttiensis*, [*Pseudomonas*] *lanceolata*, [*Aquaspirillum*] *delicatum* and [*Aquaspirillum*] *autotrophicum* as *Herbaspirillum huttiense* comb. nov., *Curvibacter lanceolatus* comb. nov., *Curvibacter delicatus* comb. nov. and *Herbaspirillum autotrophicum* comb. nov. *Int. J. Syst. Evol. Microbiol.* **54**, 2223–2230.

Döbereiner, J. 1995. Isolation and identification of aerobic nitrogen-fixing bacteria from soil and plants. *In* Methods in Applied Soil Microbiology and Biochemistry, pp. 134–141. *In* Alef, K. and Nannipieri, P. (eds.). Academic Press, London, UK.

Dobritsa, A.P., Reddy, M.C.S., and Samadpour, M. 2010. Reclassification of *Herbaspirillum putei* as a later heterotypic synonym of *Herbaspirillum huttiense*, with the description of *H. huttiense* subsp. *huttiense* subsp. nov. and *H. huttiense* subsp. *putei* subsp. nov., comb. nov., and description of *Herbaspirillum aquaticum* sp. nov. *Int. J. Syst. Evol. Microbiol.* **60**, 1418–1426.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376.

Fernandes, C., Rainey, F.A., Nobre, M.F., Pinhal, I., Folhas, F., and Da Costa, M.S. 2005. *Herminiimonas fonticola* gen. nov., sp. nov., a *Betaproteobacterium* isolated from a source of bottled mineral water. *Syst. Appl. Microbiol.* **28**, 596–603.

Hiraishi, A., Shin, Y.K., and Sugiyama, J. 1997. Proposal to reclassify *Zoogloea ramigera* IAM 12670 (P. R. Dugan 115) as *Duganella zoogloeoides* gen. nov., sp. nov. *Int. J. Syst. Bacteriol.* **47**, 1249–1252.

Höppener-Ogawa, S., De Boer, W., Leveau, J.H.J., Van Veen, J.A., De Brandt, E., Vanlaere, E., Sutton, H., Dare, D.J., and Vandamme, P. 2008. *Collimonas arenae* sp. nov. and *Collimonas pratensis* sp. nov., isolated from (semi-)natural grassland soils. *Int. J. Syst. Evol. Microbiol.* **58**, 414–419.

Im, W.T., Bae, H.S., Yokota, A., and Lee, S.T. 2004. *Herbaspirillum chlorophenolicum* sp. nov., a 4-chlorophenol-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* **54**, 851–855.

Jendrossek, D. 2001. Transfer of [*Pseudomonas*] *lemoignei*, a Gram-negative rod with restricted catabolic capacity, to *Paucimonas* gen. nov. with one species, *Paucimonas lemoignei* comb. nov. *Int. J. Syst. Evol. Microbiol.* **51**, 905–908.

Jung, S.Y., Lee, M.H., Oh, T.K., and Yoon, J.H. 2007. *Herbaspirillum rhizosphaerae* sp. nov., isolated from rhizosphere soil of *Allium victorialis* var. *platyphyllum*. *Int. J. Syst. Evol. Microbiol.* **57**, 2284–2288.

Kämpfer, P., Busse, H.J., and Falsen, E. 2006. *Herminiimonas aquatilis* sp. nov., a new species from drinking water. *Syst. Appl. Microbiol.* **29**, 287–291.

Kämpfer, P., Wellner, S., Lohse, K., Martin, K., and Lodders, N. 2012. *Duganella phyllosphaerae* sp. nov., isolated from the leaf surface of *Trifolium repens* and proposal to reclassify *Duganella violaceinigra* into a novel genus as *Pseudoduganella violceinigra* gen. nov., comb. nov. *Syst. Appl. Microbiol.* **35**, 19–23.

Kirchhof, G., Eckert, B., Stoffels, M., Baldani, J.I., Reis, V.M., and Hartmann, A. 2001. *Herbaspirillum frisingense* sp. nov., a new nitrogen-fixing bacterial species that occurs in C4-fibre plants. *Int. J. Syst. Evol. Microbiol.* **51**, 157–168.

Kluge, A.G. and Farris, J.S. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18**, 1–32.

Lang, E., Swiderski, J., Stackbrandt, E., Schumann, P., Spröer, C., and Sahin, N. 2007. *Herminiimonas saxobsidens* sp. nov., isolated from a lichen-colonized rock. *Int. J. Syst. Evol. Microbiol.* **57**, 2618–2622.

Loveland-Curtez, J., Miteva, V.I., and Brenchley, J.E. 2009. *Herminiimonas glaciei* sp. nov., a novel ultramicrobacterium from 3042 m deep Greenland glacial ice. *Int. J. Syst. Evol. Microbiol.* **59**, 1272–1277.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., and *et al.* 2004. ARB: a software environment for sequence data. *Nucleic Acids Res.* **32**, 1363–1371.

Mergaert, J., Schirmer, A., Hauben, L., Mau, M., Hoste, B., Kersters, K., Jendrossek, D., and Swings, J. 1996. Isolation and identification of poly(3-hydroxyvalerate)-degrading strains of *Pseudomonas lemoignei*. *Int. J. Syst. Bacteriol.* **46**, 769–773.

Mesbah, M., Premachandran, U., and Whitman, W.B. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39**, 159–167.

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

Muller, D., Simeonova, D.D., Riegel., P., Mangenot, S., Koechler, S., Lièvremont, D., Bertin, P.N., and Lett, M.C. 2006. *Herminiimonas arsenicoxydans* sp. nov., a metalloresistant bacterium. *Int. J. Syst. Evol. Microbiol.* **56**, 1765–1769.

Prusse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glöckner, F.O. 2007. Silva: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**, 7188–7196.

Rothballer, M., Schmid, M., Klein, I., Gattinger, A., Grundmann, S., and Hartmann, A. 2006. *Herbaspirillum hiltneri* sp. nov., isolated from surface-sterilized wheat roots. *Int. J. Syst. Evol. Microbiol.* **56**, 1341–1348.

Sahin, N., Gonzalez, J.M., Iizuka, T., and Hill, J.E. 2010. Characterization of two aerobic ultramicrobacteria isolated from urban soil and a description of *Oxalicibacterium solurbis* sp. nov. *FEMS Microbiol. Lett.* **307**, 25–29.

Sahin, N., Portillo, M.C., Kato, Y., and Schumann, P. 2009. Description of *Oxalicibacterium horti* sp. nov. and *Oxalicibacterium faecigallinarum* sp. nov., new aerobic, yellow-pigmented, oxalotrophic bacteria. *FEMS Microbiol. Lett.* **296**, 198–202.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Seldin, L. and Dubnau, D. 1985. Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int. J. Syst. Bacteriol.* **35**, 151–154.

Smibert, R.M. and Krieg, N.R. 1994. Phenotypic characterization. *In* Methods for General and Molecular Bacteriology, pp. 607–654. *In* Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. (eds.). American Society for Microbiology, Washington, D.C., USA.

Tamer, A.Ü., Aragno, M., and Şahin, N. 2002. Isolation and characterization of a new type of aerobic, oxalic acid utilizing bacteria, and proposal of *Oxalicibacterium flavum* gen. nov., sp. nov. *Syst. Appl. Microbiol.* **25**, 513–519.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. Mega5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Ueda, T., Suga, Y., Yahiro, N., and Matsuguchi, T. 1995. Remarkable N_2-fixing bacterial diversity detected in rice roots by molecular evolutionary analysis of *nifH* gene sequences. *J. Bacteriol.* **177**, 1414–1417.

Valverde, A., Velázquez, E., Gutiérrez, C., Cervantes, E., Ventosa, A., and Igual, J.M. 2003. *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. *Int. J. Syst. Evol. Microbiol.* **53**, 1979–1983.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**, 697–703.