Dokdonella ginsengisoli sp. nov., isolated from soil from a ginseng field, and emended description of the genus *Dokdonella*

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A Gram-negative, aerobic, rod-shaped, non-motile, non-spore-forming bacterial strain, designated Gsoil 191^T, was isolated from a soil sample from a ginseng field in Pocheon Province, South Korea, and was characterized taxonomically by using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that strain Gsoil 191^T belongs to the family *Xanthomonadaceae* and is related to *Dokdonella fugitiva* LMG 23001^T (97.8% sequence similarity) and *Dokdonella koreensis* KCTC 12396^T (96.9%). The G + C content of the genomic DNA was 68.7 mol%. The major respiratory quinone was Q-8 and the major fatty acids were iso-C_{17:1}ω9c (30.6%), iso-C_{17:0} (21.6%) and iso-C_{15:0} (13.0%), supporting the affiliation of strain Gsoil 191^T to the genus *Dokdonella*. DNA–DNA hybridization experiments showed that the DNA–DNA relatedness values between strain Gsoil 191^T and its closest phylogenetic neighbours were below 40%. The results of physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain Gsoil 191^T from recognized species of the genus *Dokdonella*. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 191^T represents a novel species of the genus *Dokdonella*, for which the name *Dokdonella ginsengisoli* sp. nov. is proposed. The type strain is Gsoil 191^T (=KCTC 12564^T=DSM 17954^T=CCUG 52462^T).

The genus *Dokdonella* was first described by Yoon *et al.* (2006) and, at the time of writing, comprises the two species *Dokdonella koreensis* (Yoon *et al.*, 2006) and *Dokdonella fugitiva* (Cunha *et al.*, 2006). The genus *Dokdonella* comprises Gram-negative, non-spore-forming, rod-shaped, strictly aerobic, urease-negative, catalase-positive, oxidase-positive bacteria. The predominant ubiquinone is Q-8 and the major fatty acids are branched ones such as iso-C_{17:1}ω9c, iso-C_{17:0} and iso-C_{15:0}.

During the course of a study on the culturable aerobic bacterial community in the soil of a ginseng field in

Pocheon Province, South Korea, a large number of novel bacterial strains were isolated. In this study, we have characterized one of these isolates, which was designated strain Gsoil 191^T. Phenotypic, chemotaxonomic and phylogenetic analyses established the affiliation of this isolate to the genus *Dokdonella*. On the basis of the results obtained in this study, we propose that strain Gsoil 191^T represents a novel species of the genus *Dokdonella*.

Strain Gsoil 191^T was obtained from a soil sample that was thoroughly suspended in 50 mM sodium phosphate buffer (pH 7.0). The suspension was spread on plates containing 1/5th-strength modified R2A agar (1⁻¹: 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, 15 g agar) after serial dilution with 50 mM sodium phosphate buffer (pH 7.0). The plates were incubated at

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 191^T is AB245362.

A transmission electron micrograph of a cell of strain Gsoil 191^T and two-dimensional thin-layer chromatograms of the polar lipids of the strain are available as supplementary material with the online version of this paper.

30 °C for 1 month. Single colonies on these cultures were purified by transferring them onto new plates and subjecting them to an additional incubation on half-strength modified R2A. The purified colonies were tentatively identified by means of partial sequencing of the 16S rRNA gene (Im *et al.*, 2005). Strain Gsoil 191^T formed one of the dominant colonies on the modified R2A agar plates under aerobic conditions. It was routinely cultured on R2A agar (Difco) at 30 °C and maintained as a glycerol suspension (20 %, w/v) at –70 °C.

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1000, with cells grown on R2A agar at 30 °C. Flagellation was investigated using transmission electron microscopy (model JEM 1010; JEOL), using cells from an exponentially growing culture. Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Enzyme activities, the assimilation of substrates as sole carbon sources, acid production from substrates and other physiological and biochemical characteristics were determined with API 20E, API 20NE, API 32GN and API 50 CH galleries (bioMérieux). Tests involving these commercial systems were performed according to the manufacturer's instructions. Results were recorded after 24 h, 72 h and 4 days incubation at 30 °C. Additional substrates (not included in the API 20NE and API 32GN tests) were tested as sole carbon sources, using a defined liquid medium with the following composition (l⁻¹): 1.8 g K₂HPO₄, 1.08 g KH₂PO₄, 0.5 g NaNO₃, 0.5 g NH₄Cl, 0.1 g KCl, 0.1 g MgSO₄, 0.05 g CaCl₂. A vitamin solution (Widdel & Bak, 1992), a trace-element solution (SL-10; Widdel *et al.*, 1983) and a selenite/tungstate solution (Tschech & Pfennig, 1984) were added to this medium and the pH was then adjusted to 6.8. Aliquots of this liquid medium were added to 96-well trays and then filter-sterilized carbon sources were added to each well (individually, at 0.1 %, w/v). The trays were incubated at 30 °C for up to 7 days and then growth in the wells was examined visually. The negative-control well did not contain a carbon source. The positive-control culture was grown in a well containing R2A broth. Anaerobic growth was tested in serum bottles by adding thioglycolate (1 g l⁻¹) to R2A broth and substituting the air atmosphere of the bottle with nitrogen gas. The use of nitrate as an electron acceptor was tested anaerobically under the same conditions, but this time the R2A broth was also supplemented with 10 mM KNO₃. Aerobic nitrate reduction was assayed by inoculating three 25 ml serum bottles each containing 12 ml R2A broth supplemented with 10 mM KNO₃. The reduction of nitrate was determined using an ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and an anion-exchange column (Metrosep Anion Supp 4; Metrohm). Tests for the degradation of DNA [in which DNase agar (Scharlau) plates were flooded with 1 M HCl

to detect DNase activity], casein, chitin, starch (Atlas, 1993), xylan and hydroxyethyl cellulose (Ten *et al.*, 2004) were performed; evaluations were carried out after 5 days. Growth at various temperatures (4, 15, 25, 30, 37, 42 and 45 °C) and pH values (pH 5.0–10.0, using increments of 0.5 pH units) was assessed after incubation for up to 5 days. The effect of pH on growth was determined in modified R2A broth media using three different buffers (final concentration, 50 mM): sodium acetate buffer (for pH 5.0–5.5), sodium phosphate buffer (for pH 6.0–8.0) and Tris/HCl buffer (for pH 8.5–10.0). Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar and MacConkey agar (all from Difco) was evaluated after incubation at 30 °C for 1 week.

An almost-complete 16S rRNA gene sequence for strain Gsoil 191^T was determined as described below. Extraction of the genomic DNA was achieved using a commercial genomic DNA-extraction kit (Core Bio System). The 16S rRNA gene was amplified from the chromosomal DNA using a universal bacterial primer set, comprising primers 9F and 1512R, and the purified PCR products were sequenced by Solgent Co. Ltd (Daejeon, South Korea) (Ten *et al.*, 2008). The full sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed using the CLUSTAL_X program (Thompson *et al.*, 1997), gaps were edited in the BioEdit program (Hall, 1999) and evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) in the MEGA3 program (Kumar *et al.*, 2004), with bootstrapping based on 1000 replications (Felsenstein, 1985).

Transmission electron microscopy of negative stained cells of Gsoil 191^T revealed single rods that were approximately 0.5–0.6 µm wide and 0.8–1.0 µm long (Supplementary Fig. S1, available in IJSEM Online). Unlike cells of *Dokdonella koreensis*, those of strain Gsoil 191^T did not exhibit polar flagella. Morphological, cultural, physiological and biochemical characteristics of strain Gsoil 191^T are given in the species description or are shown in Table 1, which lists the characteristics that serve to differentiate strain Gsoil 191^T from its closest phylogenetic relatives (*Dokdonella fugitiva* LMG 23001^T and *Dokdonella koreensis* KCTC 12396^T).

The 16S rRNA gene sequence of strain Gsoil 191^T was a continuous stretch of 1473 bp. Comparative 16S rRNA gene sequence analyses showed that the strain is phylogenetically affiliated to members of the genus *Dokdonella*. The phylogenetic tree based on the neighbour-joining algorithm placed strain Gsoil 191^T within the genus *Dokdonella*, joining with *Dokdonella fugitiva* LMG 23001^T (Fig. 1). Together with *Dokdonella koreensis* KCTC 12396^T, they formed a monophyletic clade with a high bootstrap

Table 1. Differential phenotypic characteristics of strain Gsoil 191^T and phylogenetically related species of the genus *Dokdonella*

All data are from this study, except where indicated otherwise. All of the strains are positive for oxidase, casein and gelatin hydrolysis, but negative for nitrate reduction to N₂, indole production, urease, D-glucose acidification and starch hydrolysis. In API 20NE and API 32GN tests, all of the strains assimilate acetate, L-proline (weakly) and L-serine (weakly), but not L-arabinose, maltose, gluconate, caprate, adipate, malate, citrate, phenyl acetate, melibiose, L-fucose, D-sorbitol, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, D-ribose, inositol, sucrose, itaconate, suberate, malonate or 5-ketogluconate. In API CH 50 tests, all of the strains are negative for acid production from all substrates except those indicated in this table. +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	Strain Gsoil 191 ^T	<i>Dokdonella fugitiva</i> LMG 23001 ^T	<i>Dokdonella koreensis</i> KCTC 12396 ^T
Motility	–	–*	+†
Growth at/with:			
pH 9.0	+	–*	+†
4 % (w/v) NaCl	+	–*	–†
42 °C	+	+*	–†
Optimum growth temperature (°C)	30	40*	30†
Reduction of nitrate to nitrite	–	–	+
Phosphatidylcholine production	+	ND	–†
Catalase	+	–*	+†
Aesculin hydrolysis	+	–	–
β-Galactosidase	+	+	–
Assimilation of (API 20NE and API 32GN):			
D-Glucose	+	–	–
D-Mannose	–	+	–
L-Rhamnose	–	w	–
N-Acetylglucosamine	+	+	–
D-Mannitol	–	+	–
Salicin	–	+	–
Propionate	–	–	+
Valerate	w	–	–
2-Ketogluconate	–	w	–
3-Hydroxybutyrate	–	w	+
Lactate	+	–	+
L-Alanine	–	w	–
Glycogen	–	w	–
Acid production from (API 50 CH):			
D-Arabinose	+	–	–
L-Xylose	+	–	–
Erythritol	+	–	–
Aesculin	+	–	–
DNA G + C content (mol%)	68.7 ± 0.3	68.3*	71.0†

*Data from Cunha *et al.* (2006).

†Data from Yoon *et al.* (2006).

value (100 %) and which was supported by both of the tree-making methods employed in this study. Strain Gsoil 191^T exhibited the highest level of 16S rRNA gene sequence similarity with respect to *Dokdonella fugitiva* LMG 23001^T (97.8 %) and *Dokdonella koreensis* KCTC 12396^T (96.9 %). The 16S rRNA gene sequence similarities between Gsoil 191^T and other recognized species within the family *Xanthomonadaceae* were below 95 %. To verify the taxonomic position of strain Gsoil 191^T, DNA–DNA hybridization was performed with the type strains of other species of the genus *Dokdonella*.

To determine the G + C content of chromosomal DNA of strain Gsoil 191^T, genomic DNA was extracted and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides. The DNA G + C content was then determined as described by Mesbah *et al.* (1989), using reversed-phase HPLC; the range was obtained by means of duplicate experiments. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under a vacuum and re-extracted in n-hexane/water (1 : 1, v/v). The crude n-hexane-quinone solution was purified using silica Sep-Pak Vac cartridges (Waters) and subse-

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain Gsoil 191^T relative to phylogenetically related and representative strains of the family Xanthomonadaceae. Bootstrap percentages (based on 1000 replications) greater than 50% are shown at branch points. Accession numbers are shown in parentheses. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.02 substitutions per nucleotide position.

quently analysed using HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for strains Gsoil 191^T, *Dokdonella fugitiva* LMG 23001^T and *Dokdonella koreensis* KCTC 12396^T grown on trypticase soy agar at 30 °C for 6 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were then analysed by GC (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and were identified using two-dimensional TLC followed by spraying with the appropriate detection reagents (Komagata & Suzuki, 1987).

The DNA G+C content of strain Gsoil 191^T was 68.7 ± 0.3 mol%, which lies within the range observed for the two recognized members of the genus *Dokdonella* (Cunha *et al.*, 2006; Yoon *et al.*, 2006). The predominant respiratory lipoquinone detected in strain Gsoil 191^T was a ubiquinone with eight isoprenoid units (Q-8), as in the closest relatives, i.e. *Dokdonella fugitiva* (Cunha *et al.*, 2006), *Dokdonella koreensis* (Yoon *et al.*, 2006), *Lysobacter enzymogenes* (Bae *et al.*, 2005), *Luteimonas mephitis* (Finkmann *et al.*, 2000), *Pseudoxanthomonas broegbernensis* (Finkmann *et al.*, 2000), *Dyella koreensis* (An *et al.*, 2005), *Fulvimonas soli* (Yoon *et al.*, 2006) and *Rhodanobacter fulvus* (Im *et al.*, 2004). The fatty acid profiles of strain

Gsoil 191^T and the two recognized species of the genus *Dokdonella* are shown in Table 2. Strain Gsoil 191^T contained large amounts of iso-branched, unsaturated iso-branched, straight-chain and hydroxy fatty acids; the major fatty acids were iso-C_{17:1}ω₉C, iso-C_{17:0}, iso-C_{15:0}, C_{16:0} and iso-C_{11:0} 3-OH, a profile that is typical of those of members of the genus *Dokdonella*. However, some minor qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 191^T and its phylogenetically closest relatives. In particular, strain Gsoil 191^T differed from recognized *Dokdonella* species by the presence of iso-C_{19:0} and iso-C_{17:0} 3-OH and it could be differentiated from *Dokdonella koreensis* by the absence of anteiso-C_{15:0} and anteiso-C_{17:0}. The result of a two-dimensional TLC analysis of polar lipids extracted from strain Gsoil 191^T is shown in Supplementary Fig. S2 (available in IJSEM Online). Strain Gsoil 191^T produced large amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, two unknown aminophospholipids (APL1 and APL2), three unknown aminolipids [AL1, AL3 (which showed the same chromatographic and staining behaviour as an aminolipid detected in *Dokdonella koreensis*) and AL4], an unknown phospholipid (PL1) and smaller amounts of phosphatidylcholine, an unknown phospholipid (PL2), an unknown aminolipid (AL2) and three unknown polar lipids (L1, L2 and L3). This aminolipid profile supported the affiliation of strain Gsoil 191^T to the genus *Dokdonella*. The proportions of the

Table 2. Cellular fatty acid profiles of strain Gsoil 191^T and phylogenetically related species of the genus *Dokdonella*

All data are from this study. –, Not detected or <1.0%.

Fatty acid	Gsoil 191 ^T	<i>Dokdonella fugitiva</i> LMG 23001 ^T	<i>Dokdonella koreensis</i> KCTC 12396 ^T
Saturated			
C _{16:0}	8.2	5.5	6.4
iso-C _{11:0}	3.5	3.4	6.9
iso-C _{15:0}	13.0	16.2	9.3
iso-C _{16:0}	2.8	1.4	2.1
iso-C _{17:0}	21.6	25.1	16.6
iso-C _{19:0}	1.7	–	–
anteiso-C _{15:0}	–	–	2.6
anteiso-C _{17:0}	–	–	4.5
Unsaturated			
C _{17:1ω6c}	–	–	1.0
iso-C _{17:1ω9c}	30.6	29.1	39.4
Hydroxy			
iso-C _{11:0} 3-OH	8.9	11.1	6.2
iso-C _{17:0} 3-OH	1.3	–	–
Summed feature 4*	4.4	–	1.2
Unknown (ECL 11.798)†	3.4	5.6	1.0

*Summed features are groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 4 contains C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

†The unknown fatty acid had no name listed in the peak library file of the MIDI system and therefore cannot be identified; ECL, equivalent chain-length.

common polar lipids together with the presence of 10 unknown polar lipids represent a unique polar lipid profile that is very helpful for differentiating strain Gsoil 191^T from recognized species of the genus *Dokdonella* (Yoon *et al.*, 2006).

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes (Sigma) and microdilution wells (Greiner), with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as the DNA–DNA hybridization values. Strain Gsoil 191^T exhibited relatively low levels of DNA–DNA relatedness with respect to *Dokdonella fugitiva* LMG 23001^T (39%) and *Dokdonella koreensis* KCTC 12396^T (12%), indicating that it is not related to them at the species level (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

The results obtained from the phenotypic and phylogenetic characterizations indicated that strain Gsoil 191^T belongs to the genus *Dokdonella*. The phylogenetic distinctiveness and the DNA–DNA hybridization data, in addition to some phenotypic differences (Table 1), confirmed that strain Gsoil 191^T represents a species that is distinct from the recognized

Dokdonella species. Therefore, on the basis of the data presented, strain Gsoil 191^T should be classified as representing a novel species of the genus *Dokdonella*, for which the name *Dokdonella ginsengisoli* sp. nov. is proposed.

Emended description of the genus *Dokdonella*

The characteristics are the same as those given in the original description (Yoon *et al.*, 2006), but with the following amendment. Cells are non-motile or motile by means of polar peritrichous flagella.

Description of *Dokdonella ginsengisoli* sp. nov.

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

Cells are Gram-negative, aerobic, non-spore-forming, non-motile and rod-shaped. Colonies grown on R2A agar plates for 2 days are smooth, circular, convex and yellowish. Growth is observed at 15 and 42 °C but not at 4 or 45 °C. Minimum pH for growth lies between 5.5 and 6.0, optimum pH is 6.5–7.0 and the maximum pH lies between 9.5 and 10.0. Growth occurs on trypticase soy agar and nutrient agar, but not on MacConkey agar. Tolerates 4% (w/v) NaCl, but not 5%. Hydrolyses aesculin, gelatin and casein, but not hydroxyethyl cellulose, xylan, chitin, starch or DNA. Oxidase-positive. The following substrates are utilized for growth: D-glucose, D-fructose, D-lyxose, *N*-acetylglucosamine, acetate, valerate, lactate, L-aspartate, L-asparagine, L-glutamate, L-glutamine, L-proline, L-serine, L-tryptophan and L-tyrosine. The following substrates are not utilized for growth: D-galactose, D-mannose, L-fucose, L-rhamnose, L-sorbose, D-arabinose, L-arabinose, D-ribose, D-xylose, D-lactose, maltose, melibiose, sucrose, trehalose, raffinose, pyruvate, formate, propionate, maleate, fumarate, phenyl acetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, salicin, caprate, citrate, gluconate, malate, malonate, succinate, glutarate, tartrate, itaconate, adipate, 3-hydroxybutyrate, suberate, 2-ketogluconate, 5-ketogluconate, oxalate, suberate, D-adonitol, dulcitol, ethanol, inositol, D-mannitol, D-sorbitol, xylitol, amygdalin, glycerol, methanol, glycogen, inulin, dextran, L-alanine, L-cysteine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine and L-valine. Positive for β-galactosidase, arginine dihydrolase, tryptophan deaminase and acetoin production. Negative for indole production, urease, lysine decarboxylase, ornithine decarboxylase and hydrogen sulphide production. Acid is not produced from glycerol, L-arabinose, D-ribose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, inositol, dulcitol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, D-lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, L-fucose, D-fucose, D-arabitol, L-

arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Major isoprenoid quinone is Q-8, and the major fatty acids are iso-C_{17:1}ω₉, iso-C_{17:0}, iso-C_{15:0}, C_{16:0} and iso-C_{17:0} 3-OH. Major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, an unknown phospholipid, two unknown aminophospholipids and three unknown aminolipids. The DNA G+C content of the type strain is 68.7 mol%.

The type strain, Gsoil 191^T (=KCTC 12564^T=DSM 17954^T=CCUG 52462^T), was isolated from soil from a ginseng field in Pocheon Province, South Korea.

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