Dyella ginsengisoli sp. nov., isolated from soil of a ginseng field in South Korea

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A Gram-negative, aerobic, yellow-pigmented, non-spore-forming, motile, rod-shaped bacterium, designated strain Gsoil 3046^T, was isolated from soil from a ginseng field in Pocheon Province, South Korea, and was characterized taxonomically by using a polyphasic approach. A comparative analysis of 16S rRNA gene sequences revealed that strain Gsoil 3046^T belongs to the family *Xanthomonadaceae* in the *Gammaproteobacteria*. The greatest sequence similarity was found with respect to *Dyella koreensis* KCTC 12359^T (97.7%), *Dyella japonica* IAM 15069^T (97.4%), *Frateuria aurantia* DSM 6220^T (96.7%), *Fulvimonas soli* LMG 19981^T (96.2%) and *Luteibacter rhizovicinus* DSM 16549^T (96.0%). The phylogenetic distances from other recognized species within the family *Xanthomonadaceae*, including *Dyella yejuensis* KACC 11405^T, were greater than 4.0% (i.e. the sequence similarities were less than 96.0%). DNA–DNA hybridization experiments showed that the levels of DNA–DNA relatedness between strain Gsoil 3046^T and its phylogenetically closest neighbours were below 25%. The G+C content of the genomic DNA was 66.6 mol%. In addition, the presence of ubiquinone Q-8 as the predominant respiratory quinone, iso-C_{17:1}ω₉C, iso-C_{16:0}, iso-C_{15:0} and iso-C_{17:0} as the major cellular fatty acids and iso-C_{13:0} 3-OH and iso-C_{11:0} 3-OH as the major hydroxy fatty acids supported the affiliation of strain Gsoil 3046^T to the genus *Dyella*. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 3046^T represents a novel species in the genus *Dyella*, for which the name *Dyella ginsengisoli* sp. nov. is proposed. The type strain is Gsoil 3046^T (=KCTC 12599^T=DSM 18387^T).

Xie & Yokota (2005) isolated three bacterial strains from garden soil and proposed that they represented a novel genus, *Dyella*, with *Dyella japonica* as the type species. Cells of the isolates were Gram-negative, yellow-coloured, rod-shaped, catalase-positive and urease-negative. These strains were shown to be distinguishable from representatives of other genera within the family *Xanthomonadaceae* of the *Gammaproteobacteria* [i.e. *Frateuria aurantia* (Swings *et al.*, 1984), *Fulvimonas soli* (Mergaert *et al.*, 2002) and *Rhodanobacter lindaniclasticus* (Nalin *et al.*, 1999)]. In addition to *D. japonica*, there are currently two other species with validly published names, namely *Dyella koreensis* (An *et al.*, 2005) and *Dyella yejuensis* (Kim *et al.*, 2006).

During the course of a study on the culturable bacterial community living in the soil of a ginseng field in Pocheon Province (South Korea), a large number of novel bacterial strains were isolated (Im *et al.*, 2005). One of these strains, designated Gsoil 3046^T, was subjected to taxonomic characterization and 16S rRNA gene sequence analysis: on the basis of the data from these investigations, the strain was found to belong to the genus *Dyella*. The results from phenotypic, chemotaxonomic and phylogenetic analyses, in combination with the data from DNA–DNA hybridization experiments, showed that Gsoil 3046^T represents a novel species of the genus *Dyella*.

The soil sample from which strain Gsoil 3046^T was obtained was thoroughly suspended in 50 mM phosphate buffer (pH 7.0) and serial decimal dilutions of the suspension were spread onto a modified version of R2A solid medium containing the following (l⁻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 (Difco), 0.5 g glucose, 0.3 g soluble starch, 0.2 g

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xylan, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4 , 0.05 g $MgSO_4$, 0.05 g $CaCl_2$ and 15 g agar. The plates were incubated at 30 °C for 2 weeks. Single colonies on the plates were purified by transferring them onto fresh plates of the modified R2A agar for further incubation. Purified colonies were tentatively identified from partial sequences of the 16S rRNA gene (Im *et al.*, 2005). One isolate, Gsoil 3046^T, was cultured routinely on R2A agar (Difco) at 30 °C and preserved in a glycerol solution (20 %, w/v) at –70 °C.

The Gram reaction was performed using the non-staining method, as described by Buck (1982). Cell morphology and motility were investigated under a Nikon light microscope ($\times 1000$ magnification) using the hanging drop technique with cells allowed to grow on R2A agar for 3 days at 30 °C. Catalase activity was determined by assessing bubble production in 3 % (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Enzyme activities and other physiological and biochemical characteristics were determined with API ZYM, API 20NE and API 50 CH galleries (bioMérieux). Tests involving commercial systems were generally performed according to the manufacturer's instructions and under the same conditions as those described by Kim *et al.* (2006). In addition to analysis using API 20NE, the assimilation of other substrates as sole carbon sources was determined, as described by Ten *et al.* (2006). Anaerobic growth was determined under a nitrogen atmosphere in serum bottles containing R2A broth (Difco) supplemented with thioglycolate (1 g l^{–1}). Degradation of DNA (DNase activity) was tested using DNase agar (Scharlau) and flooding plates with 1 M HCl; results were evaluated after 5 days. Tests for degradation of casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan (Ten *et al.*, 2004) and cellulose (Ten *et al.*, 2004) were also evaluated after 5 days. Growth at various temperatures (4, 15, 20, 25, 30, 37, 42, 45 and 50 °C) and pH values (pH 4.5–10.0, using increments of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation.

For the phylogenetic analysis of strain Gsoil 3046^T, genomic DNA was extracted using a commercial genomic DNA-extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with the CLUSTAL_X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA3 program (Kumar *et al.*, 2004); bootstrap analyses were based on 1000 replications (Felsenstein, 1985).

For measurement of the G + C content of the chromosomal DNA, genomic DNA of strain Gsoil 3046^T was extracted and purified as described by Moore & Dowhan (1995) and enzymically degraded into nucleosides. The G + C content was then determined as described by Mesbah *et al.* (1989), using reversed-phase HPLC. 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 quinone in *n*-hexane solution was purified using Sep-Pak Vac silica cartridges (Waters) and analysed subsequently using HPLC, as described by Hiraishi *et al.* (1996). The cellular fatty acid profile was determined for strain Gsoil 3046^T grown on R2A agar for 48 h. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analysed by means of gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). The value range was obtained using duplicate experiments.

Cells of strain Gsoil 3046^T were Gram-negative, aerobic, motile and rod-shaped (0.2–0.5 µm wide and 1.5–2.1 µm long). After 3 days growth on R2A agar, colonies were 1.0–3.0 mm in diameter, yellow-pigmented, smooth, circular, entire and transparent with clear edges. On R2A agar, the optimal temperature for growth for the isolate was 30 °C; growth occurred at 15–42 °C, but not at 4 or 45 °C. Like recognized species of the genus *Dyella*, strain Gsoil 3046^T was positive for catalase, alkaline phosphatase, leucine arylamidase, valine arylamidase, D-glucose assimilation and acid production from D-glucose and D-fucose, but negative for arginine dihydrolase, urease and indole production. Phenotypic and chemotaxonomic characteristics that serve to differentiate strain Gsoil 3046^T from recognized *Dyella* species and *Frateruia aurantia* are listed in Table 1. In particular, strain Gsoil 3046^T could be readily differentiated from the above-mentioned species from its ability to produce α -mannosidase, its ability to produce acid from arbutin, by the absence of esterase (C4) activity and by the absence of acid production from D-mannose and D-galactose.

The 16S rRNA gene sequence of strain Gsoil 3046^T was a continuous stretch of 1491 bp (base position 27–1512 with respect to the *Escherichia coli* numbering system). Comparative 16S rRNA gene sequence analyses showed that strain Gsoil 3046^T is phylogenetically affiliated with species of the genus *Dyella*. Phylogenetic trees obtained using neighbour-joining and maximum-parsimony methods placed strain Gsoil 3046^T in a distinct position within the radiation of the genus *Dyella* (Fig. 1). On the basis of 16S rRNA gene sequence similarities, the closest phylogenetic neighbours of strain Gsoil 3046^T were *D. koreensis* KCTC 12359^T (97.7 % similarity), *D. japonica* IAM 15069^T (97.4 %), *Frateruia aurantia* DSM 6220^T (96.7 %), *Fulvimonas soli* LMG 19981^T (96.2 %) and *Luteibacter rhizovicinus* DSM 16549^T (96.0 %). The phylogenetic

Table 1. Differential characteristics of strain Gsoil 3046^T (*Dyella ginsengisoli* sp. nov.) and members of the genera *Dyella* and *Frateruria*

Strains: 1, Gsoil 3046^T (data from this study); 2, *D. koreensis* KCTC 12359^T; 3, *D. japonica* IAM 15069^T; 4, *D. yeosuensis* KACC 11405^T; 5, *Frateruria aurantia* DSM 6220^T. All data for taxa 2–5 are from Kim *et al.* (2006), except where indicated otherwise. All strains are positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase activities (API ZYM). All strains are negative for lipase (C14), trypsin, α -chymotrypsin and β -glucuronidase activities (API ZYM) and for indole production and arginine dihydrolase and urease activities (API 20NE). All strains utilize D-glucose and do not assimilate L-arabinose, D-mannitol, gluconate, caprate, adipate, citrate or phenyl acetate. All strains produce acids from D-glucose and D-fucose and do not produce acids from glycerol, erythritol, D-arabinose, L-xylose, D-adonitol, methyl β -D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, D-mannitol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamine, amygdalin, salicin, D-lactose, melibiose, sucrose, inulin, melezitose, raffinose, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Motility	+	–*	+†	+‡	+§
Oxidase	+	+*	–†	+‡	–§
Growth at 4 % NaCl	–	w*	–†	+‡	–*
Cellulose hydrolysis	–	+*	–†	–‡	–*
Enzymic activities (API ZYM)					
Cystine arylamidase	+	–	–	+	–
Esterase (C4)	–	+	+	+	+
Acid phosphatase	+	+	+	+	–
α -Mannosidase	+	–	–	–	–
α -Galactosidase	+	–	–	+	–
β -Galactosidase	+	+	–	+	–
α -Glucosidase	+	+	+	+	–
β -Glucosidase	+	+	+	+	–
N-Acetyl- β -glucosaminidase	+	+	–	+	–
α -Fucosidase	+	+	–	–	–
Biochemical characteristics (API 20NE)					
Nitrate reduction	–	+	+	–	–
Aesculin hydrolysis	+	+	–	+	–
Gelatin hydrolysis	+	+	–	+	–
β -Galactosidase	+	+	–	+	–
Assimilation (API 20NE)					
D-Mannose	+	+	+	+	–
N-Acetylglucosamine	+	+	+	+	–
Maltose	+	+	+	+	–
Malate	–	+	–	–	–
Acid production (API 50 CH) from:					
Arbutin	+	–	–	–	–
D-Mannose	–	+	+	+	+
L-Arabinose	–	+	+	–	+
D-Ribose	–	–	–	–	+
D-Xylose	–	–	–	–	+
D-Galactose	–	+	+	+	+
D-Fructose	–	+	+	+	–
Inositol	–	–	–	–	+
Aesculin	+	+	+	+	–
Cellobiose	–	–	+	–	–
Maltose	–	–	+	–	–
Trehalose	–	–	+	–	–
Starch	–	–	+	–	–
DNA G + C content (mol%)	66.6	63.8*	63.4–64.0†	63.0‡	63.5§

*Data from An *et al.* (2005).

†Data from Xie & Yokota (2005).

‡Data from Kim *et al.* (2006).

§Data from Mergaert *et al.* (2002).

Fig. 1. Rooted neighbour-joining tree based on 16S rRNA gene sequences of strain Gsoil 3046^T and related type strains in the Gammaproteobacteria. GenBank accession numbers are shown in parentheses. Bootstrap percentages (based on 1000 replications) >50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony algorithm. The 16S rRNA gene sequence of *E. coli* ATCC 11775^T (X80725) was used as an outgroup (not shown). Bar, 0.02 substitutions per nucleotide position.

distances from other recognized species within the family *Xanthomonadaceae*, including *D. yeosuensis* KACC 11405^T, were greater than 4% (i.e. the sequence similarities were less than 96%). It is generally accepted that strains with <70% DNA–DNA relatedness (as measured by hybridization) or with >3% 16S rRNA gene sequence dissimilarity can be considered as belonging to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). On the basis of these criteria, the data for strain Gsoil 3046^T indicate that this strain represents a novel species of the genus *Dyella*. To verify the taxonomic position of strain Gsoil 3046^T, DNA–DNA hybridization was performed with its two closest relatives in the genus *Dyella* and with *Frateuria aurantia*.

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 3046^T exhibited relatively low levels of DNA–DNA relatedness with respect to *D. koreensis* KCTC 12359^T (24.8%), *D. japonica* IAM 15069^T (18.5%) and *Frateuria aurantia* DSM 6220^T (11.4%), indicating that it is not related to them at species level (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

The DNA G+C content of strain Gsoil 3046^T was 66.6 mol%, which is slightly higher than the values

reported for *Dyella* species (63.0–64.0 mol%) (An *et al.*, 2005; Xie & Yokota, 2005; Kim *et al.*, 2006). However, the value still lies within the range expected for members of the same genus and the G+C content range of the genus *Dyella* should be extended to take account of the value obtained in our study. The cellular fatty acid profiles of isolate Gsoil 3046^T and related strains are presented in Table 2. Strain Gsoil 3046^T contained large amounts of iso-branched and unsaturated iso-branched fatty acids such as iso-C_{17:1ω9c}, iso-C_{15:0}, iso-C_{16:0} and iso-C_{17:0}, which are the major fatty acids found in members of the genus *Dyella* (Kim *et al.*, 2006). The presence of iso-C_{13:0} 3-OH and iso-C_{11:0} 3-OH as the major hydroxy fatty acids also supported the affiliation of strain Gsoil 3046^T to the genus *Dyella* (Xie & Yokota, 2005). However, some minor qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 3046^T and its phylogenetically closest relatives. In particular, our strain differed from recognized *Dyella* species in possessing C_{17:0} cyclo and containing less iso-C_{11:0} 3-OH. Strain Gsoil 3046^T contained ubiquinone Q-8 as the major respiratory quinone; this was also the case for recognized *Dyella* species (An *et al.*, 2005; Xie & Yokota, 2005; Kim *et al.*, 2006).

All of the characteristics determined for strain Gsoil 3046^T are in accordance with those of the genus *Dyella*. The phylogenetic distinctiveness of strain Gsoil 3046^T, together with the DNA–DNA hybridization data, confirmed that this strain represents a species that is distinct from the recognized *Dyella* species. There are some phenotypic

Table 2. Cellular fatty acid profiles of strain Gsoil 3046^T (*Dyella ginsengisoli* sp. nov.) and its phylogenetically closest relatives

Strains: 1, Gsoil 3046^T (data from this study); 2, *D. koreensis* KCTC 12359^T; 3, *D. japonica* IAM 15069^T; 4, *D. yeosuensis* KACC 11405^T; 5, *Frateriuria aurantia* DSM 6220^T. Data for taxa 2–5 are from Kim *et al.* (2006). All strains were grown on R2A agar at 30 °C for 48 h. –, Not detected or <1 % of total fatty acid content.

Fatty acid	1	2	3	4	5
Saturated					
C _{14:0}	–	–	–	–	3.1
C _{16:0}	3.4	2.5	1.5	1.8	7.5
C _{18:0}	–	–	–	–	1.2
iso-C _{11:0}	5.2	4.5	5.0	3.7	–
iso-C _{14:0}	1.0	1.1	–	1.1	–
iso-C _{15:0}	16.0	11.1	14.6	14.5	41.2
iso-C _{16:0}	16.1	19.2	17.5	21.3	–
iso-C _{17:0}	8.6	6.7	8.5	7.9	5.3
iso-C _{18:0}	1.0	2.6	1.3	1.2	–
anteiso-C _{15:0}	6.5	3.6	5.1	6.9	2.4
anteiso-C _{17:0}	1.5	2.9	1.8	1.6	–
C _{17:0} cyclo	2.4	–	–	–	9.8
Hydroxy					
C _{12:0} 2-OH	–	–	–	–	2.2
C _{12:0} 3-OH	–	–	–	–	5.7
iso-C _{11:0} 3-OH	1.3	5.5	6.0	4.2	–
iso-C _{12:0} 3-OH	1.0	1.4	1.4	1.0	–
iso-C _{13:0} 3-OH	3.4	2.3	4.0	2.4	1.2
iso-C _{17:0} 3-OH	–	–	1.5	–	–
Unsaturated					
C _{14:1} ω5c	–	–	–	–	1.0
iso-C _{17:1} ω9c	19.9	19.0	20.3	21.5	1.8
Unknown (ECL 11.799)*	3.5	2.5	3.1	2.3	–
Summed feature 3†	8.9	7.9	5.0	5.2	10.5

*ECL, Equivalent chain-length.

†Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

differences between strain Gsoil 3046^T and phylogenetically related *Dyella* species (Table 1). Therefore, on the basis of the data presented, strain Gsoil 3046^T should be classified within the genus *Dyella* as representing a novel species, for which the name *Dyella ginsengisoli* sp. nov. is proposed.

Description of *Dyella ginsengisoli* sp. nov.

Dyella ginsengisoli (gin.seng.i.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil from a ginseng field, the source of the organism).

Cells are Gram-negative, aerobic, non-spore-forming, motile and rod-shaped (0.2–0.5 µm wide and 1.5–2.1 µm long). pH range for growth is 5.0–8.5. Tolerates 2 % (w/v) NaCl, but not 3 %. Hydrolyses aesculin, gelatin and starch but not cellulose, chitin, DNA, xylan, skimmed milk or

olive oil. The following substrates are utilized for growth: D-glucose, D-mannose, *N*-acetylglucosamine, maltose, D-xylose, L-xylose, cellobiose, trehalose, glycogen, pyruvate, acetate, DL-3-hydroxybutyrate, valerate, L-glutamic acid and L-glutamine. The following substrates are not utilized for growth: D-galactose, D-lactose, D-fucose, ethanol, L-rhamnose, L-sorbose, D-arabinose, L-arabinose, D-lyxose, D-ribose, formate, propionate, caprate, maleate, fumarate, phenyl acetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, salicin, citrate, lactate, malate, malonate, succinate, glutarate, tartrate, itaconate, adipate, suberate, oxalate, gluconate, D-adonitol, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, amygdalin, glycerol, methanol, inulin, dextran, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-histidine, glycine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. Q-8 is the major isoprenoid quinone. Major fatty acids are iso-C_{17:1}ω9c, iso-C_{16:0}, iso-C_{15:0} and iso-C_{17:0}. The G+C content of the genomic DNA of the type strain is 66.6 mol%.

The type strain, Gsoil 3046^T (=KCTC 12599^T=DSM 18387^T), was isolated from soil from a ginseng field in Pocheon Province, South Korea.

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