Burkholderia soli sp. nov., isolated from soil cultivated with Korean ginseng

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A polyphasic study was carried out to clarify the taxonomic position of a Gram-negative bacterium isolated from soil cultivated with Korean ginseng in the Eumseong region of Korea. The novel strain, GP25-8^T, grew optimally at pH 6–7, 28 °C and 0–1 % NaCl (w/v). The major fatty acids were $C_{18:1}\omega7c$, summed feature 3 ($C_{16:1}\omega7c/C_{15:0}$ iso 2-OH) and $C_{16:0}$ (together representing 71.2 % of the total). The 16S rRNA gene sequence similarities between strain GP25-8^T and members of the genus *Burkholderia* ranged from 94.7 to 97.4 %, indicating that this novel strain was phylogenetically related to members of that genus. The novel strain showed the highest sequence similarities to *Burkholderia caryophylli* ATCC 25418^T (97.4 %) and *Burkholderia phenazinium* LMG 2247^T (97.2 %); the levels of DNA–DNA hybridization with these strains were 28 and 12 %, respectively. These results support the conclusion that strain GP25-8^T represents a novel species within the genus *Burkholderia*, for which the name *Burkholderia soli* sp. nov. is proposed. The type strain is GP25-8^T (=KACC 11589^T=DSM 18235^T).

The genus *Burkholderia* (Yabuuchi *et al.*, 1992) was created through the transfer of the former *Pseudomonas* rRNA homology group II. *Burkholderia* species are isolated from very diverse ecological niches (Coenye & Vandamme, 2003) and, to date, more than 30 species with validly published names have been reported, *Burkholderia cepacia* being the type species.

In the course of a study of bacterial diversity in fields cultivated with Korean ginseng (*Panax ginseng* C. A. Meyer), a bacterial strain was isolated in the Eumseong region of Korea. A soil sample was serially diluted with 0.85 % NaCl (w/v) and suitable 10-fold dilutions were plated onto R2A agar (Difco). The plates were incubated at 28 °C for 4 days and strain GP25-8^T was isolated.

The almost-complete 16S rRNA gene of strain GP25-8^T (approx. 1400 bp) was amplified and sequenced as described by Weon *et al.* (2005). Alignment of 16S rRNA gene sequences was performed with the CLUSTAL W program (Thompson *et al.*, 1994). A phylogenetic tree (Fig. 1) was constructed by using the neighbour-joining method (Saitou & Nei, 1987) with Kimura's two-parameter calculation model (Kimura, 1980). The phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences clearly indicated that strain GP25-8^T was related to members of the genus *Burkholderia*. Strain GP25-8^T showed the highest levels of sequence similarity (100 and 99.1 %, respectively) with *Burkholderia* species A6.2 (GenBank accession number AF247491), isolated from a polycyclic aromatic hydrocarbon-contaminated soil in the USA (Friedrich *et al.*, 2000) and *Burkholderia* species Ellin155 (AF408997), isolated from a pasture soil in Australia (Schoenborn *et al.*, 2004). The sequence similarities between strain GP25-8^T and the validly described *Burkholderia* species ranged from 94.7 to 97.4 %, showing >97 % sequence similarity only with *Burkholderia caryophylli* (97.4 %) and *Burkholderia phenazinium* (97.2 %). The phylogenetic tree indicated that strain GP25-8^T clustered with *B. caryophylli* ATCC 25418^T with a relatively high bootstrap value (90 %) and this cluster grouped with another cluster, including *Burkholderia phenazinium* LMG 2247^T, at a bootstrap level of 88 %.

The level of DNA–DNA relatedness was determined using the filter hybridization method, as described by Seldin &

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GP25-8^T is DQ465451.

A transmission electron micrograph of a cell of strain GP25-8^T is available as a supplementary figure in IJSEM Online.

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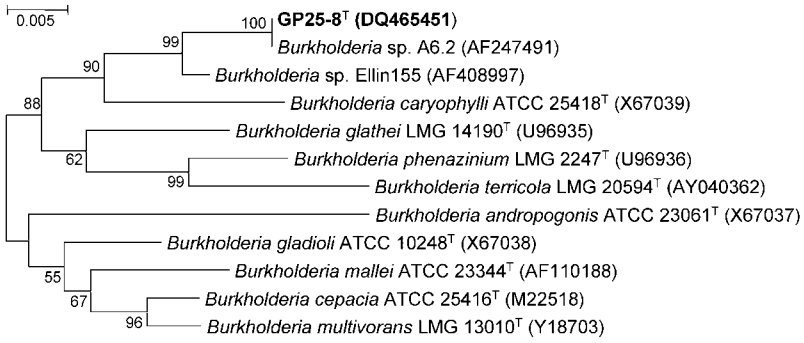

Fig. 1. Unrooted neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain GP25-8^T. Bootstrap values based on 1000 replications are shown at the nodes of the tree. Bar, 0.005 substitutions per nucleotide position.

Dubnau (1985). Probe-labelling was conducted by using the non-radioactive DIG-High prime system (Roche), and hybridized DNA was visualized using the DIG luminescence detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad). The value for DNA–DNA hybridization between strains GP25-8^T and *B. caryophylli* LMG 2155^T was 28 %, while that between strain GP25-8^T and *B. phenazinium* DSM 10684^T was 12 %.

Cellular fatty acid contents were determined for cell mass grown on trypticase soy agar at 28 °C for 48 h. Fatty acid methyl esters were extracted and prepared according to standard protocols, as described for the MIDI Microbial Identification System (Sasser, 1990), and analysed with the MIDI Microbial Identification system. The fatty acid content of strain GP25-8^T was generally consistent with that of *B. caryophylli* LMG 2155^T, although strain GP25-8^T differs in that it contains a small amount of $C_{12:0}$ and lacks $C_{14:0}$ (Table 1). DNA G+C contents were determined by using an HPLC-based method, as described by Mesbah *et al.* (1989), with a reverse-phase column (Supelcosil LC-18-S; Supelco). The DNA G+C content of strain GP25-8^T was 64.9 mol%.

For physiological and biochemical tests, the isolate was routinely cultivated on R2A medium at 28 °C. Gram reaction was determined according to the method described by Smibert & Krieg (1994). Cell morphology was observed by transmission electron microscopy (model 912AB; LEO) and phase-contrast microscopy (Axio; Zeiss) after incubation for 1 day on R2A agar. Growth under anaerobic conditions was determined in BBL anaerobic agar (Becton Dickinson). Catalase activity, oxidase activity, indole production, the Voges–Proskauer reaction and the hydrolysis of casein, DNA, gelatin, starch and Tween 80 were tested by using standard procedures (Smibert & Krieg, 1994). The hydrolysis of carboxymethylcellulose (0.1 %), chitin from crab shells (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was also tested. Growth at different temperatures and pH values was assessed after 14 days incubation. Salt tolerance was tested on R2A medium supplemented with 1–10 % (w/v) NaCl after 14 days incubation. Several other physiological characteristics and the utilization of various substrates as sole carbon sources were determined with the API ID 32GN, API 20NE and API ZYM galleries, according to the manufacturer's instructions (bioMérieux). The oxidation of various substrates was also determined by using the Biolog GN2 MicroPlate assay as recommended by the manufacturer. API ZYM tests were read after 4 h incubation at 37 °C; the other API tests and Biolog microplates were read after 48 h at 28 °C.

The cells of strain GP25-8^T were 0.5–0.75 × 1.6–3.9 μm in size, Gram-negative, aerobic and non-motile (see Supplementary Fig. S1, available in IJSEM Online). The colonies were milky, convex and circular with clear margins. Growth was observed at temperatures in the range 10–40 °C and at pH 4–8. The isolate grew on R2A, nutrient agar (Difco) and trypticase soy agar (Difco), but did not grow on MacConkey agar (Difco). The differential phenotypic characteristics of strain GP25-8^T and closely related *Burkholderia* species are shown in Table 1.

On the basis of the above results, it is proposed that strain GP25-8^T represents a novel species in the genus *Burkholderia*, for which the name *Burkholderia soli* sp. nov. is proposed.

Description of *Burkholderia soli* sp. nov.

Burkholderia soli (so′li. L. gen. n. *soli* of soil, the source of the type strain).

Colonies are milky, convex and circular with clear margins. Cells are approximately 0.5–0.75 μm wide and 1.6–3.9 μm long. Gram-negative, strictly aerobic, rod-shaped and non-motile. Growth occurs at 10–40 °C (optimum, 28 °C), pH 4–8 (optimum, pH 6–7) and 0–2 % (w/v) NaCl. Catalase- and oxidase-positive. Negative for indole production and in the Voges–Proskauer test. Tween 80 and tyrosine are hydrolysed, but casein, chitin, carboxymethylcellulose, DNA, gelatin, pectin and starch are not. The following are oxidized: Tweens 40 and 80, *N*-acetyl-D-glucosamine, D-fructose, α-D-glucose, D-mannitol, pyruvic acid methyl ester, itaconic acid, bromosuccinic acid, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, L-histidine, L-phenylalanine, urocanic acid, glycerol and D-glucose 6-phosphate. The following are not oxidized: α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, i-erythritol, L-fucose, D-galactose, gentiobiose, *myo*-inositol, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl

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Table 1. Phenotypic comparison of strain GP25-8^T, *B. caryophylli* LMG 2155^T and *B. phenazinium* DSM 10684^T

Strains: 1, GP25-8^T; 2, *B. caryophylli* LMG 2155^T; 3, *B. phenazinium* DSM 10684^T. According to the API 20NE and API ZYM strips, all strains test positive for β-galactosidase, alkaline phosphatase, esterase C4, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. All strains are negative for indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis, gelatin hydrolysis, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. All strains assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, potassium gluconate and malic acid. None of the strains assimilate D-maltose. Fatty acids that account for <1.0 % of the total are not shown.

Characteristic	1	2	3
DNA–DNA hybridization (%) with GP25-8^T	100	28	12
Nitrate reduction (API 20NE)	+	+	−
Assimilation (API 20NE):			
Adipic acid	+	−	+
Capric acid	−	−	+
Phenylacetic acid	+	−	+
Trisodium citrate	−	+	+
API ZYM:			
Esterase lipase C8	+	+	−
β-Glucosidase	+	−	−
Lipase C14	−	+	−
Valine arylamidase	+	−	−
Fatty acids (% of total):			
$C_{12:0}$	1.8	−	−
$C_{13:1}$ AT 12-13	1.0	1.0	1.0
$C_{14:0}$	−	4.8	4.3
$C_{16:0}$	15.3	18.3	15.7
$C_{16:0}$ 2-OH	1.7	2.4	2.4
$C_{16:0}$ 3-OH	4.2	4.3	4.2
$C_{16:1}$ 2-OH	4.8	2.6	2.9
$C_{17:0}$ cyclo	1.0	2.1	13.7
$C_{18:1}$ 2-OH	2.7	3.2	1.8
$C_{18:1}\omega7c$	33.5	36.8	24.0
$C_{19:0}\omega8c$ cyclo	−	1.0	13.5
Summed feature 2*	6.6	6.1	5.9
Summed feature 3*	23.8	16.1	8.3

*Summed feature 2 comprises $C_{14:0}$ 3-OH or $C_{16:1}$ iso I, or both; summed feature 3 comprises $C_{16:1}\omega7c$ or iso-$C_{15:0}$ 2-OH, or both.

β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, succinamic acid, glucuronamide, L-alaninamide, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, DL-α-glycerol phosphate and α-D-glucose 1-phosphate (Biolog GN microplate). Assimilates *N*-acetylglucosamine, D-ribose, inositol, itaconic acid, lactic acid, L-alanine, glycogen, D-mannitol, D-glucose, L-fucose, D-sorbitol, L-arabinose, propionic acid, L-histidine, 3-hydroxybutyric acid and L-proline, but does not assimilate L-rhamnose, D-sucrose, D-maltose, suberic acid, sodium malonate, sodium acetate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, D-melibiose, capric acid, valeric acid, trisodium citrate, potassium 2-ketogluconate or 4-hydroxybenzoic acid (API ID 32GN; bioMérieux). The major fatty acids are $C_{18:1}\omega7c$, summed feature 3 and $C_{16:0}$ (representing 71.2 % of the total). The DNA G+C content is 64.9 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, GP25-8^T (=KACC 11589^T=DSM 18235^T), was isolated in Korea from soil cultivated with Korean ginseng.

Acknowledgements

This study was carried out with the support of the program of international co-research work between the Rural Development Administration (South Korea) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany).

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