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Burkholderia ginsengisoli sp. nov., a β-glucosidase-producing bacterium isolated from soil of a ginseng field

Ho-Bin Kim,[1] Min-Ju Park,[1] Hee-Chan Yang,[1] Dong-Shan An,[2] Hai-Zhu Jin[3] and Deok-Chun Yang[1]

[1]Department of Oriental Medicinal Material and Processing, College of Life Science, Kyung Hee University, 1 Seocheon-dong, Giheung-gu, Yongin-si, Gyeonggi-do 446-701, South Korea

[2]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, South Korea

[3]Institute of Food Science and Engineering, Yantai University, Yantai 264005, People's Republic of China

Correspondence
Deok-Chun Yang
dcyang@khu.ac.kr

A bacterial strain (designated KMY03^T) that possesses β-glucosidase activity was isolated from soil from a ginseng field in South Korea and was characterized in order to determine its taxonomic position. The bacterium was found to comprise Gram-negative, rod-shaped, motile cells with unipolar polytrichous flagella. On the basis of 16S rRNA gene sequence similarity, strain KMY03^T was shown to belong to the family *Burkholderiaceae* of the *Betaproteobacteria*, being most closely related to *Burkholderia caledonica* LMG 19076^T (97·8 %), *Burkholderia terricola* LMG 20594^T (97·5 %), *Burkholderia xenovorans* LMG 21463^T (97·4 %) and *Burkholderia phytofirmans* LMG 22146^T (97·3 %). Chemotaxonomic data (major ubiquinone, Q-8; major fatty acids, $C_{17:0}$ cyclo, $C_{16:0}$, $C_{19:0}$ cyclo ω8*c* and summed feature 2) supported the affiliation of the novel strain with the genus *Burkholderia*. The results of DNA–DNA hybridizations and physiological and biochemical tests allowed the strain to be differentiated genotypically and phenotypically from *Burkholderia* species with validly published names. On the basis of these data, strain KMY03^T represents a novel species of the genus *Burkholderia*, for which the name *Burkholderia ginsengisoli* sp. nov. is proposed. The type strain is KMY03^T (=KCTC 12389^T=NBRC 100965^T).

During a study of bacterial populations (from diverse environmental soils near Daejeon in South Korea) that metabolize ginsenoside Rb1 to Rg3, Rh2 or compound K, a large number of novel β-glucosidase-producing bacterial strains were isolated (An *et al.*, 2005). In this study, one of these isolates, designated strain KMY03^T, was subjected to phenotypic, genotypic and chemotaxonomic investigations in order to determine its taxonomic position. The results provide evidence that strain KMY03^T represents a novel species within the genus *Burkholderia*. This genus was first described by Yabuuchi *et al.* (1992) and currently contains 39 species, which have been isolated from diverse ecological niches ranging from contaminated soils to the respiratory tracts of humans (Coenye & Vandamme, 2003).

Strain KMY03^T was isolated, via direct plating onto R2A agar (Difco), from soil from a ginseng field near Daejeon in South Korea. The resulting colonies were purified by transferring them onto new plates and subjecting them to an additional incubation for 3 days at 30 °C. Purified colonies were tentatively identified from partial 16S rRNA gene sequences.

The Gram reaction was performed by using the non-staining method described by Buck (1982). Cell morphology was examined by using light microscopy (Nikon) and transmission electron microscopy (Carl Zeiss) after negative staining with 1 % (w/v) phosphotungstic acid. Catalase and oxidase tests were performed by using the procedures outlined by Cappuccino & Sherman (2002). Utilization of substrates as sole carbon sources and several other physiological characteristics were determined with the API 32GN, API 20NE and API ZYM galleries according to the instructions of the manufacturer (bioMérieux). Tests for anaerobic growth were performed in a serum bottle containing R2A broth supplemented with thioglycolate (1 g l^{-1}) and in which the upper air layer was substituted with nitrogen gas. Tests for the reduction of nitrate and nitrite were performed in serum bottles containing R2A broth supplemented with KNO_3

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMY03^T is AB201286.

A negatively stained transmission electron micrograph of strain KMY03^T is available as a supplementary figure in IJSEM Online.

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(10 mM) and $NaNO_2$ (10 mM), respectively; reduction was monitored on an ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). Nitrogen-fixing ability was determined from growth in 50 ml nitrogen-free medium (DSMZ medium no. 3) contained in a 500 ml Erlenmeyer flask. Acetylene reduction was examined for all liquid cultures by injecting purified acetylene into appropriate containers closed with rubber stoppers to yield 15 % acetylene (v/v); this was followed by incubation for up to 24 h. Ethylene was measured using a Hewlett Packard 5890A gas chromatograph equipped with a flame-ionization detector and a prepacked column (HayeSep N; Supelco). The primer system PolF–PolR (Poly *et al.*, 2001) was used to amplify a *nifH* gene, according to the methods described by Im *et al.* (2004). Degradation of DNA [using DNA agar (Difco) supplemented with 0·01 % toluidine blue (Merck)], chitin, CM-cellulose, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987) and xylan (Ten *et al.*, 2004) was also investigated; reactions were read after 5 days. Growth at different temperatures and pH values was assessed after 5 days incubation. Salt tolerance was tested on R2A medium supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Antibiotic-sensitivity tests were performed using filter-paper discs containing the following: streptomycin (5, 10 and 15 μg ml^{-1}), tetracycline (5, 10 and 15 μg ml^{-1}), kanamycin (1·0, 1·5 and 2·0 mg ml^{-1}) and ampicillin (20, 30 and 50 μg ml^{-1}) (Sigma). Discs were placed on R2A plates spread with culture of strain KMY03^T and were then incubated at 30 °C for 5 days. All of the phenotypic tests described above were performed in duplicate.

Genomic DNA was extracted using a commercial kit (Solgent), and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to the methods described by 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 the GenBank database. Multiple alignments were performed by using the CLUSTAL_X program (Thompson *et al.*, 1997) and gaps were edited with the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using a neighbour-joining method (Saitou & Nei, 1987) and with the maximum-parsimony method (Fitch, 1971) using MEGA 3 (Kumar *et al.*, 2004) and with bootstrap values based on 1000 replications (Felsenstein, 1985).

Quinones were extracted from cells grown on nutrient broth (Difco) and then analysed as described by Komagata & Suzuki (1987) by using reversed-phase HPLC. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by GC (Hewlett Packard 6890) were identified using the Microbial Identification software package (Sasser, 1990).

Total DNA for determination of the G+C content was extracted from cells grown on a nutrient agar plate (Difco), using the method described by Ausubel *et al.* (1995). RNA in the DNA solution was removed by incubation with a mixture of RNase A and RNase T1 (20 U ml^{-1} in each case) at 30 °C for 1 h. The G+C content of the total DNA was analysed as described by Mesbah *et al.* (1989), using reversed-phase HPLC. DNA–DNA reassociation was performed fluorometrically at 50 °C according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. The prehybridization solution and the hybridization solution contained 2× SSC (1× SSC is 0·15 M NaCl plus 0·015 M sodium citrate), Denhardt's solution and 50 % formamide.

The cells of strain KMY03^T were found to be Gram-negative, rod-shaped and motile by means of unipolar polytrichous flagella (see Supplementary Fig. S1 available in IJSEM Online). Colonies grown on R2A were circular, convex and cream-coloured. The temperature range for growth was 25–42 °C; no growth occurred at 45 °C. An internal region of the *nifH* gene (about 360 bp) was clearly amplified, and strain KMY03^T was able to grow well in a nitrogen-free liquid medium. The physiological characteristics of strain KMY03^T are summarized in the species description; Table 1 presents a comparison between selected characteristics of strain KMY03^T and the most closely related type strains of the genus *Burkholderia*.

An almost-complete 16S rRNA gene sequence of strain KMY03^T was obtained (1462 bp). Preliminary sequence comparisons with 16S rRNA gene sequences deposited in the GenBank database indicated that our isolate belonged to the family *Burkholderiaceae* of the *Betaproteobacteria*. On the basis of 16S rRNA gene sequence similarity, the closest cultured relatives were *Burkholderia caledonica* LMG 19076^T (97·8 %), *Burkholderia terricola* LMG 20594^T (97·5 %), *Burkholderia xenovorans* LMG 21463^T (97·4 %) and *Burkholderia phytofirmans* LMG 22146^T (97·3 %). Relationships between strain KMY03^T and other members of the genus *Burkholderia* were also evident in the phylogenetic tree (Fig. 1). Lower levels of sequence similarity (<97·0 %) were found with respect to all other recognized species of the genus *Burkholderia*.

Ubiquinone Q-8 was found to be the predominant quinone system in this strain; this is similar to the situation in other species of the genus *Burkholderia*. The cellular fatty acids of strain KMY03^T included $C_{17:0}$ cyclo (24·9 %), $C_{16:0}$ (16·1 %), $C_{19:0}$ cyclo $\omega 8c$ (15·1 %) and summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I, 11·7 %). Significant differences in fatty acid profiles were observed between strain KMY03^T and the other type strains of *Burkholderia* species, e.g. the production of a large amount of the fatty acid $C_{17:0}$ cyclo (Table 2). The DNA G+C content of strain KMY03^T was 61·6 mol%, which is quite

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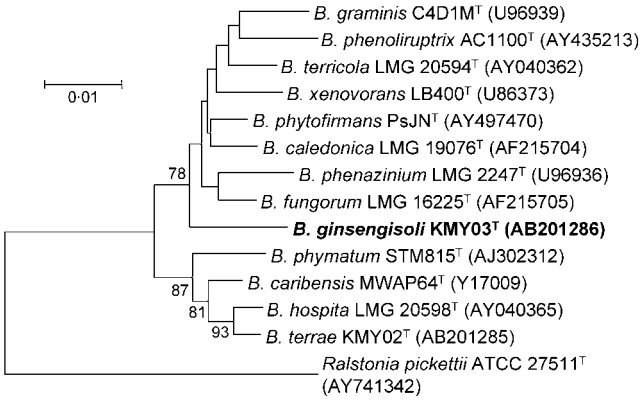

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic positions of KMY03^T and related taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Bar, 0·01 substitutions per nucleotide position.

similar to the values for members of the genus *Burkholderia* (Table 1).

The values for DNA–DNA relatedness between strain KMY03^T and species of the genus *Burkholderia* were in the range 21–45 % (45 % for *B. caledonica* LMG 19076^T, 42 % for *B. terricola* LMG 20594^T, 35 % for *B. xenovorans* LMG 21463^T and 21 % for *B. phytofirmans* LMG 22146^T), which are low enough to permit the assignment of strain KMY03^T to a novel species of the genus *Burkholderia*.

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparisons, strain KMY03^T represents a novel member of the genus *Burkholderia*, for which the name *Burkholderia ginsengisoli* sp. nov. is proposed.

Description of *Burkholderia ginsengisoli* sp. nov.

Burkholderia 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 organism).

Cells are Gram-negative rods, 1·5–2·0 μm long by 0·3–0·6 μm wide, and motile by means of unipolar polytrichous flagella. Colonies grown on R2A are circular, convex and cream-coloured. Temperature range for growth is 25–42 °C; no growth occurs at 45 °C. Optimal growth temperature is 25–30 °C. Growth occurs in the absence of NaCl and in the presence of 1·5 % (w/v) NaCl, but not at NaCl concentrations above 3·0 % (w/v). Nitrate is not reduced. Catalase, arginine dihydrolase, urease, *β*-galactosidase and *β*-glucosidase activities are present. Oxidase and tryptophanase activities are absent. The type strain is positive for the assimilation of adipate, L-malate, phenylacetate, salicin, L-fucose, D-sorbitol, propionate, histidine, 2-ketogluconate, L-proline, rhamnose, inositol, DL-lactate, L-alanine and L-serine and negative for the assimilation of caprate, citrate, D-melibiose, valerate, 3-hydroxybutyrate, 3-hydroxybenzoate, 4-hydroxybenzoate, D-ribose, sucrose, itaconate, suberate, acetate, 5-ketogluconate and glycogen. Alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *β*-glucuronidase activities are present; esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, α-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase and α-fucosidase are not detected. Resistant to (ml^{-1}) ampicillin (20 μg) and tetracycline (5 μg) and sensitive to kanamycin (1 mg) and streptomycin (5 μg). Predominant ubiquinone is Q-8. The major fatty acids are $C_{17:0}$ cyclo, $C_{16:0}$, $C_{19:0}$ cyclo *ω*8*c* and summed feature 2. The G+C content of the genomic DNA is 61·6 mol%.

The type strain, KMY03^T (=KCTC 12389^T=NBRC 100965^T), was isolated from soil from a ginseng field near Daejeon in South Korea.

Table 1. Differential phenotypic characteristics of strain KMY03^T and the type strains of phylogenetically related *Burkholderia* species

Strains: 1, KMY03^T; 2, *B. caledonica* LMG 19076^T; 3, *B. terricola* LMG 20594^T; 4, *B. xenovorans* LMG 21463^T; 5, *B. phytofirmans* LMG 22146^T; 6, *Burkholderia fungorum* LMG 16225^T; 7, *Burkholderia phenazinium* DSM 10684^T; 8, *Burkholderia phenoliruptrix* LMG 22037^T. Data for reference species were taken from Coenye *et al.* (2001, 2004), Goris *et al.* (2002, 2004) and Sessitsch *et al.* (2005). The following features are present in all strains investigated: motility, growth at 30 °C and assimilation of *N*-acetyl-D-glucosamine, D-glucose, D-gluconate, L-malate, mannitol, mannose and phenylacetate. The following features are absent in all strains investigated: assimilation of mannosidase and α-fucosidase. +, Positive; −, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Oxidase	−	−	−	+	+	+	+	+
Growth at 42 °C	+	−	−	−	−	−	−	−
Growth in O–F medium with D-glucose	−	+	+	+	+	+	−	+
Nitrate reduction	−	−	+	−	−	+	−	−
Growth in 1·5 % NaCl	+	+	−	−	+	+	−	ND
Hydrolysis of arginine, aesculin and urea	+	−	−	−	−	−	−	−
Assimilation of:								
Adipate	+	−	−	+	ND	+	−	+
Caprate	−	−	−	+	ND	+	−	−
Citrate	−	−	−	+	ND	+	−	+
Sucrose	−	−	+	−	ND	−	+	ND
Activity of:								
Esterase (C4)	−	−	+	+	+	+	+	ND
Cysteine arylamidase	−	−	−	+	ND	+	−	ND
β-Galactosidase	+	−	+	−	−	−	−	+
β-Glucuronidase	+	−	−	−	ND	−	−	ND
β-Glucosidase	+	−	−	−	ND	−	−	ND

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Table 2. Cellular fatty acid composition (%) of strain KMY03^T and phylogenetically related *Burholderia* species

Taxa: 1, KMY03^T; 2, *B. caledonica* ($n=7$); 3, *B. terricola* ($n=17$); 4, *B. xenovorans* ($n=1$); 5, *B. phytofirmans* ($n=1$); 6, *B. fungorum* ($n=9$); 7, *B. phenazinium* ($n=2$); 8, *B. phenoliruptrix* ($n=1$). Data for reference species were taken from Coenye *et al.* (2001, 2004) (*B. caledonica*, *B. fungorum*, *B. phenazinium* and *B. phenoliruptrix*), Goris *et al.* (2002, 2004) (*B. terricola* and *B. xenovorans*) and Sessitsch *et al.* (2005) (*B. phytofirmans*). Type strains were included for all species. The values shown are mean percentages of total fatty acids ($\pm$SD, as appropriate). Fatty acids for which the mean amount in all taxa was less than 1 % are not given; −, <1 %.

Fatty acid	1	2	3	4	5	6	7	8
$C_{14:0}$	5·9	4·7±0·2	4·8±0·8	4·7	3·6	4·6±0·1	5·1±0·5	4·0
$C_{16:0}$	16·1	13·6±1·6	14·3±1·3	18·2	13·8	14·7±0·9	15·7±2·7	19·7
$C_{18:1}\omega7c$	4·2	34·2±1·7	27·8±8·8	27·3	44·3	35·6±2·1	30·1±0·1	38·3
$C_{16:0}$ 2-OH	5·6	2·4±0·4	3·5±1·4	2·2	2·1	3·6±0·5	2·0±1·0	1·8
$C_{16:1}$ 2-OH	2·9	2·7±0·4	3·1±1·2	2·2	2·3	3·5±0·7	3·0±1·8	1·6
$C_{16:0}$ 3-OH	8·9	6·0±0·4	6·6±1·5	7·1	4·1	5·6±0·5	4·2±2·0	4·7
$C_{18:1}$ 2-OH	1·2	1·1±0·3	1·5±0·7	−	1·4	1·7±0·2	1·6±1·0	−
$C_{17:0}$ cyclo	24·9	8·4±1·5	14·0±5·2	5·1	2·1	5·1±1·6	8·4±0·5	1·7
$C_{19:0}$ cyclo $\omega8c$	15·1	3·7±0·7	5·5±3·0	3·6	1·8	2·5±0·7	6·3±0·1	−
Summed feature 2*	11·7	7·4±0·9	8·2±2·8	8·5	5·6	8·1±1·1	7·3±0·7	5·9
Summed feature 3*	2·3	14·5±1·8	9·1±4·9	19·1	17·6	13·6±1·9	12·6±0·1	18·2

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2 comprises $C_{14:0}$ 3-OH, iso-$C_{16:1}$ I, an unidentified fatty acid with an equivalent chain length of 10·928 and $C_{12:0}$ ALDE, or any combination of these fatty acids. Summed feature 3 comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

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