Kribbella ginsengisoli sp. nov., isolated from soil of a ginseng field

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A Gram-positive, non-spore-forming, aerobic bacterium, designated Gsoil 001^T, was isolated from the soil of a ginseng field in Pocheon province, South Korea. On the basis of 16S rRNA gene sequence analysis, strain Gsoil 001^T was shown to belong to the genus *Kribbella*. The closest phylogenetic relatives were *Kribbella koreensis* LM 161^T (99.0% 16S rRNA gene sequence similarity), *Kribbella flavida* KACC 20248^T (98.8%) and *Kribbella alba* (98.8%). Lower similarity (98.4–96.9%) was found with the other recognized species of the genus *Kribbella*. The major quinone was MK-9(H₄), the major fatty acids were anteiso-C_{15:0} and iso-C_{16:0} and the major polar lipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. The cell-wall peptidoglycan contained LL-2,6-diaminopimelic acid. These chemotaxonomic data and the high genomic DNA G + C content (66.3 mol%) supported the affiliation of strain Gsoil 001^T to the genus *Kribbella*. The results of physiological and biochemical tests differentiated strain Gsoil 001^T genotypically and phenotypically from the *Kribbella* species with validly published names. Therefore, this isolate represents a novel species, for which the name *Kribbella ginsengisoli* sp. nov. is proposed, with the type strain Gsoil 001^T (=KCTC 19134^T =DSM 17941^T).

The genus *Kribbella* was established by Park *et al.* (1999) as a result of the reclassification of two strains, '*Nocardioides fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419, as the type strains of *Kribbella flavida* and *Kribbella sandramycini*, respectively. At the time of writing, the genus included a further 11 species: *Kribbella koreensis* (Sohn *et al.*, 2003), *K. antibiotica* (Li *et al.*, 2004), *K. lupini* (Trujillo *et al.*, 2006), *K. solani* (Song *et al.*, 2004), *K. jejuensis* (Song *et al.*, 2004), *K. yunnanensis* (Li *et al.*, 2006), *K. alba* (Li *et al.*, 2006), *K. karoensis* (Kirby *et al.*, 2006), *K. swartbergensis* (Kirby *et al.*, 2006), *K. aluminosa* (Carlsohn *et al.*, 2007) and *K. hippodromi* (Everest & Meyers, 2008). In this study, we report on the taxonomic characterization of a novel *Kribbella* strain, Gsoil 001^T.

Strain Gsoil 001^T was isolated from the soil of a ginseng field in Pocheon Province, South Korea. The soil sample was thoroughly suspended and serially diluted in 50 mM phosphate buffer (pH 7.0) and spread on one-fifth-strength modified-R2A agar plates (Cui *et al.*, 2007).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 001^T is AB245391.

Supplementary tables showing the characteristics that differentiate strain Gsoil 001^T from *K. koreensis* KACC 20250^T and detailed DNA–DNA hybridization results are available with the online version of this paper.

Strain Gsoil 001^T was isolated after incubation at 30 °C for 1 month. The strain was subsequently cultured on R2A agar (Difco) at 30 °C and maintained as a glycerol suspension (20%, w/v) at –70 °C.

Gram-reaction testing was performed by the non-staining method as described by Buck (1982). Cell morphology was observed at ×1000 magnification with a light microscope (Nikon) using cells grown for 24 h at 30 °C on modified R2A agar. Catalase activity was determined by bubble production in 3% (v/v) H₂O₂ and oxidase activity was determined using 1% (w/v) tetramethyl *p*-phenylenediamine. For studies on sole-carbon-source utilization, we performed the 96-well-tray method (Cui *et al.*, 2007). Some physiological characteristics were determined with API 20E, API 20NE and API ID32 GN galleries (bioMérieux), according to the manufacturer's instructions. Aerobic nitrate reduction was confirmed in three 25 ml serum bottles containing 12 ml R2A broth supplemented with 10 mM KNO₃. The reduction of nitrate was monitored by 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 [by flooding DNase agar (Scharlau) plates with 1 M HCl], casein, chitin, starch (Atlas 1993), lipid (Kouker & Jaeger, 1987), xylan

and cellulose (Ten *et al.*, 2004) were evaluated after 10 days. Growth at 4, 6, 8, 10, 12, 15, 20, 25, 30, 37, 42 and 45 °C and pH 5.0–10.0 (at intervals of 0.5 pH units) was assessed after 5 days of incubation. Salt tolerance was tested on modified R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days of incubation. Growth on nutrient agar, trypticase soy agar and MacConkey agar was also evaluated at 30 °C. Sensitivity to antibiotics was tested on R2A agar using discs containing the following (µg): streptomycin sulfate (2, 4, 6 and 12), gentamicin sulfate (2, 4, 6 and 12), tetracycline hydrochloride (4.5, 7.2, 10 and 15), chloramphenicol (5, 10, 15 and 30), kanamycin (2, 4, 6 and 12), penicillin (6, 12, 18 and 24) and erythromycin (2, 4, 6 and 9).

Extraction of genomic DNA was performed with 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 Ten *et al.* (2008). An almost-complete sequence was compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed with CLUSTAL X (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA3 program (Kumar *et al.*, 2004) and bootstrap values were calculated on the basis of 1000 replications (Felsenstein, 1985).

For the measurement of chromosomal DNA G+C content, genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995) and the G+C content was determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Cellular fatty acid profiles were determined for strains grown on trypticase soy broth (TSB; Difco) for 5 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 analysed with a gas chromatograph (Hewlett Packard 6890) and identified by the Microbial Identification software package (Sasser, 1990). For chemotaxonomic analysis (isoprenoid quinones, cell-wall peptidoglycan types, polar lipids and cell-wall sugars), strain Gsoil 001^T was grown in modified R2A broth for 7 days at 30 °C. Collected cells were lyophilized for 24 h. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed by HPLC as described by Hiraishi *et al.* (1996). The cell-wall peptidoglycan was determined by using TLC after hydrolysis with 6 M HCl at 100 °C for 18 h, as described by Komagata & Suzuki (1987). Polar lipids were extracted, examined by two-dimensional TLC

and identified as described by Minnikin *et al.* (1977). Cell-wall sugars were analysed as described by Stanek & Roberts (1974).

The 16S rRNA gene sequence of strain Gsoil 001^T determined in this study was a continuous stretch of 1482 nt (base positions 9–1512 with respect to the *Escherichia coli* numbering system). Sequence similarity calculations after a neighbour-joining analysis indicated that the closest relatives of strain Gsoil 001^T were *K. koreensis* LM 161^T (99.0 % similarity), *K. flavida* KACC 20248^T (98.8 %), *K. alba* YIM 31075^T (98.8 %), *K. sandramycini* ATCC 39419^T (98.4 %), *K. karoensis* Q41^T (98.4 %), *K. antibiotica* YIM 31530^T (98.3 %), *K. yunnanensis* YIM 30006^T (98.2 %), *K. swartbergensis* HMC25^T (98.2 %), *K. lupini* LU14^T (97.9 %), *K. hippodromi* S1.4^T (97.8 %), *K. aluminosa* HKI 0478^T (97.7 %), *K. solani* DSA1^T (97.4 %) and *K. jejuensis* HD9^T (96.9 %). Lower sequence similarities (<94.1 %) were found with all other recognized species of the family *Nocardioideae*. This relationship between strain Gsoil 001^T and other members of the genus *Kribbella* was also evident in the phylogenetic tree, which used over 1300 nt (Fig. 1). Strain Gsoil 001^T and 13 recognized species of genus *Kribbella* formed a monophyletic clade with a high bootstrap value (100 %), which was supported by both tree-making methods employed in this study.

The results of the morphological and physiological characterization are given in the species description and in Table 1. Comparison of some physiological characteristics of strain Gsoil 001^T with those of related type strains are also shown in Table 1 and, for *K. koreensis* KACC 20250^T, in Supplementary Table S1 (available in IJSEM Online). Strain Gsoil 001^T could be differentiated easily from *K. koreensis* KACC 20250^T on the basis of sole-carbon-source utilization patterns.

DNA–DNA hybridization experiments were performed between strain Gsoil 001^T and type strains of the genus *Kribbella* with the method described by Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was performed 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 were converted to percentage DNA–DNA relatedness values. DNA–DNA relatedness values between strain Gsoil 001^T and the type strains of the 13 *Kribbella* species were in the range 5–45 % (*K. koreensis*, 45 %; *K. flavida*, 22 %; *K. alba*, 24 %; *K. sandramycini*, 22 %; *K. karoensis*, 12 %; *K. antibiotica*, 24 %; *K. yunnanensis*, 17 %; *K. swartbergensis*, 10 %; *K. lupini*, 18 %; *K. hippodromi*, 18 %; *K. aluminosa*, 20 %; *K. solani*, 11 %; *K. jejuensis*, 5 %). Detailed results of the DNA–DNA relatedness analysis are shown in Supplementary Table S2. These values are low enough to assign strain Gsoil 001^T to a novel species of genus *Kribbella* (Wayne *et al.*, 1987).

Results of chemotaxonomic analyses are given in the species description. The respiratory quinone system of

Fig. 1. Neighbour-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strain Gsoil 001^T (*Kribbella ginsengisoli* sp. nov.) with other related species. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony algorithm. Bootstrap values (>60%) based on 1000 replications are shown at branch nodes. Bar, 0.005 substitutions per nucleotide position.

strain Gsoil 001^T is MK-9(H₄), which was consistent with the quinone system of the genus *Kribbella*. The presence of LL-diaminopimelic acid as the cell-wall peptidoglycan also supports the placement of the strain Gsoil 001^T in the genus *Kribbella*. The fatty acid profile of strain Gsoil 001^T comprised anteiso-C_{15:0} (20.6%), iso-C_{16:0} (11.5%), C_{16:0} (8.8%), C_{16:0} 2-OH (8.1%), iso-C_{15:0} (6.4%), C_{18:0} (6.3%), iso-C_{17:0} (5.0%), ante-C_{18:0} and/or C_{18:2}ω6,9c (3.9%), anteiso-C_{17:0} (3.4%), C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (3.3%), anteiso-C_{19:0} (3.1%), C_{18:1}ω9c (2.9%), C_{17:1}ω8c (2.9%), iso-C_{17:1}ω9c (2.8%), C_{15:0} (2.1%), C_{18:1}ω7c/ω9t/ω12t (2.1%), C_{20:2}ω6,9c (2.0%), C_{10:0} (1.8%) and C_{17:0} (1.8%).

In summary, the characteristics of strain Gsoil 001^T are consistent with the description of the genus *Kribbella* with regard to morphological, biochemical and chemotaxonomic properties. However, on the basis of the phylogenetic distances between strain Gsoil 001^T and recognized *Kribbella* species, indicated by the 16S rRNA gene sequence similarity values, and the combination of unique phenotypic characteristics (Table 1), it is demonstrable that strain Gsoil 001^T should be assigned to the genus *Kribbella* in a novel species, for which the name *Kribbella ginsengisoli* sp. nov. is proposed.

Description of *Kribbella ginsengisoli* sp. nov.

Kribbella 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).

Gram-positive, strictly aerobic, non-spore-forming actinomycete. Extensively branched substrate mycelium penetrating into the agar and aerial mycelium are observed on R2A agar plates (Difco) and most media tested. Grows at 8–37 °C (no growth at 6 or 42 °C) and pH 5.5–8.5. Growth occurs with up to 4.0% (w/v) NaCl. Grows on nutrient agar but not on MacConkey agar. Catalase- and oxidase-positive. Unable to reduce nitrate or nitrite under either aerobic or anaerobic conditions. Negative for production of β-galactosidase, arginine dihydrolase, lysine decarbox-

ylase, ornithine decarboxylase, urease and tryptophan deaminase. Gelatinase- and Voges–Proskauer test-positive. Utilizes glucose as a sole carbon source but does not produce acid or gas from glucose. Indole is not produced from tryptophan; H₂S is not produced from sodium citrate. Degrades chitin, starch and casein, but not xylan, DNA or cellulose. The following compounds are utilized as sole carbon sources: *N*-acetylglucosamine, amygdalin, asparagine, aspartic acid, cellobiose, fructose, fumaric acid, gluconate, glutamic acid, glutamine, glycerol, glycogen, histidine, lactate, malate, melibiose, oxalic acid, phenylalanine, pyruvic acid, raffinose, sodium acetate, succinic acid, threonine, trehalose and valerate, but not adipate, adonitol, alanine, arginine, benzoic acid, caprate, cysteine, dextran, dulcitol, ethanol, formic acid, D-fucose, glutaric acid, glycine, 3- and 4-hydroxybenzoates, 3-hydroxybutyrate, inulin, isoleucine, itaconate, leucine, lysine, D-lyxose, maleic acid, malonate, methanol, methionine, phenyl acetate, proline, propionate, D-ribose, salicin, serine, sorbitol, L-sorbose, suberate, tartaric acid, tryptophan, tyrosine, valine or xylitol. Grows with antibiotic discs containing the following (μg): streptomycin sulfate (2, 4), gentamicin sulfate (2), tetracycline hydrochloride (4.5, 7.2), chloramphenicol (5, 10), kanamycin (2, 4), penicillin (6, 12) and erythromycin (2, 4); does not grow with streptomycin sulfate (6, 12), gentamicin sulfate (4, 6, 12), tetracycline hydrochloride (10, 15), chloramphenicol (15, 30), kanamycin (6, 12), penicillin (18, 24) or erythromycin (6, 9). MK-9(H₄) is the predominant menaquinone and anteiso-C_{15:0} and iso-C_{16:0} are the major cellular fatty acids (>10%). The cell-wall peptidoglycan contains LL-diaminopimelic acid, glycine, alanine and glutamate. The polar lipids detected are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol. The cell-wall sugars detected are ribose, xylose and galactose. The DNA G+C content of the type strain is 66.3 mol%.

The type strain, Gsoil 001^T (=KCTC 19134^T =DSM 17941^T), was isolated from the soil of a ginseng field in Pocheon Province, South Korea.

Table 1. Physiological characteristics of strain Gsoil 001^T and related type strains of the genus *Kribbella*

Strains: 1, *K. ginsengisoli* sp. nov. Gsoil 001^T (data from this study); 2, *K. yunnanensis* DSM 15499^T (Li *et al.*, 2006); 3, *K. alba* DSM 15500^T (Li *et al.*, 2006); 4, *K. sandramycini* KCTC 9609^T (Park *et al.*, 1999); 5, *K. koreensis* KACC 20250^T (Lee *et al.*, 2000); 6, *K. flavida* KCTC 9580^T (Park *et al.*, 1999); 7, *K. antibiotica* KCTC 19026^T (Li *et al.*, 2004); 8, *K. swartbergensis* DSM 17345^T (Kirby *et al.*, 2006); 9, *K. karoonensis* DSM 17344^T (Kirby *et al.*, 2006); 10, *K. lupini* DSM 16683^T (Trujillo *et al.*, 2006); 11, *K. solani* KACC 20196^T (Song *et al.*, 2004); 12, *K. jejuensis* KACC 20266^T (Song *et al.*, 2004); 13, *K. hippodromi* DSM 19227^T (Everest & Meyers, 2008); 14, *K. aluminosa* DSM 18824^T (Carlssohn *et al.*, 2007). All strains contained LL-diaminopimelic acid in the cell-wall peptidoglycan and could utilize sucrose as a sole carbon source. +, Positive; w, weakly positive; D, doubtful growth; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Growth conditions														
Temperature (°C)	8–37	15–35	15–35	20–37	10–37	20–37	ND	20–45	20–37	12–37	20–30	20–42	20–37	20–37
pH	5.5–8.5	5.5–8.5	5.5–8.5	5.0–9.0	7.0–10.0	5.0–9.0	ND	7.0–9.0	5.0–9.0	6–9	5.5–8.5	5.5–8.5	5.0–8.0	5.0–9.0
Catalase	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Oxidase	+	+	–	+	–	+	ND	ND	ND	+	ND	ND	–	ND
Urease	–	ND	ND	+	+	+	ND	ND	ND	–	–	+	+	–
Nitrate reduction	–	–	–	–	+	+	–	+	+	–	–	–	–	+
H ₂ S production	–	–	–	–	–	–	–	+	+	ND	ND	ND	+	+
Hydrolysis of:														
Casein	+	ND	ND	+	+	–	+	+	+	+	–	w	+	+
Cellulose	–	ND	ND	–	–	–	–	ND	ND	ND	ND	ND	ND	ND
Starch	w	+	+	w	+	–	+	+	+	–	–	–	+	w
Sole carbon sources														
Arabinose	+	+	+	+	+	–	+	w	D	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	ND	ND	ND	+	+
Galactose	+	–	+	+	+	w	+	ND	ND	+	–	+	ND	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	w	+	ND	ND	ND	ND	+
Inositol	+	+	+	+	+	+	+	w	+	ND	–	–	+	+
Inulin	–	ND	ND	+	+	+	ND	–	–	ND	–	+	ND	+
Lactose	+	+	+	–	+	+	+	+	+	ND	+	+	ND	D
Mannitol	+	+	+	+	+	+	+	w	+	+	–	–	+	+
Mannose	+	+	+	–	+	+	+	+	+	+	+	–	ND	+
Maltose	+	+	+	+	+	+	+	+	D	+	ND	ND	ND	+
D-Rhamnose	+	ND	ND	+	+	–	+	+	+	+	+	+	+	+
Sorbitol	–	+	+	ND	ND	+	+	+	ND	+	ND	ND	ND	ND
Xylose	+	+	+	+	+	–	+	+	+	ND	+	+	+	D
Sodium acetate	+	+	+	–	+	–	+	w	D	ND	ND	ND	+	w
Trisodium citrate	+	+	–	–	+	+	+	w	D	–	ND	ND	+	w
Polar lipid(s)*	DPG, PC, PG, PI	DPG, PC, PG	DPG, PC, PG, PI	PC	DPG, PC, PG, PI	PC	DPG, PC, PG, PI	ND	ND	DPG, PG, PC, PI	DPG, PC, PI	DPG, PC, PI	DPG, PC, PI, UL	ND
Cell-wall sugar(s)†	Gal, Rib, Xyl	Gal, GlcN, Man, Rib	Gal, Man, Rib	Man, Gal, GlcN	Gal, GlcN, Man, Rib	Gal, GlcN, Man	Man, Rib, Xyl	ND	ND	Gal	ND	ND	ND	ND
DNA G + C content (mol%)	66.3	68.6	67.9	68.3	71.3	70	67	ND	ND	68	69	68	ND	ND

*DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; UL, unknown lipids.

†Gal, Galactose; GlcN, glucosamine; Man, mannose; Rib, ribose; Xyl, xylose.

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