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

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A Gram-reaction-negative, chemo-organotrophic, non-motile, non-spore-forming, rod-shaped bacterium (strain Gsoil 080^T) was isolated from soil collected in a ginseng field in Pocheon Province, South Korea, and was investigated by using a polyphasic taxonomic approach. Comparative 16S rRNA gene sequence analysis showed that strain Gsoil 080^T was related most closely to *Inquilinus limosus* strains AU0476^T and AU1979 (98.9% similarity to both). Strain Gsoil 080^T shared ≤91.3% 16S rRNA gene sequence similarity with the type strains of other recognized species examined. The genus *Inquilinus* belongs to the family *Rhodospirillaceae* in the order *Rhodospirillales*, class *Alphaproteobacteria*. The predominant ubiquinone was Q-10 and the major fatty acids were summed feature 7 (C_{18:1}ω9c/ω12t/ω7c) and C_{19:0} cyclo ω8c. The G+C content of the genomic DNA of strain Gsoil 080^T was 69.9 mol%. The level of DNA–DNA relatedness between strain Gsoil 080^T and *I. limosus* LMG 20952^T was 12%. The results of genotypic analyses in combination with chemotaxonomic and physiological data demonstrated that strain Gsoil 080^T represents a novel species of the genus *Inquilinus*, for which the name *Inquilinus ginsengisoli* sp. nov. is proposed. The type strain is Gsoil 080^T (=KCTC 12574^T =LMG 23638^T).

The genus *Inquilinus* was described by Coenye *et al.* (2002) and, at the time of writing, comprises just one species, *Inquilinus limosus*. This species was isolated from respiratory secretions of cystic fibrosis patients. It was characterized as a Gram-reaction-negative, strictly aerobic, chemo-organotrophic, non-motile, rod-shaped bacterium. Based on 16S rRNA gene sequence analysis, the genus *Inquilinus* belongs to the family *Rhodospirillaceae* in the order *Rhodospirillales*, class *Alphaproteobacteria*.

During the course of a study on the culturable aerobic and facultatively anaerobic bacterial community of ginseng field soil in Pocheon Province, South Korea, a large number of bacteria were isolated (Im *et al.*, 2005). One of these isolates, designated strain Gsoil 080^T, was identified as belonging to the *Alphaproteobacteria* and was the subject of further taxonomic investigation. Based on genotypic,

chemotaxonomic and classical phenotypic characteristics, used here to establish its phylogenetic affiliation, we propose that strain Gsoil 080^T represents a novel species of the genus *Inquilinus*.

Strain Gsoil 080^T was isolated as described by Liu *et al.* (2006). It was one of several isolates that appeared on modified R2A agar plates under aerobic conditions and was routinely cultured on R2A agar (Difco) at 30 °C and maintained as a glycerol suspension (20%, w/v) at –70 °C.

For phylogenetic analysis of strain Gsoil 080^T, DNA was extracted by using a 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). The almost-complete sequence of the 16S rRNA gene was assembled by using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed by using the CLUSTAL X program (Thompson *et al.*, 1997). Gaps were edited in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 080^T is AB245352.

A supplementary table is available with the online version of this paper.

the BioEdit program (Hall, 1999). Evolutionary distances were calculated by using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods by using the MEGA 3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

The length of the almost-complete 16S rRNA gene sequence of strain Gsoil 080^T was 1393 bp (base positions 24–1485 with respect to the *Escherichia coli* numbering system). 16S rRNA gene sequence similarity calculations via the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) indicated that strain Gsoil 080^T was related most closely to *I. limosus* strains AU0476^T and AU1979 (98.9% similarity to both); it shared ≤91.3% similarity with the type strains of other recognized species examined here. This relationship between strain Gsoil 080^T and representatives of the order *Rhodospirillales* was also evident in the phylogenetic tree (Fig. 1). Strain Gsoil 080^T and *I. limosus* AU0476^T formed a clade supported by a high bootstrap value (100%) that was supported by both tree-making methods employed.

Cell morphology and motility were observed under a Nikon light microscope (×1000 magnification) by using cells grown for 2 days at 30 °C on R2A agar. The Gram reaction was assessed by the non-staining method as described by Buck (1982). Catalase activity was determined by bubble production in 3% (v/v) H₂O₂ and oxidase activity was determined by using 1% (w/v) tetramethyl *p*-phenylenediamine. Growth at 4, 15, 20, 25, 30, 37, 42 and 45 °C was assessed after 5 days of incubation. Salt tolerance was tested on R2A agar supplemented with 1–5% (w/v) NaCl after 5 days of incubation. Utilization of various substrates as sole carbon sources was investigated, together with some physiological characteristics, by using API 32GN

Fig. 1. Unrooted neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain Gsoil 080^T and related taxa. Filled circles at nodes indicate generic branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) ≥70% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). Anaerobic growth was tested in serum bottles by adding thioglycolate (1 g l⁻¹) to R2A broth and replacing the upper air layer with nitrogen gas.

The physiological, biochemical and genotypic characteristics of strain Gsoil 080^T are summarized in the species description, and characteristics that differentiate it from *I. limosus* LMG 20952^T are detailed in Table 1.

For measurement of the G + C content of the chromosomal DNA, genomic DNA of strain Gsoil 080^T was extracted and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides and the DNA G + C content was then determined as described by Mesbah *et al.* (1989) by using reversed-phase HPLC. 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

Table 1. Comparison of selected characteristics of strain Gsoil 080^T and *I. limosus* LMG 20952^T

Data are from this study (API 20 NE and API ID32GN kits), unless indicated otherwise. The two strains were Gram-negative, non-spore-forming, non-motile, rod-shaped, strict aerobes. Colonies were somewhat similar: creamy white and slightly irregular, raised and with a greasy surface. The two strains were positive for production of catalase, oxidase and β-galactosidase, but negative for reduction of nitrate to nitrite, indole production, acidification of glucose and production of urease, arginine dihydrolase, β-glucosidase and protease. The two strains were positive for assimilation of D-mannitol, D-glucose, salicin, melibiose, L-fucose, D-sorbitol, L-arabinose, 2-ketogluconate, 3-hydroxybutyrate, L-proline, L-rhamnose, N-acetyl-D-glucosamine, D-ribose, inositol, sucrose, maltose, L-alanine, 5-ketogluconate, L-serine and D-mannose, but negative for assimilation of propionate, 4-hydroxybenzoate, itaconate, suberate, malonate, acetate, 3-hydroxybenzoate and phenylacetate. +, Positive; –, negative; w, weakly positive or delayed response.

Characteristic	Gsoil 080 ^T	<i>I. limosus</i> LMG 20952 ^T
Growth in/at:		
3% (w/v) NaCl	–	+
37 °C	–	+
Utilization of:		
Gluconate	–	+
Adipate	–	w
Malate	–	+
Caprate	–	+
Valerate	–	+
Citrate	+	–
L-Histidine	+	–
DL-Lactate	+	–
Glycogen	+	–
DNA G + C content (mol%)	69.9	69.7*

*Data from Coenye *et al.* (2002).

solution was purified by using Sep-Pak Vac cartridges silica (Waters) and was subsequently analysed by HPLC as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for strain Gsoil 080^T and *I. limosus* LMG 20952^T grown on R2A agar for 3 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 on a Hewlett Packard 6890 gas chromatograph and were identified via the Microbial Identification software package (Sasser, 1990).

The G + C content of the genomic DNA of strain Gsoil 080^T was 69.9 mol%. Q-10 was the predominant respiratory ubiquinone. The fatty acid profiles of strain Gsoil 080^T and *I. limosus* LMG 20952^T are shown in Supplementary Table S1. The major fatty acids of strain Gsoil 080^T were summed feature 7 (C_{18:1}ω9c/ω12t/ω7c; 23.3 %) and C_{19:0} cyclo ω8c (12.3 %). However, some minor qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 080^T and *I. limosus* LMG 20952^T.

Strain Gsoil 080^T showed 98.9 % 16S rRNA gene similarity with respect to *I. limosus* LMG 20952^T. A similarity of 97 % or higher is indicative of possible species relatedness (Stackebrandt & Goebel, 1994) and, in this case, DNA–DNA hybridizations need to be performed. DNA–DNA hybridization experiments were performed between Gsoil 080^T and *I. limosus* LMG 20952^T according to the method described by Ezaki *et al.* (1989) by using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the mean (±SD) of the remaining three values was quoted as the percentage DNA–DNA relatedness. Strain Gsoil 080^T exhibited 12±3.2 % DNA–DNA relatedness with *I. limosus* LMG 20952^T, whereas reciprocal hybridization resulted in a higher value of 15±2.7 %. Given the level of DNA–DNA relatedness of 70 % generally accepted as the limit for species delineation (Wayne *et al.*, 1987), this level is sufficiently low to permit the classification of strain Gsoil 080^T in a species distinct from *I. limosus* (Wayne *et al.*, 1987).

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparisons, we consider that strain Gsoil 080^T represents a novel species of the genus *Inquilinus*, for which the name *Inquilinus ginsengisoli* sp. nov. is proposed.

Description of *Inquilinus ginsengisoli* sp. nov.

Inquilinus 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-reaction-negative, chemo-organotrophic, strictly aerobic, non-spore-forming and rod-shaped (0.6–0.8 µm in diameter and 2.5–4.0 µm long when grown for

2 days at 30 °C on R2A agar). After 2 days of incubation on R2A agar, colonies are creamy white, round to slightly irregular and 1.0–5.0 mm in diameter. Growth occurs at 15–30 °C, but not at 5 or 37 °C. Grows in the presence of 2 % (w/v) NaCl. Substrate utilization and other physiological characteristics are detailed in Table 1. Q-10 is the predominant ubiquinone and summed feature 7 (C_{18:1}ω9c/ω12t/ω7c) and C_{19:0} cyclo ω8c are the major cellular fatty acids. The G + C content of the genomic DNA of the type strain is 69.9 mol% (as determined by HPLC).

The type strain, Gsoil 080^T (=KCTC 12574^T =LMG 23638^T), was isolated from soil collected in a ginseng field in Pocheon Province, South Korea.

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