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 080T) 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 080T was related most closely to Inquilinus limosus strains AU0476T and AU1979 (98.9 % similarity to both). Strain Gsoil 080T 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 (C18 : 1ω9cω12tω7c) and C19 : 0 cyclo ω8c. The G+C content of the genomic DNA of strain Gsoil 080T was 69.9 mol%. The level of DNA–DNA relatedness between strain Gsoil 080T and L. limosus LMG 20952T was 12 %. The results of genotypic analyses in combination with chemotaxonomic and physiological data demonstrated that strain Gsoil 080T represents a novel species of the genus Inquilinus, for which the name Inquilinus ginsengisoli sp. nov. is proposed. The type strain is Gsoil 080T (=KCTC 12574T =LMG 23638T).

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 080T, 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 080T represents a novel species of the genus Inquilinus.

Strain Gsoil 080T 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 080T, 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 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 080T 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 080T was related most closely to I. limosus strains AU0476T 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 080T and representatives of the order Rhodospirillales was also evident in the phylogenetic tree (Fig. 1). Strain Gsoil 080T and I. limosus AU0476T 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 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 080T are summarized in the species description, and characteristics that differentiate it from I. limosus LMG 20952T are detailed in Table 1.

For measurement of the G+C content of the chromosomal DNA, genomic DNA of strain Gsoil 080T 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 080T and I. limosus LMG 20952T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gsoil 080T</th>
<th>I. limosus LMG 20952T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth in/at:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 % (w/v) NaCl</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>37 °C</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gluconate</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Adipate</td>
<td>−</td>
<td>w</td>
</tr>
<tr>
<td>Malate</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Caprate</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Valerate</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>l-Histidine</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DL-Lactate</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Glycogen</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>69.9</td>
<td>69.7*</td>
</tr>
</tbody>
</table>

*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 080T and *I. limosus LMG 20952T* 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 080T was 69.9 mol%. Q-10 was the predominant respiratory ubiquinone. The fatty acid profiles of strain Gsoil 080T and *I. limosus LMG 20952T* are shown in Supplementary Table S1. The major fatty acids of strain Gsoil 080T 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 080T and *I. limosus LMG 20952T*.

Strain Gsoil 080T showed 98.9 % 16S rRNA gene similarity with respect to *I. limosus LMG 20952T*. 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 080T and *I. limosus LMG 20952T* 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 080T exhibited 12 ± 3.2 % DNA–DNA relatedness with *I. limosus LMG 20952T*, 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 080T 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 080T 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 physiologival 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 080T (=KCTC 12574T =LMG 23638T), was isolated from soil collected in a ginseng field in Pocheon Province, South Korea.

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**References**


