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Lysobacter pocheonensis sp. nov., isolated from soil of a ginseng field

Muhammad Zubair Siddiqi^{1,2} · Wan-Taek Im^{1,2}

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Abstract A Gram-staining-negative, non-spore-forming, non-flagellated, rod-shaped, catalase- and oxidase-negative bacterium, designated as Gsoil 193^T, was isolated from the soil of ginseng field in Pocheon province, South Korea. 16S rRNA gene sequence analysis showed that strain Gsoil 193^T belonged to family *Xanthomonadaceae* and was most closely related to *Lysobacter daecheongensis* KCTC 12,600^T (96.4 %), *Lysobacter panaciterrae* KCTC 12601^T (96.3 %), *Lysobacter dokdonensis* DSM 17958^T (96.3 %) and *Lysobacter oligotrophicus* JCM 18257^T (95.6 %). Strain Gsoil 193^T grew at temperatures between 20 and 30 °C with an optimum of 30 °C. The pH range for growth was 5–9 pH (optimum 6–7 pH). The predominant respiratory quinone was ubiquinone Q-8 and a fatty acid profile with iso-C_{15:0}, iso-C_{16:0} and summed feature 9 (iso-C_{17:1} ω₉/C_{16:0} 10-methyl) as the major fatty acids supported the affiliation of strain Gsoil 193^T to the genus *Lysobacter*. The

genomic DNA G+C content was 64.8 mol %. On the basis of the genotypic analysis, physiological and chemotaxonomic results indicate that strain Gsoil 193^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter pocheonensis* sp. nov. is proposed. The type strain is Gsoil 193^T (= DSM 18338^T = KCTC 12624^T).

Keywords 16S rRNA gene · Polyphasic taxonomy · *Lysobacter pocheonensis*

Introduction

The genus *Lysobacter* was first described by Christensen and Cook (1978), and the description was emended by Park et al. (2008). Members of the genus *Lysobacter*, in the family *Xanthomonadaceae*, contain ubiquinone Q-8 as the major respiratory quinone and have a high DNA G+C content. (Park et al. 2008; Wang et al. 2009). Members of the genus *Lysobacter* are Gram-negative, aerobic, non-fruiting, gliding organisms and with colonies that were very mucoid and creamy, pink or yellow-brownish in color. Members of this genus are strongly proteolytic and were found to have great potential antibiotic compounds against human pathogens (Ahmed et al. 2003; Hashizume et al. 2004) and may be used as biocontrol agents for plant fungal pathogens (Islam et al. 2005; Park et al. 2008). There are 33 recognized species in the genus *Lysobacter* (<http://www.bacterio.net>), and most of the species of this genus were isolated from soil, *Lysobacter thermophilus* (Wei et al. 2012), *Lysobacter arseniciresistens* (Luo et al. 2012), *Lysobacter panacisoli* (Choi et al. 2014), *Lysobacter terrae* (Ngo et al. 2014) and *Lysobacter novalis* [(Singh et al. 2015) IJSEM in press]. Some of them are also isolated from sludge: *Lysobacter caeni* (Ye et al. 2015) and *Lysobacter mobilis* (Yang

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The NCBI GenBank accession number for 16S rRNA gene sequence of strain Gsoil 193^T is EU273938.

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et al. 2015). Until now 19 species were isolated from variety of soil samples.

During the course of a study on the culturable aerobic and facultative anaerobic bacterial community living in the soil of a ginseng field in Pocheon province [(37°91'96"N, 127°22.4'59.1"E) May 2005, South Korea], a large number of bacterial strains were isolated on modified R2A agar plates (Ten et al. 2009). Here, we report on the taxonomic characterization of one of these strains, designated Gsoil 193^T, which appeared to be a member of the genus *Lysobacter*.

Materials and methods

Isolation of bacterial strain

Strain Gsoil 193^T was cultured routinely on R2A agar at 30 °C and preserved, as a suspension in R2A broth with 20 % (v/v) glycerol, at –70 °C. The type strains, *Lysobacter daecheongensis* KCTC 12600^T, *Lysobacter panaciterae* KCTC 12601^T, *Lysobacter dokdonensis* DSM 17958^T and *Lysobacter oligotrophicus* JCM 18257^T, respectively, were obtained from our laboratory's storage box or culture collections (KCTC and JCM), grown under the same conditions and used as reference strains with Gsoil 193^T.

Phylogenetic tree construction and DNA G+C content analysis

The genomic DNA of strain Gsoil 193^T was extracted using commercial DNA extraction kit (Solgent) and a PCR assay with the universal bacterial primer pair 9F and 1512R to amplify the 16S rRNA gene (Weisburg et al. 1991). The purified PCR products were sequenced by Solgent Co. Ltd (Im et al. 2010). The almost complete (1501 nt) sequence of the 16S rRNA gene was assembled using SeqMan software (DNASTAR) and compared with the 16S rRNA gene sequences of related taxa, which were obtained from the GenBank database or [http://www.ezbiocloud.net/eztaxon; Kim et al. (2012)]. Multiple alignments were made using CLUSTAL_X program (Thompson et al. 1997) with gaps edited using the BioEdit program (Hall 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura 1983), neighbor-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood method using the MEGA6 program (Tamura et al. 2013) with bootstrap values based on 1000 replications (Felsenstein 1985).

For the measurement of the DNA G+C content, the genomic DNA of the novel strain was extracted and purified as described by Moore and Dowhan (1995) and enzymatically degraded into nucleosides, and the base composition was determined as described by Mesbah et al. (1989) using a reverse-phase HPLC.

Physiological and biochemical characteristics

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1000, with cells grown on R2A agar for 2 days at 30 °C. Cell morphology was examined with the scanning electron microscope (Hitachi SU-3500), using cells grown for 2 days at 30 °C on R2A agar (BD). Gliding motility was investigated using the hanging drop method described by Bernardet et al. (2002). Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman (2002). Biochemical tests were carried out by using API 20NE, API ID 32GN and API ZYM kits according to the instructions of the manufacturer (bioMérieux). Tests for degradation of DNA (using DNase agar from Scharlau, with DNase activity by flooding plates with 1 N HCl), casein, starch (Atlas 1993), olive oil (Kouker and Jaeger 1987), xylan and carboxyl methyl cellulose (Ten et al. 2004) were performed and evaluated after 7 days. Growth at different temperatures (4, 10, 15, 25, 30, 37 and 42 °C) and various pH values (pH 4–10 at intervals of 0.5 pH units) was assessed after 5-day incubation at 30 °C. Three different buffers (final concentration, 50 mM) were used to adjust the pH of R2A broth. Acetate buffer was used for pH 4.0–5.5, phosphate buffer was used for pH 6.0–8.0, and Tris buffer was used for pH 8.5–10.0. Salt tolerance was tested on R2A medium supplemented with 1–10 % (w/v at intervals of 1 % unit) NaCl, and growth was assessed after 7 days of incubation. Growth on nutrient agar (NA, BD), trypticase soy agar (TSA, BD), LB agar (BD) and MacConkey agar (BD) was also evaluated at 30 °C.

Chemotaxonomic analysis

Analysis of respiratory quinone

Cells were grown in R2A broth at 30 °C, shaken at 160 rpm for 2 days and then centrifuged. The pellets were dissolved in chloroform/methanol (2/1, v/v). Isoprenoid quinone was extracted, and afterward concentrated at 40 °C using vacuum rotary evaporator, then the residue was subsequently extracted with n-hexane/water (1/1, v/v). The crude n-hexane–quinone solution was purified using Sep-Pak Vac Cartridges Silica (Waters) and analyzed by HPLC as previously described (Hiraishi et al. 1996).

Fatty acids analysis

Cellular fatty acid profile was determined for strain grown on R2A agar (BD) for 48 h at 30 °C. The fatty acids were extracted, methylated and saponified by the described method of Sherlock Microbial Identification System (MIDI).

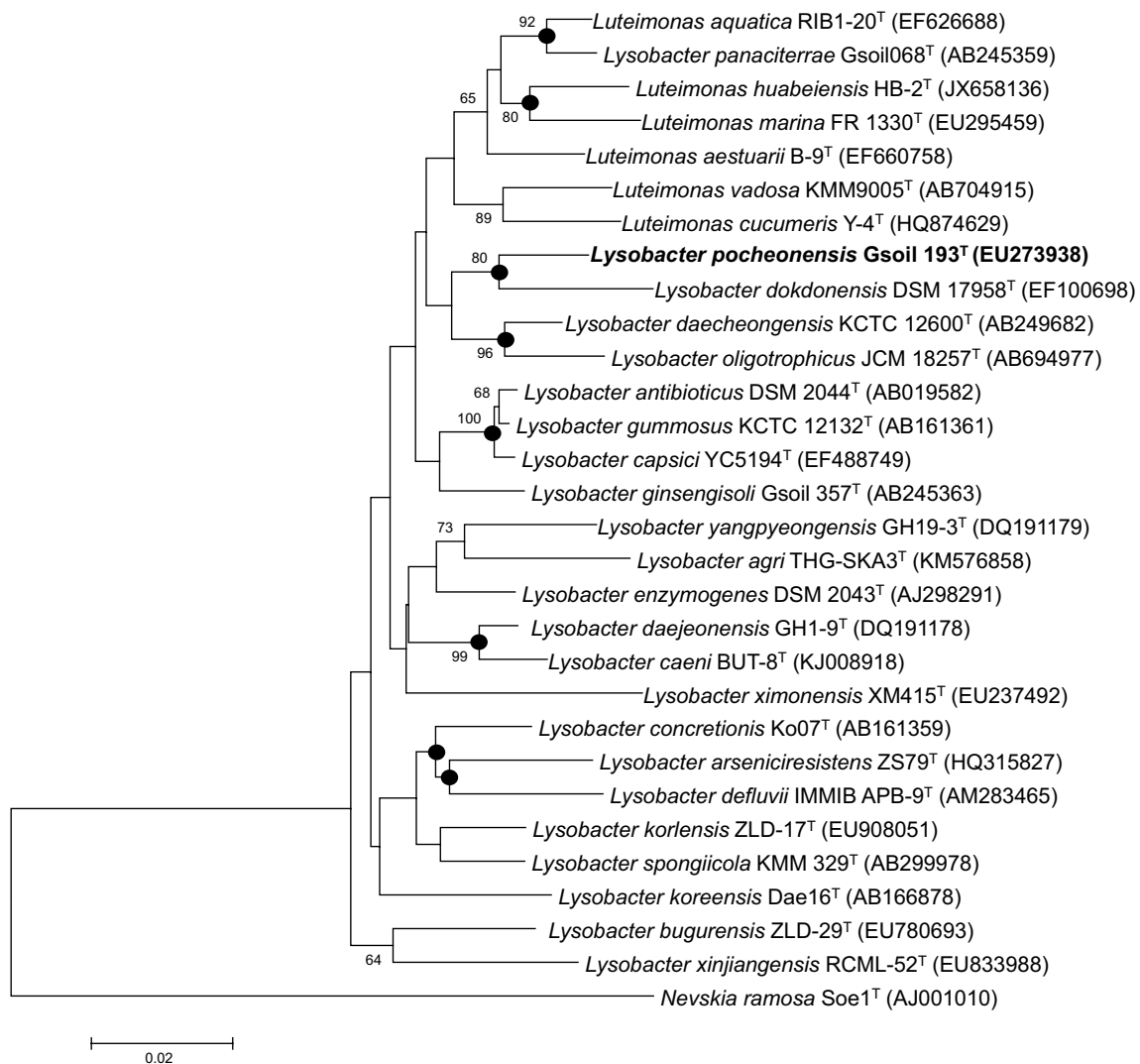


Fig. 1 Phylogenetic relationship of strain Gsoil 193^T with recognized *Lysobacter* species. The tree was constructed by using the neighbor-joining method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) >60 % are shown at

branch points. *Nevskia ramosa* Soe1^T (AJ001010) was used as an out group. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with maximum-parsimony algorithms and maximum likelihood. Bar 0.02 substitutions per nucleotide position

Then, it was analyzed by capillary GLC (Hewlett Packard 6890) using the TSBA library (version 6.1) Sasser (1990).

Results and discussion

Phylogenetic tree and DNA G+C content

The 16S rRNA gene sequence of strain Gsoil 193^T determined in this study is a continuous stretch of 1,501 bp, which has been deposited in the GenBank database (accession number EU273938). A sequence similarity calculation from using the EzTaxon-e server indicated that strain Gsoil 193^T shows highest sequence similarity *Lysobacter daecheongensis* KCTC 12600^T (96.4 %),

Lysobacter panaciterrae KCTC 12601^T (96.3 %), *Lysobacter dokdonensis* DSM 17958^T (96.3 %) and *Lysobacter oligotrophicus* JCM 18257^T (95.6 %). However, in the phylogenetic tree constructed by the neighbor-joining algorithm (Fig. 1), strain Gsoil 193^T clustered with *Lysobacter dokdonensis* DSM 17958^T. This phylogenetic relationship was also confirmed in the trees generated with the maximum-likelihood (Supplementary Fig. S2) and maximum-parsimony algorithms. Strain Gsoil 193^T and *Lysobacter dokdonensis* DSM 17958^T formed a monophyletic group with a bootstrap high value (80 %), which was supported by all three trees making methods used in this study.

On the basis of these phylogenetic results, *L. dokdonensis* DSM 17958^T, *L. daecheongensis* KCTC 12600^T, *L.*

Table 1 Physiological and biochemical characteristics of strain Gsoil 193^T and related type species of the genus *Lysobacter*

Characteristics	1	2	3	4	5
Temperature range	20–30 °C	20–30 ^a °C	15–45 ^b °C	4–38 ^c °C	5–25 ^d °C
pH range	5–9	5–9 ^a	5–8.5 ^b	6–7.5 ^c	6–9 ^d
Esculin hydrolysis	–	+	+	–	+
Enzymes activity					
β-Galactosidase	–	–	+	+	–
Nitrate reduction to nitrite	–	–	+	–	–
Esterase	+	–	+	–	–
Esterase lipase	+	+	+	–	–
Trypsin	+	–	+	–	+
α-Chymotrypsin	+	–	+	–	+
Arginine dihydrolase	+	–	–	–	+
α-Glucosidase	–	–	+	–	–
β-D-Galactopyranosidase	–	–	+	+	–
Assimilation of					
D-Glucose	–	–	+	–	–
D-Mannose	–	–	–	–	–
N-Acetyl-glucosamine	–	–	+	–	–
Adipic acid	–	–	–	+	+
Capric acid	–	+	–	–	–
Malic acid	–	–	+	–	–
D-Saccharose	–	–	+	–	–
D-Maltose	–	–	–	+	–
Glycogen	–	–	+	–	–
L-Serine	–	–	+	–	+
Salicin	–	–	+	–	+
L-Fucose	–	–	+	–	+
Propionic acid	–	–	+	–	+
Valeric acid	–	–	+	–	+
3-Hydroxybutyric acid	–	–	+	–	+
DNA G+C mol %	64.8	69.3 ^a	67.0 ^b	68.1 ^c	66.1 ^d

Strains: 1, Gsoil 193^T; 2, *L. daecheongensis* KCTC 12600^T; 3, *L. panaciterrae* KCTC 12601^T; 4, *L. dokdonensis* DSM 17958^T; 5, *L. oligotrophicus* JCM 18257^T (all data are from this study where indicated)

All strains were positive for urea, alkaline phosphatase, gelatin, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, while all strains were negative for indole production, potassium gluconate, L-rhamnose, L-arabinose, D-ribose, inositol, itaconic acid, suberic acid, lactic acid, L-alanine, potassium-5-ketogluconate, D-mannitol, D-melibiose, D-sorbitol, phenyl acetic acid, L-proline, β-glucuronidase, β-glucoside, N-acetyl-p-glucosaminidase, α-mannosidase and α-fucosidase

^a *Lysobacter daecheongensis* KCTC 12600 (Ten et al. 2008), ^b *Lysobacter panaciterrae* KCTC 12601^T (Ten et al. 2009), ^c *Lysobacter dokdonensis* DSM 17958^T (Oh et al. 2011), ^d *Lysobacter oligotrophicus* JCM 18257^T (Fukuda et al. 2013)

panaciterrae KCTC 12601^T and *L. oligotrophicus* JCM 18257^T were selected as the closest recognized neighbors of strain Gsoil 193^T.

The genomic DNA G+C content of strain Gsoil 193^T was 64.8 mol %, which lies within the range observed for members of the genus *Lysobacter* (61.7–69.2 mol %, respectively).

Physiological biochemical tests

Cells of strain Gsoil 193^T were Gram-reaction-negative, aerobic, non-spore-forming and non-motile. Cells were rod shaped (measuring 0.3–0.4 and 2.5–5 μm) shown in Supplementary Fig. S1. Colonies grown on the R2A agar plates for 2 days at 30 °C were convex, translucent, circular, light

Table 2 Fatty acid profiles of strain Gsoil 193^T and related type species of the genus *Lysobacter*

Fatty acids	1	2	3	4	5
<i>Saturated</i>					
C _{16:0}	tr	2	1.1	tr	2.0
<i>Hydroxyl</i>					
iso-C _{11:0} 3OH	7.3	5.6	5.9	6.5	8.7
iso-C _{12:0} 3OH	tr	–	tr	2.7	tr
<i>Branched fatty acid</i>					
iso-C _{11:0}	5.1	5.3	4.8	3.4	6.9
iso-C _{14:0}	8.0	1.3	2.9	2.4	3.7
iso-C _{15:0}	28.9	35.0	23.1	15.8	23.9
anteiso-C _{15:0}	3.2	1.8	2.5	14.0	2.0
iso-C _{16:0}	25.2	11.9	25.6	24.9	12.1
iso-C _{17:0}	1.4	9.0	4.1	tr	tr
anteiso-C _{17:0}	tr	tr	tr	3.0	tr
C _{17:0} cyclo	–	–	–	5.7	–
<i>Unsaturated fatty acid</i>					
Iso-C _{15:1} ω9c	–	–	–	–	5.7
iso-C _{15:1} (F)	1.6	1.1	tr	–	–
iso-C _{16:1} (H)	2.1	tr	–	3.7	tr
<i>Summed feature*</i>					
1; C _{15:1} (H)/C _{13:0} 3OH	1.2	tr	tr	1.1	tr
3; C _{16:1} ω6c/C _{16:1} ω7c	tr	2.4	1.6	2.7	7.6
9; iso-C _{17:1} ω9c/C _{16:0} 10-methyl	12.4	21.6	24.0	6.2	7.6

Strains: 1. Gsoil 193^T; 2. *Lysobacter daecheongensis* KCTC 12600^T; 3. *Lysobacter panaciterrae* KCTC 12601^T; 4. *Lysobacter dokdonensis* DSM 17958^T; 5. *Lysobacter oligotrophicus* JCM 18257^T

Cells of all strains were collected after 48 h on R2A medium (BD) at 30 °C. All data are obtained from this study. The data shown in this table are more than 1 % for all strains. tr traces <1 %, – not detected

* Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system

yellowish in color and 0.3–1.0 mm in diameter after incubation at 30 °C for 2 days. The morphological, physiological and biochemical characteristics of strain Gsoil 193^T are given in the species description and in Table 1. Some physiological characteristics of strain Gsoil 193^T compared with those of the three reference type strains are given in Table 1.

Quinone and fatty acids

The fatty acid profile of strain Gsoil 193^T (Table 2) was compared with those of the type strains of recognized *Lysobacter* species. The major components were branched fatty acids iso-C_{15:0} (28.9 %), iso-C_{16:0} (25.2 %) and summed feature 9 [(iso-C_{17:1} ω9c/C_{16:0} 10-methyl), 12.4 %], as the major, which is a profile typical of members of the genus *Lysobacter* (Bae et al. 2005; Weon et al. 2006, 2007;

Romanenko et al. 2007). However, some minor qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 193^T and its phylogenetically closest relatives. Strain Gsoil 193^T contained ubiquinone Q-8 as the major respiratory quinone. These data are in good agreement with those of other members of the genus *Lysobacter* (Bae et al. 2005; Weon et al. 2006, 2007; Park et al. 2008; Romanenko et al. 2007; Yassin et al. 2007).

The results obtained from the phenotypic and phylogenetic characterizations indicated that strain Gsoil 193^T belongs to the genus *Lysobacter*. The phylogenetic distinctiveness confirmed that this isolate represents a species that is distinct from recognized *Lysobacter* species. There are some phenotypic differences between strain Gsoil 193^T and phylogenetically related *Lysobacter* species (Table 1). Therefore, on the basis of the data presented, strain Gsoil 193^T should be classified within the genus *Lysobacter* as representing a novel species, for which the name *Lysobacter pocheonensis* sp. nov. is proposed.

Description of *Lysobacter pocheonensis* sp. nov

Lysobacter pocheonensis (po.che.on.en'sis. N.L. mac. adj. *pocheonensis* pertaining to Pocheon province in South Korea, the location of the soil sample from which the type strain was isolated).

Cells are Gram-reaction-negative, aerobic, non-motile, non-spore-forming, rod-shaped (0.3–0.4 μm in diameter and 2.5–5 μm in length), catalase- and oxidase-negative. Colonies grown on the R2A agar plates for 2 days at 30 °C were convex, translucent, circular, light yellowish in color and 0.3–1.0 mm in diameter. Growth occurs at 20–30 °C with pH 5–9 (optimum at 30 °C with pH 6–7.0) without additional NaCl supplement. Growth is inhibited in the presence of 0.5 % (w/v) NaCl. Hydrolyze gelatin and casein, but not chitin, starch, CM-cellulose, xylan, olive oil and DNase. Using the API kit (API 32 GN, API ZYM and API 20 NE), the following substrates are utilized: urea, alkaline phosphatase, esterase, esterase, lipase, leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, gelatin and naphthol-AS-BI-phosphohydrolase. List of all negative traits of commercial kits is given in Table S1. Ubiquinone 8 (Q-8) is the predominant quinone. The major fatty acids are iso-C_{15:0}, iso-C_{16:0} and summed feature 9 (iso-C_{17:1} ω9c). The G+C content of genomic DNA is 64.8 mol %.

The type strain Gsoil 193^T (= DSM 18338^T = KCTC 12624^T) was isolated from soil from a ginseng field in Pocheon Province, South Korea.

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