Two novel species, Lysobacter daejeonensis sp nov and Lysobacter yangpyeongensis sp nov., isolated from Korean greenhouse soils

Article *in* International Journal of Systematic and Evolutionary Microbiology · June 2006

DOI: 10.1099/ijs.0.64095-0 · Source: PubMed

Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov., isolated from Korean greenhouse soils

Hang-Yeon Weon,[1] Byung-Yong Kim,[2] Youn-Kyung Baek,[2] Seung-Hee Yoo,[2] Soon-Wo Kwon,[2] Erko Stackebrandt[3] and Seung-Joo Go[2]

Correspondence
Soon-Wo Kwon
swkwon@rda.go.kr

[1]Applied Microbiology Division, National Institute of Agricultural Science and Technology, Rural Development Administration (RDA), Suwon 441-707, Republic of Korea

[2]Korean Agricultural Culture Collection (KACC), Genetic Resources Division, National Institute of Agricultural Biotechnology, RDA, Suwon 441-707, Republic of Korea

[3]Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

Two bacterial strains were isolated from greenhouse soils of Daejeon and Yangpyeong regions in Korea. The strains, designated GH1-9^T and GH19-3^T, were Gram-negative and aerobic, with rod-shaped cells. Their DNA G+C contents were 61·7 and 67·3 mol%, respectively. The major fatty acids of strain GH1-9^T were iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{14:0}$, iso-$C_{17:1}\omega9c$ and iso-$C_{11:0}$ 3-OH and the major components of strain GH19-3^T were iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol, iso-$C_{17:1}\omega9c$ and iso-$C_{11:0}$ 3-OH. None of the species of the genus *Lysobacter* with validly published names showed 16S rRNA gene sequence similarity values of more than 97 % with respect to the novel isolates. The closest sequence similarity of strain GH1-9^T was with *Lysobacter concretionis* DSM 16239^T (96·4 %), whereas strain GH19-3^T showed the highest sequence similarity with *Lysobacter enzymogenes* DSM 2043^T (96·6 %). Polyphasic taxonomic studies indicated that the two strains should be classified as representing novel members of the genus *Lysobacter*. The names *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov. are proposed, with strains GH1-9^T (=KACC 11406^T=DSM 17634^T) and GH19-3^T (=KACC 11407^T=DSM 17635^T), respectively, as the type strains.

Although the genus *Lysobacter* was first proposed by Christensen & Cook (1978), the taxonomic properties of its species have not been fully characterized. Recently, Bae *et al.* (2005) evaluated these properties more deeply and carried out physiological, biochemical, chemotaxonomic and phylogenetic analyses. At the time of writing, the genus *Lysobacter* includes six species, *Lysobacter enzymogenes*, *Lysobacter antibioticus*, *Lysobacter brunescens*, *Lysobacter concretionis*, *Lysobacter gummosus* and *Lysobacter koreensis*.

Two novel bacterial strains, GH1-9^T and GH19-3^T, were isolated from greenhouse soil cultivated with lettuce (*Lactuca sativa* L.). Soil samples were suspended in sterilized water and diluted solutions were spread on R2A agar (Difco) and incubated at 28 °C. Purified colonies were obtained from subcultures. Cellular morphology was determined by using phase-contrast microscopy with 2-day-old cells. Motility was examined by using 1/10 strength R2A medium, and gliding motility was observed by oil-immersion phase-contrast microscopy of the edges of colonies of cells in exponential phase. The temperature range (4–50 °C), pH range (4–10 at intervals of 1 pH unit) and the requirement for 0, 1, 2, 3, 5 and 7 % NaCl (w/v) for growth were determined using R2A medium. Gram staining, and tests for catalase and oxidase, indole production and hydrolysis of casein, chitin, DNA, gelatin and starch were conducted according to the methods of Smibert & Krieg (1994). Carboxymethylcellulose (CM-cellulose; 0·1 %, w/v; Sigma) and Whatman powder CF11 (0·1 %, w/v) were used to test for cellulase. Hydrolysis of chitin from crab shells (1 %, w/v; Sigma) and tyrosine (0·5 %, w/v) was also tested. API 20NE, API ID 32 GN and API ZYM kits (bioMérieux) were used to determine biochemical properties, utilization of carbohydrates and enzymic activities, according to the manufacturer's instructions. The API ZYM tests were read after 4 h incubation at 37 °C and the other API tests after 72 h at 28 °C.

Published online ahead of print on 23 December 2005 as DOI 10.1099/ijs.0.64095-0.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains GH1-9^T and GH19-3^T are DQ191178 and DQ191179, respectively.

The two strains were aerobic, with rod-shaped cells (0·4–0·6 × 3·0–4·0 μm). Colonies of both strains were yellow, circular and convex, with clear margins after 2 days incubation on R2A agar. Both strains grew well on R2A and nutrient agar (Difco), but did not grow on MacConkey agar (Difco). Growth on trypticase soy agar (Difco) was observed on initial inoculation, but no growth was observed after the strains had been subcultured three or four times. With a longer incubation time (>1 week), the centre of the colonies of strain GH19-3^T developed a brown colour. Using the API 20NE and API ID 32 GN kits, strain GH1-9^T tested positive for nitrate reduction and aesculin hydrolysis, and assimilated D-maltose, sodium acetate, glycogen, D-glucose, valeric acid and 3-hydroxybutyric acid. Strain GH19-3^T tested positive for gelatin hydrolysis and assimilated glycogen and 3-hydroxybutyric acid. Using API ZYM, strain GH1-9^T was positive for alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, and weakly positive for lipase C14, valine arylamidase and β-glucosidase. Strain GH19-3^T was positive for alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, and weakly positive for α-chymotrypsin and *N*-acetyl-β-glucosaminidase. Phenotypic characteristics of the two strains are given in the species descriptions. Phenotypic characteristics that differentiated the two strains and other related *Lysobacter* species are shown in Table 1.

Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). Analysis of the respiratory lipoquinones indicated that the two isolates contained ubiquinone-8 (Q-8). This quinone system is a characteristic feature of members of the genus *Lysobacter* (Bae *et al.*, 2005). The DNA G+C content was determined as described by Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18-S; Supelco). The G+C contents of strains GH1-9^T and GH19-3^T were 61·7 and 67·3 mol%, respectively.

Table 1. Phenotypic characteristics of strains GH1-9^T, GH19-3^T and other *Lysobacter* species

Taxa: 1, GH1-9^T; 2, GH19-3^T; 3, *L. enzymogenes* DSM 2043^T; 4, *L. antibioticus* DSM 2044^T; 5, *L. brunescens* ATCC 29482^T; 6, *L. concretionis* DSM 16239^T; 7, *L. gummosus* ATCC 29489^T; 8, *L. koreensis* KCTC 12204^T. Data from Christensen (1989), Bae *et al.* (2005), Lee *et al.* (2006) and this study. +, Positive; −, negative; W, weak; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Cell size (μm)	0·4–0·6 × 3·0–4·0	0·4–0·6 × 3·0–4·0	0·5 × 38·0	0·4–6·5	0·3–11·0	0·7 × 1·0 –13·5	0·4 × 2·0	0·5–0·8 × 1·5–2·0
Gliding motility	−	−	+	+	+	+	+	ND
Catalase/oxidase	W/+	−/+	+/+	+/+	+/+	+/+	+/ND	+/−
Aesculin hydrolysis	+	−	+	+	+	−	+	−
Indole production	−	−	−	−	+	−	−	−
Glucose acidification	−	−	+	+	−	−	−	−
Arginine dihydrolase	−	−	−	−	+	−	−	−
Urease	−	−	−	−	+	−	−	−
β-Galactosidase	−	−	+	+	−	−	+	−
Growth on:								
N-Acetylglucosamine	−	−	+	−	−	−	+	−
D-Maltose	+	−	+	+	−	−	+	−
Acetate	+	−	−	−	+	+	+	−
Glycogen	+	+	+	+	−	+	+	−
L-Serine	−	−	+	−	−	−	−	+
D-Glucose	+	−	+	−	−	−	+	−
Salicin	−	−	+	−	−	−	−	−
D-Melibiose	−	−	+	−	−	−	+	−
Arabinose	−	−	−	−	−	−	+	+
Valerate	+	−	−	+	−	+	+	+
Citrate	−	−	+	−	−	−	+	+
Histidine	−	−	−	−	−	−	+	−
3-Hydroxybutyrate	+	+	+	+	−	+	+	−
L-Proline	−	−	+	+	−	+	+	−
Mannose	−	−	+	+	−	−	−	−
Malate	−	−	+	−	−	−	−	−
G+C content (mol%)	61·7	67·3	69·0	69·2	67·7	63·8	65·7	68·9

After growth of the cells on R2A agar for 48 h at 28 °C, fatty acid methyl esters were extracted and prepared by using the standard protocol of the Microbial Identification System (MIDI; Microbial ID). The major fatty acids of strain GH1-9^T were iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{14:0}$, iso-$C_{17:1}\omega9c$ and iso-$C_{11:0}$ 3-OH and the major fatty acids of strain GH19-3^T were iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol, iso-$C_{17:1}\omega9c$ and iso-$C_{11:0}$ 3-OH. The characteristic fatty acid of the two isolates that differentiated them from the other *Lysobacter* species was iso-$C_{12:0}$ 3-OH. A unique fatty acid component of GH1-9^T was iso-$C_{15:1}$ F. Unique fatty acid components of strain GH19-3^T were anteiso-$C_{17:0}$ and $C_{10:0}$ 3-OH. A comparison of fatty acid profiles among *Lysobacter* species is shown in Table 2.

The 16S rRNA gene was amplified by PCR (Kwon *et al.*, 2003). The products were sequenced directly using an ABI PRISM BigDye Primer cycle sequencing kit (Applied Biosystems) with an ABI 3700 DNA sequencer (Applied Biosystems). The 16S rRNA gene sequences of the two isolates were aligned with reference sequences of *Lysobacter* species and members of related genera. *Escherichia coli* (GenBank accession no. J01695) was used as an outgroup. Sequences were aligned using the multiple sequence alignment program CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic distances were determined according to Jukes & Cantor (1969) and a tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) as implemented in MEGA version 2.1.

According to the phylogenetic tree (Fig. 1), the two isolates were clearly grouped in a cluster composed of *Lysobacter* species except for *L. brunescens* ATCC 29482^T. The sequence similarity between strains GH1-9^T and GH19-3^T was 95·3 %. The neighbour-joining tree indicated that strain GH1-9^T was most highly related to *L. concretionis* DSM 16239^T (96·4 % sequence similarity) and strain GH19-3^T showed the highest sequence similarity with *L. enzymogenes* DSM 2043^T (96·6 %). The low level of 16S rRNA gene sequence similarity (<97 %) among *Lysobacter* species with

Table 2. Cellular fatty acid content of strains GH1-9^T and GH19-3^T compared with other related species

Taxa: 1, GH1-9^T; 2, GH19-3^T; 3, *L. enzymogenes* DSM 2043^T; 4, *L. antibioticus* DSM 2044^T; 5, *L. brunescens* ATCC 29482^T; 6, *L. concretionis* DSM 16239^T; 7, *L. gummosus* ATCC 29489^T; 8, *L. koreensis* KCTC 12204^T. Data from Bae *et al.* (2005), Lee *et al.* (2006) and this study. Results are presented as a percentage of the total fatty acids. −, Not detected.

Fatty acid	1	2	3	4	5	6	7	8
$C_{10:0}$	−	0·6	−	0·9	1·3	−	−	−
iso-$C_{10:0}$	0·9	0·9	−	−	−	−	−	0·7
$C_{10:0}$ 3-OH	−	0·8	−	−	−	−	−	−
iso-$C_{11:0}$	3·7	4·3	4·3	3·5	7·0	6·4	4·0	3·5
iso-$C_{11:0}$ 3-OH	6·0	5·5	6·0	5·2	5·6	5·6	4·4	5·6
iso-$C_{12:0}$	2·0	1·2	−	−	−	−	−	0·7
iso-$C_{12:0}$ 3-OH	0·6	1·0	−	−	−	−	−	−
$C_{14:0}$	0·9	−	1·2	1·7	0·7	0·8	−	−
iso-$C_{14:0}$	11·2	4·5	−	2·3	6·7	2·6	−	2·7
iso-$C_{15:0}$	13·1	14·5	43·0	19·9	23·8	36·1	39·3	17·0
iso-$C_{15:1}$ AT5	−	3·1	−	−	−	−	−	2·7
anteiso-$C_{15:0}$	3·2	5·1	1·9	5·4	1·7	1·2	5·1	−
iso-$C_{15:1}$ F	3·2	−	−	−	−	−	−	−
$C_{16:0}$	1·4	3·1	5·1	10·5	1·5	2·4	7·8	2·0
iso-$C_{16:0}$	33·7	27·5	3·0	12·1	21·9	19·9	4·7	33·0
iso-$C_{16:0}$ H	2·6	1·1	−	−	2·7	−	−	1·5
$C_{16:1}\omega7c$ alcohol	−	8·8	−	3·0	0·8	−	1·1	4·1
$C_{16:1}\omega11c$	−	2·2	−	5·5	−	−	−	−
iso-$C_{17:0}$	0·5	1·9	4·4	1·9	0·7	2·9	13·0	2·5
iso-$C_{17:1}\omega9c$	6·7	6·7	8·8	4·5	11·5	13·9	12·6	19·9
anteiso-$C_{17:0}$	−	1·1	−	−	−	−	−	−
$C_{17:0}$ cyclo	−	−	10·6	8·1	−	2·5	−	−
$C_{18:0}$	−	−	−	−	−	−	−	1·0
iso-$C_{18:0}$	−	0·8	−	−	−	−	−	0·9
Summed feature 4*	6·1	3·3	8·3	11·2	9·0	0·9	4·9	2·1
Summed feature 7*	−	−	1·6	1·3	−	−	−	−

*Summed feature 4 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; summed feature 7 comprises $C_{18:1}\omega7c/\omega9t/\omega12t$ and/or $C_{18:1}\omega7c/\omega9c/\omega12t$, which could not be separated by the MIDI system.

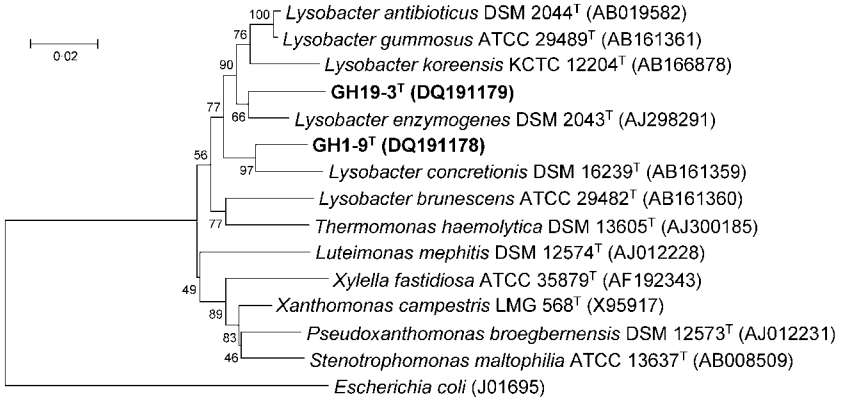

Fig. 1. Phylogenetic relatedness of strains GH1-9^T and GH19-3^T and the type strains of recognized *Lysobacter* species based on 16S rRNA gene sequence comparison. Some related genera were also used and *E. coli* served as a root. The dendrogram was generated by using neighbour-joining analysis (Saitou & Nei, 1987). Numbers indicate percentages of occurrence of the branching order in 1000 bootstrapped trees. Bar, 2 substitutions per 100 nt.

validly published names and the novel isolates indicated that the two isolates each represented novel genomic species of the genus *Lysobacter*. Consequently, DNA–DNA reassociation studies were not necessary (Stackebrandt & Goebel, 1994). Therefore, based on the polyphasic taxonomic approach described here, we propose the name *Lysobacter daejeonensis* sp. nov. for isolate GH1-9^T and *Lysobacter yangpyeongensis* sp. nov. for isolate GH19-3^T.

Description of *Lysobacter daejeonensis* sp. nov.

Lysobacter daejeonensis (dae.je.on.en′sis. N.L. masc. adj. *daejeonensis* pertaining to Daejeon, a city in Korea, from where the type strain was isolated).

Cells are aerobic, Gram-negative, non-motile and rod-shaped (0·4–0·6 × 3·0–4·0 μm in size). Colonies are yellow, circular and convex, with clear margins after 2 days incubation on R2A agar. NaCl, temperature and pH ranges for growth are 0–3 % (w/v), 10–37 °C and 6–8, respectively. Does not hydrolyse chitin, CM-cellulose, Whatman powder CF11 or starch, but does hydrolyse casein, DNA, gelatin and tyrosine. Major fatty acids are iso-$C_{16:0}$ (33·7 %), iso-$C_{15:0}$ (13·1 %), iso-$C_{14:0}$ (11·2 %), iso-$C_{17:1}\omega9c$ (6·7 %) and iso-$C_{11:0}$ 3-OH (6·0 %). Contains Q-8. The G+C content of the genomic DNA is 61·7 mol% (HPLC). Additional characteristics are listed in Table 1.

The type strain, GH1-9^T (=KACC 11406^T=DSM 17634^T), was isolated from greenhouse soil in Korea.

Description of *Lysobacter yangpyeongensis* sp. nov.

Lysobacter yangpyeongensis (yang.pye.ong.en′sis. N.L. masc. adj. *yangpyeongensis* pertaining to Yangpyeong, a province in Korea, from where the type strain was isolated).

Cells are aerobic, Gram-negative, motile and rod-shaped (0·4–0·6 × 3·0–4·0 μm in size). Colonies are yellow, circular and convex, with clear margins after 2 days incubation on R2A agar. Temperature and pH ranges for growth are 15–40 °C and 5–8, respectively. Does not grow in 1 % (w/v) NaCl. Hydrolyses casein, DNA, gelatin, starch and tyrosine, but not chitin, CM-cellulose or Whatman powder CF11. Major fatty acids are iso-$C_{16:0}$ (27·5 %), iso-$C_{15:0}$ (14·5 %), $C_{16:1}\omega7c$ alcohol (8·8 %), iso-$C_{17:1}\omega9c$ (6·7 %) and iso-$C_{11:0}$ 3-OH (5·5 %). Contains Q-8. The G+C content of the genomic DNA is 67·3 mol% (HPLC). Additional characteristics are listed in Table 1.

The type strain, GH19-3^T (=KACC 11407^T=DSM 17635^T), was isolated from greenhouse soil in Korea.

References

Bae, H.-S., Im, W.-T. & Lee, S.-T. (2005). *Lysobacter concretionis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int J Syst Evol Microbiol* **55**, 1155–1161.

Christensen, P. (1989). Genus I. *Lysobacter* Christensen and Cook 1978, 372^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2083–2089. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.

Christensen, P. & Cook, F. D. (1978). *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* **28**, 367–393.

Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kwon, S. W., Kim, J. S., Park, I. C., Yoon, S. H., Park, D. H., Lim, C. K. & Go, S. J. (2003). *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea. *Int J Syst Evol Microbiol* **53**, 21–27.

Lee, J. W., Im, W.-T., Kim, M. K. & Yang, D.-C. (2006). *Lysobacter koreensis* sp. nov., isolated from a ginseng field. *Int J Syst Evol Microbiol* **56**, 231–235.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.