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

Sathiyaraj Srinivasan,[1] Myung Kyum Kim,[1,2] Gayathri Sathiyaraj,[1] Ho-Bin Kim,[1] Yeon-Ju Kim[1] and Deok-Chun Yang[1]

Correspondence
Deok-Chun Yang
dcyang@khu.ac.kr

[1]Korean Ginseng Center and Ginseng Genetic Resource Bank, Kyung Hee University, 1 Seocheon-dong, Giheung-gu Yongin-si, Gyeonggi-do 449-701, Republic of Korea

[2]Department of Bio and Environmental Technology, Division of Environmental and Life Science, College of Natural Science, Seoul Women's University, Seoul 139-774, Republic of Korea

Strain DCY21^T, a Gram-negative, gliding and rod-shaped aerobic bacterium was isolated from soil of a ginseng field in the Republic of Korea and characterized using a polyphasic approach in order to determine its taxonomic position. Comparative 16S rRNA gene sequence analysis revealed that strain DCY21^T clustered with the species of the genus *Lysobacter*. It was closely related to *Lysobacter gummosus* LMG 8763^T (97.9 %), *Lysobacter capsici* YC5194^T (97.6 %), *Lysobacter antibioticus* DSM 2044^T (97.5 %), *Lysobacter niastensis* DSM 18481^T (97.2 %) and *Lysobacter enzymogenes* DSM 2043^T (96.9 %). The major cellular fatty acids of strain DCY21^T were iso-$C_{15:0}$ (34.3 %), iso-$C_{17:1}\omega 9c$ (19.5 %) and iso-$C_{17:0}$ (17.2 %) and the major isoprenoid quinone was Q-8. The major polar lipids of strain DCY21^T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-*N*-methylethanolamine. The G+C content of the total DNA was 65.4 mol%. The DNA–DNA relatedness values, and biochemical and physiological characteristics strongly supported the genotypic and phenotypic differentiation of strain DCY21^T from species of the genus *Lysobacter*. Strain DCY21^T therefore represents a novel species, for which the name *Lysobacter soli* sp. nov. is proposed. The type strain is DCY21^T (=KCTC 22011^T =LMG 24126^T).

The genus *Lysobacter*, in the class *Gammaproteobacteria* and family *Xanthomonadaceae*, was first described by Christensen & Cook (1978) based on the non-fruiting, gliding nature of bacteria with high DNA G+C contents. At the time of writing, the genus *Lysobacter* consists of 16 species with validly published names. Recently, three novel species of the genus, *Lysobacter panaciterrae* Gsoil 068^T from the rhizosphere soil of a ginseng plant (Ten *et al.*, 2009), *Lysobacter ximonensis* XM415^T (Wang *et al.*, 2009) from the soil of Tibet and *Lysobacter oryzae* YC6269^T from the rhizosphere of a rice plant (Aslam *et al.*, 2009) have been described.

Strain DCY21^T was originally isolated from the soil of a ginseng field near Daejeon city in the Republic of Korea and characterized by a polyphasic approach. DCY21^T was one of the isolates dominating a 10-fold diluted sample on an R2A agar plate incubated under aerobic conditions. The strain was routinely cultured on R2A agar (Difco) at 30 °C and maintained as a glycerol suspension (20 %, w/v) at −70 °C. In this study, strain DCY21^T was characterized by a polyphasic approach, including phylogenetic analysis based on 16S rRNA gene sequences, genomic DNA relatedness and chemotaxonomic and phenotypic properties. On the basis of the results obtained in this study, we propose that strain DCY21^T should be placed in the genus *Lysobacter* as the type strain of a novel species, namely *Lysobacter soli*.

The Gram reaction was performed via the non-staining method as described by Buck (1982). Cell morphology and motility were observed under a Nikon light microscope (×1000 magnification) using the hanging drop method, with cells grown on R2A agar (Difco) for 2 days at 30 °C. Catalase activity was determined with 3 % (v/v) H_2O_2, and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. Growth at different temperatures (4, 25, 30, 37, 42 and 45 °C) and at pH 5.0–11.0 (at intervals of 0.5 pH units) was assessed after 5 days of incubation on LB agar (Difco). Growth on nutrient agar, trypticase soy agar (TSA, Difco), and LB agar was also evaluated at 30 °C. Anaerobic growth was assessed in serum bottles by adding a thioglycolate (1 g l^{-1}) to the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY21^T is EF623862.

Two-dimensional thin-layer chromatograms of polar lipids of strain DCY21^T, cellular fatty acid profiles and DNA–DNA relatedness data for strain DCY21^T and closely related type strains of the genus *Lysobacter* are available with the online version of this paper.

R2A broth and substituting the upper air layer with nitrogen gas. Substrate utilization as the sole carbon source and some enzyme activities were tested by using the API ZYM, API 50CH, API 20NE and API 32GN galleries according to the instructions of the manufacturer (bioMérieux).

Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), purified by TLC and subsequently analysed by HPLC, as previously described by Collins & Jones (1981) and Shin *et al.* (1996). For fatty acid methyl ester analysis, cells were allowed to grow on TSA for 48 h at 30 °C, and then two-loops full of well-grown cells were harvested. Fatty acid methyl esters were prepared, separated and identified by using the Sherlock Microbial Identification System (MIDI; Sasser, 1990). Polar lipids were extracted and examined by the two-dimensional TLC technique (Minnikin *et al.*, 1977).

Extraction of genomic DNA was performed by using 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 Kim *et al.* (2005). Sequences of the 16S rRNA gene were compiled 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 out with the BioEdit program (Hall, 1999). Evolutionary distances were calculated by using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods by using the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

For measurement of G+C content, chromosomal DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995). The isolated DNA was enzymically degraded into nucleosides and the nucleosides were analysed by using HPLC as described by Tamaoka & Komagata (1984) and Mesbah *et al.* (1989). DNA–DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and micro-dilution wells. Five replications were conducted for each sample. The highest and lowest values obtained for each sample were excluded and the remaining three values were used for the calculation of hybridization values. The DNA relatedness values quoted are expressed as means of these three values.

Cells of strain DCY21^T were Gram-negative, non-spore-forming, gliding, aerobic rods, 0.2–0.5 μm wide and 0.6–0.9 μm long. The strain grew well at 30 °C, and growth occurred at 42 °C. Phenotypic, biochemical and chemotaxonomic characteristics that differentiate strain DCY21^T from other related species of the genus *Lysobacter* are listed in Table 1. The major fatty acids detected (percentages of the total cellular fatty acids) from strain DCY21^T were iso-$C_{15:0}$ (34.3 %), iso-$C_{17:1}$ ω9c (19.5 %) and iso-$C_{17:0}$ (17.2 %). Although the major fatty acids were similar to those of other members of the genus *Lysobacter*, the presence of 10-methyl $C_{16:0}$ differentiated this strain from other members of the genus. A comparison of fatty acid profiles among *Lysobacter* species is shown in Supplementary Table S1 (available in IJSEM Online). The major ubiquinone detected was Q-8. This quinone system is a characteristic feature of members of the genus *Lysobacter* (Bae *et al.*, 2005).

Strain DCY21^T exhibited a polar lipid profile that was almost identical with those of other species of the genus *Lysobacter* (Park *et al.*, 2008), containing predominantly diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl-*N*-methylethanolamine and a minor amount of an unknown aminolipid (Supplementary Fig. S1).

The 16S rRNA gene sequence of strain DCY21^T determined in this study was a continuous stretch of 1496 bp. The phylogenetic tree (Fig. 1) shows that strain DCY21^T clustered within the genus *Lysobacter* of the class *Gammaproteobacteria*. The 16S rRNA gene sequence of strain DCY21^T showed high similarity with *Lysobacter gummosus* LMG 8763^T (97.9 %), *Lysobacter capsici* YC5194^T (97.6 %), *Lysobacter antibioticus* DSM 2044^T (97.5 %), *Lysobacter niastensis* DSM 18481^T (97.2 %) and *Lysobacter enzymogenes* DSM 2043^T (96.9 %). The DNA G+C content of strain DCY21^T was 65.4 mol%. DNA–DNA hybridization was performed to differentiate strain DCY21^T from the closely related species of the genus *Lysobacter*. Strain DCY21^T exhibited relatively low levels of DNA–DNA relatedness (Supplementary Table S2) with respect to *L. gummosus* (35 %), *L. capsici* (45 %), *L. antibioticus* (53 %), *L. enzymogenes* (25 %) and *L. niastensis* (28 %), indicating that they are not related at the species level (Wayne *et al.*, 1987). These data indicate that strain DCY21^T is clearly separated from other members of the genus *Lysobacter*.

As shown in Table 1 and Supplementary Table S1, the phenotypic and chemotaxonomic characteristics of strain DCY21^T are enough to differentiate it from those species of the genus *Lysobacter* that form a consistent phylogenetic cluster (except *L. niastensis*, which forms polyphyletic relationships with other species). Therefore, on the basis of the data presented, strain DCY21^T clearly represents a novel member of the genus *Lysobacter*, for which the name *Lysobacter soli* sp. nov. is proposed.

Description of *Lysobacter soli* sp. nov.

Lysobacter soli (so′li. L. neut. gen. n. *soli* of soil, the source of the organism).

Cells are Gram-negative, aerobic, gliding and rod-shaped, approximately 0.2–0.5 μm wide and 0.6–0.9 μm long. Colonies grown on R2A agar (Difco) for 2 days are

Table 1. Differential characteristics of strain DCY21^T and closely related species of the genus *Lysobacter*

Strains: 1, *Lysobacter soli* DCY21^T sp. nov.; 2, *L. antibioticus* DSM 2044^T; 3, *L. capsici* YC5194^T; 4, *L. enzymogenes* DSM 2043^T; 5, *L. gummosus* LMG 8763^T; 6, *L. niastensis* DSM 18481^T. +, Positive; −, negative; W, weakly positive; C, cream; DY, deep yellow; LB, light beige; PY, pale yellow, Y, yellow; YC, yellow–cream.

Characteristic	1	2	3	4	5	6
Cell size (μm)						
Length	0.6–0.9	6.5*	2.0–20*	38.0*	2.0*	2.0–4.0†
Width	0.2–0.5	0.4*	0.3–0.5*	0.5*	0.4*	0.5–0.6†
Colony colour	Y	C	YC	DY–C	PY	LB
Growth on LB at:						
pH 9.5	+	W	W	W	W	W
pH 10	+	−	−	−	W	−
pH 11	W	−	−	−	−	−
Nitrate reduction to nitrite	+	−	−	−	−	+
Enzyme activity (API ZYM)						
N–Acetyl-*β*-glucosaminidase	+	−	+	+	+	−
Acid phosphatase	+	−	+	+	W	+
Cystine arylamidase	+	−	−	+	−	−
Esterase (C4)	+	W	−	−	W	+
Esterase lipase (C8)	+	+	−	+	W	+
α-Galactosidase	−	−	+	+	−	−
α-Glucosidase (starch hydrolysis)	+	−	W	−	−	−
β-Galactosidase (PNPG)	−	−	−	+	−	−
β-Glucosidase (aesculin hydrolysis)	+	+	W	−	−	W
Lipase (C14)	+	−	−	−	−	−
Naphthol-AS-BI-phosphohydrolase	+	−	+	+	+	+
Trypsin	−	W	+	+	+	+
Valine arylamidase	+	−	−	−	−	−
Acid production from (API 50CH):						
Gluconate	−	W	−	−	W	W
L-Arabinose	+	+	−	+	−	−
Cellobiose	−	+	−	−	−	−
D-Fucose	+	−	W	−	−	−
Gentiobiose	−	+	−	W	−	−
D-Lyxose	+	−	−	−	−	−
Maltose	W	+	−	−	−	−
Melibiose	−	+	W	+	−	−
Raffinose	−	+	−	−	−	−
Sucrose	−	+	+	W	W	−
Trehalose	−	+	W	+	+	−
Turanose	W	+	−	+	−	W
D-Xylose	−	+	−	+	−	W
Methyl α-D-glucoside	−	+	W	+	−	−
L-Arabitol	+	−	−	−	−	W
Amygdalin	−	W	W	−	−	−
Salicin	−	+	−	−	−	−
Assimilation test (API 20NE and 32GN)						
3-Hydroxybenzoate	+	−	W	−	−	W
3-Hydroxybutyrate	−	+	+	+	+	−
Acetate	+	−	W	W	+	−
Citrate	−	W	+	W	+	−
Valerate	−	+	W	−	+	−
Vanillate	W	−	−	−	−	−
D-Glucose	+	W	−	+	+	W
Maltose	+	−	W	+	+	+
D-Mannose	+	+	W	+	+	−

Table 1. cont.

Characteristic	1	2	3	4	5	6
Melibiose	−	−	−	+	+	−
Sucrose	−	w	−	w	−	−
myo-Inositol	w	−	w	−	−	−
L-Histidine	−	−	w	−	+	+
Salicin	−	−	w	+	−	−
Glycogen	−	+	w	+	+	−
DNA G+C content (mol%)	65.4	69.2*	65.4*	69.0*	65.7*	66.6†

Data from other studies as indicated: *Park *et al.* (2008); †Weon *et al.* (2007).

convex, glistening, circular and yellow. The temperature range for growth is 4–42 °C, but the optimum temperature is 30 °C; no growth is observed at 45 °C. The pH range for growth is 5–10.5; weak growth is observed at pH 11 and optimum growth at pH 7.0−7.5. Oxidase and catalase-positive. Reduction of nitrates to nitrites and glucose acidification are positive; indole production is negative. The following carbon sources are utilized in the API 20NE and API 32GN tests: acetate, *N*-acetylglucosamine, D-glucose, 3-hydroxybenzoate, 4-hydroxybenzoate, maltose, D-mannose and L-proline. Utilization of the following substrates is negative: adipate, L-arabinose, L-alanine, caprate, citrate, L-fucose, glycogen, gluconate, 3-hydroxybutyrate, L-histidine, itaconate, 2-ketogluconate, 5-ketogluconate, lactate, L-malate, D-mannitol, malonate, melibiose, phenylacetate, propionate, D-ribose, salicin, L-serine, D-sorbitol, suberate, sucrose and valerate. In assays with API ZYM, *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), α-glucosidase, β-glucosidase, leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase and valine arylamidase activities are present, but α-chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and trypsin are absent. Other differential phenotypic and biochemical characteristics are summarized in Table 1. Contains predominantly diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl-*N*-methylethanolamine and a minor amount of unknown aminolipid. Q-8 is the predominant quinone. The major cellular fatty acids are iso-$C_{15:0}$ (34.3 %), iso-$C_{17:1}\omega9c$ (19.5 %), iso-$C_{17:0}$ (17.2 %), iso-$C_{16:0}$ (7.5 %) and iso-$C_{11:0}$ 3-OH (5.8 %). The DNA G+C content of the type strain is 65.4 mol%.

The type strain, DCY21^T (=KCTC 22011^T =LMG 24126^T) was isolated from soil of a ginseng field in Daejeon City, South Korea.

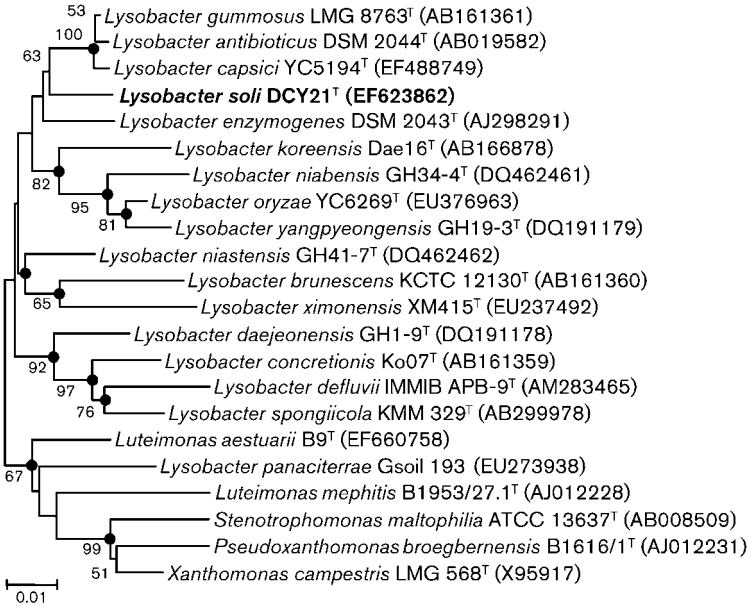

Fig. 1. Phylogenetic relationship between strain DCY21^T and related species of the genus *Lysobacter* and closely related genera. Tree reconstructed by using the neighbour-joining method. Bar, 0.01 substitutions per nucleotide position. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at the branch points. Filled circles indicate generic branches that were also recovered by using maximum-parsimony algorithms.

References

Aslam, Z., Yasir, M., Jeon, C. O. & Chung, Y. R. (2009). *Lysobacter oryzae* sp. nov., isolated from the rhizosphere of rice (*Oryza sativa* L.). *Int J Syst Evol Microbiol* **59**, 675–680.

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.

Buck, J. D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

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.

Collins, M. D. & Jones, D. (1981). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol Rev* **45**, 316–354.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a β-glucosidase producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

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.

Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.

Moore, D. D. & Dowhan, D. (1995). Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.

Park, J. H., Kim, R., Aslam, Z., Jeon, C. O. & Chung, Y. R. (2008). *Lysobacter capsici* sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*. *Int J Syst Evol Microbiol* **58**, 387–392.

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

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Shin, Y. K., Lee, J.-S., Chun, C. O., Kim, H.-J. & Park, Y.-H. (1996). Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^{T}. *J Microbiol Biotechnol* **6**, 68–69.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Ten, L. N., Jung, H.-M., Im, W.-T., Yoo, S.-A., Oh, H.-M. & Lee, S.-T. (2009). *Lysobacter panaciterrae* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **59**, 958–963.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Wang, Y., Dai, J., Zhang, L., Luo, X., Li, Y., Chen, G., Tang, Y., Meng, Y. & Fang, C. (2009). *Lysobacter ximon*ensis sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **59**, 786–789.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Weon, H.-Y., Kim, B.-Y., Kim, M.-K., Yoo, S.-H., Kwon, S.-W., Go, S.-J. & Stackebrandt, E. (2007). *Lysobacter niabensis* sp. nov. and *Lysobacter niastensis* sp. nov., isolated from greenhouse soils in Korea. *Int J Syst Evol Microbiol* **57**, 548–551.