INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

NOTE

Kim *et al.*, *Int J Syst Evol Microbiol* 2017;67:1102–1106

DOI 10.1099/ijsem.0.001729

MICROBIOLOGY SOCIETY

Lysobacter solanacearum sp. nov., isolated from rhizosphere of tomato

Soo-Jin Kim,[1] Jae-Hyung Ahn,[1] Hang-Yeon Weon,[1] Jae-Ho Joa,[2] Seung-Beom Hong,[1] Soon-Ja Seok,[1] Jeong-Seon Kim[1] and Soon-Wo Kwon[1,*]

Abstract

A bacterial strain, designated T20R-70^T, was isolated from tomato rhizosphere soil collected in Yecheon-gun, Gyeongsangbuk-do in South Korea. Growth was observed within the ranges 10–40 °C (optimally at 28–30 °C), pH 7.0–8.0 (optimally at pH 7.0) and 0–1 % NaCl (optimally at 0 %). The 16S rRNA gene sequence showed the highest similarities with those of *Lysobacter hankyongensis* KTCe-2^T (98.7 %), *Lysobacter brunescens* KCTC 12130^T (98.0 %), '*Lysobacter daecheongensis*' Dae08 (97.2 %) and *Lysobacter oligotrophicus* 107-E2^T (97.1 %). The phylogenetic tree showed that strain T20R-70^T formed a clade with *Lysobacter hankyongensis* KTCe-2^T and *Lysobacter brunescens* KCTC 12130^T. The dominant fatty acids (>10 %) were iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:1}\omega9c$ and summed feature 3 (including iso-$C_{15:0}$ 2-OH and/or iso-$C_{16:1}$ $\omega7c$). The major polar lipids were phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol. The major respiratory quinone was Q-8. DNA–DNA hybridization data revealed that strain T20R-70^T had a hybridization value of 42±4 % (mean±SD) to the most closely related species of the genus *Lysobacter*. The DNA G+C content was 63.0 mol%. The physiological, biochemical and chemotaxonomic data allowed the discrimination of the new isolate from its phylogenetic relatives. Strain T20R-70^T is thus considered to be a representative of a novel species of the genus *Lysobacter*, for which the name *Lysobacter solanacearum* sp. nov. is proposed. The type strain is T20R-70^T (=KACC 18656^T=NBRC 111881^T).

The genus *Lysobacter* belongs to the family *Xanthomonadaceae* within the class *Gammaproteobacteria*. The genus *Lysobacter* was first proposed for Gram-stain-negative, aerobic, rod-shaped, non-fruiting bacterial strains isolated from soil and fresh water [1]. The DNA G+C contents of members of this genus were found to be high (more than 60 mol%), polar lipids consisted mainly of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidyl-*N*-methylethanolamine, and the major fatty acids were iso-$C_{15:0}$ and iso-$C_{16:0}$ [2–6]. At the time of writing, the genus *Lysobacter* consisted of 36 species with validly published names (www.bacterio.net/lysobacter.html), which were isolated from various habitats, such as soil, water, sludge, municipal solid waste, freshwater sediment, plants, ores, estuary sediment and a deep-sea sponge. Here, we describe the taxonomic characterization of a novel species belonging to the genus *Lysobacter* isolated from the rhizosphere of a tomato plant.

During the course of bacterial population analysis in the rhizosphere of a tomato plant, we isolated several bacterial strains. The rhizosphere soil was sampled at Yecheon-gun, Gyeongsangbuk-do, South Korea (36° 43′ 3.33″ N 128° 29′ 55.29″ E). The soil was serially diluted in 0.85 % (w/v) NaCl solution, plated on R2A agar medium (BD) and incubated at 28 °C. A bacterial strain, designated T20R-70^T, was pure-cultured through sub-culturing. The strain was preserved at 4 °C after lyophilization using 12 % (w/v) skimmed milk solution. The reference strains, *Lysobacter brunescens* KACC 11385^T, '*Lysobacter daecheongensis*' KACC 18720, *Lysobacter dokdonensis* KACC 18711^T, *Lysobacter hankyongensis* KACC 16618^T, *Lysobacter oligotrophicus* KACC 18724^T and *Lysobacter sediminicola* KACC 16617^T were obtained from the Korean Agricultural Culture Collection (South Korea) and were grown on R2A medium at 28 °C.

Cellular morphology was determined by using transmission electron microscopy (model 912AB; LEO) and phase-contrast microscopy (AXIO; Zeiss) with cells grown on R2A agar for 2 days. Gram staining was performed using a Gram staining kit (Difco) according to the manufacturer's

Author affiliations: [1]Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration, Wanju-gun, Jeollabuk-do 55365, Republic of Korea; [2]National Institute of Horticultural and Herbal Science, Rural Development Administration, Jeju 63240, Republic of Korea.

***Correspondence:** Soon-Wo Kwon, swkwon1203@korea.kr

Keywords: *Lysobacter solanacearum*; 16S rRNA gene ; polar lipid; quinone.

The GenBank/EMBL/DDBJ number for the 16S rRNA gene sequence of strain T20R-70^T is KU379668.

Two supplementary figures and one supplementary table are available with the online Supplementary Material.

instructions. Catalase and oxidase activity was checked through bubble production in 3 % (v/v) hydrogen peroxide solution and a colour change in 1 % (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux), respectively. Hydrolysis of substrates was assessed using R2A agar medium supplemented with the following substrates: casein (1 %, w/v), chitin (1 %, w/v), CM-cellulose (1 %, w/v), hypoxanthine (0.5 %, w/v), starch (1 %, w/v), Tween 80 (1 %, w/v), tyrosine (0.1 %, w/v) or xanthine (0.5 %, w/v). The DNase test was conducted on DNase test agar (Difco). The optimum temperature range for the bacterial growth was checked on R2A agar medium at 4, 10, 15, 20, 25, 28, 30, 33, 35, 37, 40 and 45 °C. The pH range for growth was checked in R2A broth, the pH of which was adjusted with citrate/phosphate buffer (pH 3.0–7.0), Tris/hydrochloride buffer (pH 8.0–9.0) and carbonate/bicarbonate buffer (pH 10.0–11.0). After sterilization (121 °C, 15 min), the pH of the R2A broth was checked and adjusted if necessary. Tolerance to NaCl was studied in R2A broth supplemented with NaCl (0, 1.0, 2.0, 3.0, 4.0 and 5.0 %, w/v). To determine the additional physiological properties, including assimilation of carbohydrates, acid production from carbohydrate sources and enzymic activities, API 20 NE, API ID 32 GN, API 50 CH and API ZYM kits (bioMérieux) were used according to the manufacturer's instructions. The API ZYM tests were read after 4 h of incubation at 37 °C, and the other API tests after 7 days at 28 °C. Cells of strain T20R-70^T were found to be Gram-stain-negative, aerobic, flagellated rods (0.6–0.8×1.6–2.6 μm) (Fig. S1, available in the online Supplementary Material). The strain could be differentiated from the closely related species of the genus *Lysobacter* on the basis of cell shape, temperature and pH ranges for growth, tolerance of NaCl and various physiological and biochemical properties (Table 1).

Phylogenetic analysis was conducted on the basis of 16S rRNA gene sequence. The 16S rRNA gene of strain T20R-70^T was amplified by PCR using the universal primers 8F and 1512R [7]. The PCR products were sequenced by Genotech (Daejeon, Republic of Korea), and a nearly complete 16S rRNA gene sequence (1480 bp) was obtained. The sequence similarities were calculated with the BLAST program of GenBank (www.ncbi.nlm.nih.gov/) and EzBioCloud (http://eztaxon-e.ezbiocloud.net/) [8]. The sequences of strain T20R-70^T and the related taxa obtained from the GenBank database (www.ncbi.nlm.nih.gov/) were aligned using the SILVA Incremental Aligner [9]. Phylogenetic trees were reconstructed in MEGA software version 6.0 [10] using the neighbour-joining [11], maximum-likelihood [12] and maximum-parsimony algorithms [13] with 1000 bootstrap iterations. The 16S rRNA gene sequence of strain T20R-70^T displayed the highest similarity with those of *L. hankyongensis* KTCe-2^T (98.7 %), *L. brunescens* KCTC 12130^T (98.0 %), '*L. daecheongensis*' Dae08 (97.2 %) and *L. oligotrophicus* 107-E2^T (97.1 %). The neighbour-joining phylogenetic tree showed that strain T20R-70^T formed a clade with *L. hankyongensis* KTCe-2^T and *L. brunescens* KCTC 12130^T, which was also supported by the maximum-likelihood and maximum-parsimony trees (Fig. 1).

Fatty acids were analysed for strain T20R-70^T and the closely related strains belonging to the genus *Lysobacter*, including *L. brunescens* KACC 11385^T, '*L. daecheongensis*' KACC 18720, *L. dokdonensis* KACC 18711^T, *L. hankyongensis* KACC 16618^T, *L. oligotrophicus* KACC 18724^T and *L. sediminicola* KACC 16617^T. All the strains were cultivated on R2A medium at 28 °C, and the incubation time varied from 1.5–5.5 days due to the differences in the growth rate of each strain, in order to reach the exponential growth stage of each strain. The fatty acids were extracted, methylated and separated by GC (6890; Hewlett Packard) according to the protocol of the Sherlock Microbial Identification System (version 5.0, MIDI) [14] and identified by using the TSBA 50 database of the Microbial Identification System. Cell biomass for analysis of polar lipids and respiratory quinones was collected after shaking at 28 °C in R2A broth for 3 days. Quinones and polar lipids were extracted and analysed using the method described by Minnikin *et al.* [15]. For detection of polar lipids, molybdatophosphoric acid (for total lipids), phosphomolybdic acid (for phospholipids), ninhydrin (for aminolipids) and α-naphthol/sulfuric acid reagent (for glycolipids) were sprayed onto the plates. The respiratory quinones were separated by TLC and identified by HPLC. The DNA G+C content was determined with the fluorometric method [16] using SYBR Green 1 and a real-time PCR thermocycler (Bio-Rad). Genomic DNA samples from *Bacillus amyloliquefaciens* subsp. *plantarum* DSM 23117^T, *Pseudomonas stutzeri* ATCC 17588^T and *Micrococcus luteus* ATCC 4698^T were used as calibration references.

The major fatty acids of strain T20R-70^T were found to be iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:1}\omega9c$ and summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or iso-$C_{16:1}\omega7c$) (Table S1). In the fatty acid comparison among the strain T20R-70^T and its closely related *Lysobacter* species, the amount of summed feature 3 (iso-$C_{15:0}$ 2-OH and/or iso-$C_{16:1}\omega7c$) was highly variable. Also, quantitative and qualitative differences in the fatty acids were observed (Table S1). The polar lipids of strain T20R-70^T consisted of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the major polar lipids with small amounts of two unidentified aminophospholipids and two unidentified phospholipids (Fig. S2). The polar lipids diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine are the major ones commonly present in many species of the genus *Lysobacter* [3, 4, 6]. The primary respiratory quinone of strain T20R-70^T was Q-8, which is the major quinone present in members of the genus *Lysobacter* [2–6]. The DNA G+C content of strain T20R-70^T was 63.0 mol%, which is slightly lower than those of the other closely related species of the genus *Lysobacter* (Table 1).

The DNA–DNA filter hybridization method described by Seldin and Dubnau [17] was used to calculate the DNA hybridization values. Probe labelling was conducted using the non-radioactive DIG High Prime system, and the

Table 1. Differential characteristics among strain T20R-70^T and the closely related species of the genus *Lysobacter*

Strains: 1, *Lysobacter solanacearum* sp. nov. T20R-70^T; 2, *L. brunescens* KACC 11385^T; 3, '*L. daecheongensis*' KACC 18720; 4, *L. dokdonensis* KACC 18711^T; 5, *L. hankyongensis* KACC 16618^T; 6, *L. oligotrophicus* KACC 18724^T; 7, *L. sediminicola* KACC 16617^T. Data were obtained in this study unless otherwise indicated. +, Positive; −, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Isolation source	Soil	Fresh water[a*]	Stream sediment[b]	Soil[c]	Sludge[d]	Antarctic freshwater lake[e]	Freshwater sediment[d]
Cell size (μm)	0.6–0.8×1.6–2.6	0.2–0.5×7.0–70.0[a]	0.7–1.0×1.0–5.0[b]	0.4–0.8×1.0–5.0[c]	0.3–0.4×1.0–1.5 [d]	0.2–0.3×1.8–2.7[e]	0.4–0.6×1.5–3.0[d]
Temperature range for growth (°C)	10–40	10–45	10–40	10–35	10–40	10–30	15–50
Catalase/oxidase	+/+	+/−	−/+	+/+	−/+	−/+	−/−
Hydrolysis of:							
Aesculin	+	+	+	+	+	+	−[d]
Casein	+	+	+	+	−	+	−
Starch	+	+	−	−	+	+	+
Enzymic activity							
Esterase (C4)	+	−	+	+	+	−	+
Lipase (C14)	−	−	+	−	+	−	−
Valine arylamidase	−	−	−	+	−	−	−
Cystine arylamidase	−	−	−	+	−	−	−
Trypsin	+	+	+	−	−	−	−
α-Chymotrypsin	+	−	−	−	−	−	+
Acid production from:							
D-Ribose	+	−	−	+	−	−	+
D-Xylose	+	−	−	+	−	−	+
D-Galactose	+	−	−	+	−	−	+
D-Glucose	+	−	−	+	−	−	+
D-Fructose	+	−	−	+	−	−	+
N-Acetylglucosamine	+	+	+	+	−	−	+
Amygdalin	−	−	−	+	−	−	+
Arbutin	−	−	−	+	−	−	+
Aesculin	+	+	−	+	−	+	+
Cellobiose	+	−	−	+	−	−	+
Maltose	+	−	−	+	−	−	+
Melibiose	+	−	−	+	−	−	+
Sucrose	+	−	−	+	−	−	+
Trehalose	+	−	−	+	−	−	+
Raffinose	+	−	−	+	−	−	+
Starch	+	−	−	+	−	−	+
Glycogen	+	−	−	+	−	−	+
Gentiobiose	+	−	−	−	−	−	+
Potassium 5-ketogluconate	−	+	+	−	+	+	−
DNA G+C content (mol%)	63.0	67.7[a]	69.3[b]	68.1[c]	68.6[d]	66.1[e]	71.5[d]

*Data from: *a*, Christensen and Cook [1]; *b*, Ten *et al.* [19]; *c*, Oh *et al.* [20]; *d*, Siddiqi and Im [6]; *e*, Fukuda *et al.* [21].

hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified using a densitometer (Bio-Rad). Strain T20R-70^T exhibited low hybridization values as compared with the closely related species: *L. hankyongensis* KACC 16618^T (32±3 %; reciprocally, 30±5 %), *L. brunescens* KACC 11385^T (42±4 %), '*L. daecheongensis*' KACC 18720 (36±5 %) and *L. oligotrophicus* KACC 18724^T (39±2 %) (mean±SD). The hybridization values of less than 70 %, the currently accepted threshold for species definition, demonstrate that strain T20R-70^T constitutes a novel genomic species [18].

On the basis of phylogenetic analysis, phenotypic characteristics and DNA–DNA hybridization values, strain T20R-70^T is considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter solanacearum* sp. nov. is proposed.

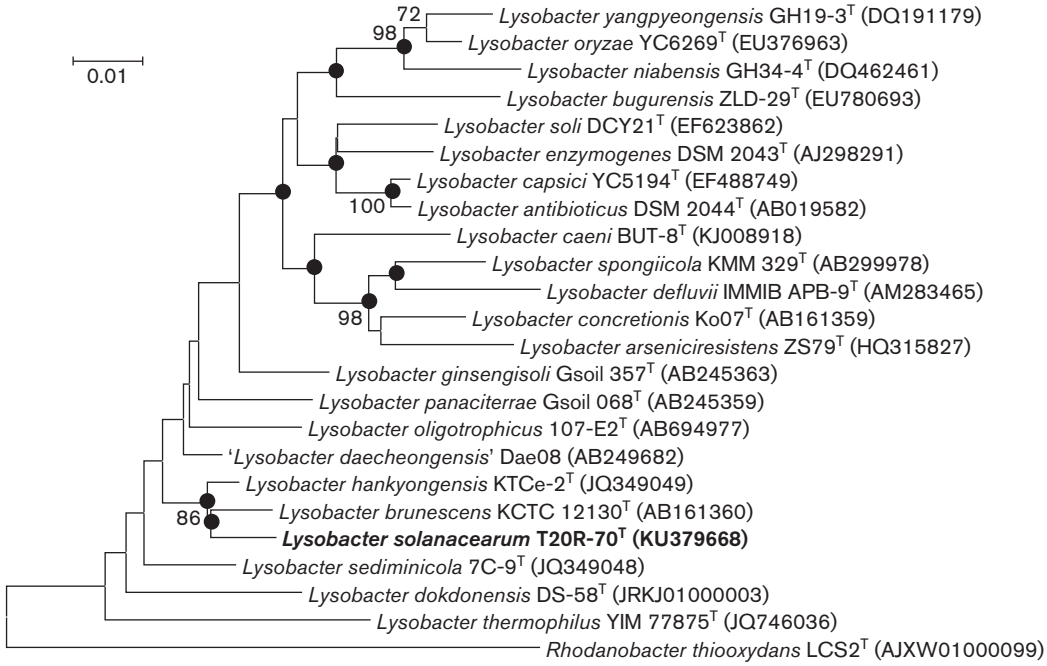

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain T20R-70^T and representative strains of some selected species of the genus *Lysobacter*. Numbers at nodes are bootstrap values (percentages of 1000 replications); only values greater than 70 % are shown. Filled circles indicate branches that were also recovered in both the maximum-likelihood and maximum-parsimony trees. Bar, 0.01 substitutions per nucleotide position.

DESCRIPTION OF *LYSOBACTER SOLANACEARUM* SP. NOV.

Lysobacter solanacearum (so.la.na.ce.a'rum. N.L. fem. pl. n. *Solanaceae*, the nightshade family; N.L. fem. pl. gen. n. *solanacearum*, of the *Solanaceae*).

C*Lysobacter rhizosphaerae*ells are Gram-stain-negative, aerobic, flagellated rods (0.6–0.8×1.6–2.6 μm). Colonies are convex, yellow and irregular on R2A medium after 2 days of incubation at 28 °C. Catalase- and oxidase-positive. Flexirubin-type pigments are not produced. Growth occurs at temperatures from 10 to 40 °C with an optimum temperature range of 28–30 °C. Growth is observed at pH 7.0 and 8.0, but not pH 6.0 or 9.0. The optimum pH for growth is pH 7.0. Able to tolerate 1 % (w/v) NaCl, and does not reduce nitrate. Negative for indole production, glucose fermentation and arginine dihydrolase. Hydrolyses aesculin, casein, gelatin, Tween 80 and starch, but not chitin, CM-cellulose, DNA, hypoxanthine, tyrosine, urea or xanthine. Positive activities for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative activities for lipase (C14), valine arylamidase, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are observed. Acid is produced from D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, *N*-acetylglucosamine, aesculin, cellobiose, maltose, melibiose, sucrose, trehalose, raffinose, starch, glycogen and gentiobiose, but not from glycerol, erythritol, D-arabinose, L-arabinose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, lactose, inulin, melezitose, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. The dominant fatty acids (>10 %) are iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:1}\omega9c$ and summed feature 3 (comprising iso-$C_{15:0}$ 2-OH and/or iso-$C_{16:1}\omega7c$). The major respiratory quinone is Q-8. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol.

The type strain, T20R-70^T (=KACC 18656^T=NBRC 111881^T), was isolated from the rhizosphere soil of tomato plants collected from Yecheon-gun, Gyeongsangbuk-do in South Korea. The DNA G+C content of the type strain is 63.0 mol%.

Funding information
This study (project no. PJ010954) was supported by the National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. **Christensen P, Cook FD.** *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* 1978; 28:367–393.
2. **Park JH, Kim R, Aslam Z, Jeon CO, Chung YR.** *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* 2008;58:387–392.
3. **Choi JH, Seok JH, Cha JH, Cha CJ.** *Lysobacter panacisoli* sp. nov., isolated from ginseng soil. *Int J Syst Evol Microbiol*. 2014;64: 2193–2197.
4. **Singh H, du J, Won KH, Yang JE, Akter S *et al.*** *Lysobacter novalis* sp. nov., isolated from fallow farmland soil. *Int J Syst Evol Microbiol* 2015;65:3131–3136.
5. **Kim SJ, Ahn JH, Weon HY, Hong SB, Seok SJ *et al.*** *Lysobacter terricola* sp. nov., isolated from greenhouse soil. *Int J Syst Evol Microbiol* 2016;66:1401–1406.
6. **Siddiqi MZ, Im WT.** *Lysobacter hankyongensis* sp. nov., isolated from activated sludge and *Lysobacter sediminicola* sp. nov., isolated from freshwater sediment. *Int J Syst Evol Microbiol* 2016;66: 212–218.
7. **Felske A, Rheims H, Wolterink A, Stackebrandt E, Akkermans AD.** Ribosome analysis reveals prominent activity of an uncultured member of the class Actinobacteria in grassland soils. *Microbiology* 1997;143:2983–2989.
8. **Kim OS, Cho YJ, Lee K, Yoon SH, Kim M *et al.*** Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
9. **Pruesse E, Peplies J, Glöckner FO.** SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
10. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
11. **Saitou N, Nei M.** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
12. **Felsenstein J.** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
13. **Fitch WM.** Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 1971;20:406–416.
14. **Sasser M.** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
15. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al.*** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2: 233–241.
16. **Gonzalez JM, Saiz-Jimenez C.** A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* 2002;4:770–773.
17. **Seldin L, Dubnau D.** Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int J Syst Bacteriol* 1985;35:151–154.
18. **Stackebrandt E, Goebel BM.** Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* 1994;44: 846–849.
19. **Ten LN, Jung HM, Im WT, Yoo SA, Lee ST.** *Lysobacter daecheongensis* sp. nov., isolated from sediment of stream near the Daechung dam in South Korea. *J Microbiol* 2008;46:519–524.
20. **Oh KH, Kang SJ, Jung YT, Oh TK, Yoon JH.** *Lysobacter dokdonensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2011;61:1089–1093.
21. **Fukuda W, Kimura T, Araki S, Miyoshi Y, Atomi H *et al.*** *Lysobacter oligotrophicus* sp. nov., isolated from an Antarctic freshwater lake in Antarctica. *Int J Syst Evol Microbiol* 2013;63:3313–3318.