

Lysobacter daecheongensis sp. nov., Isolated from Sediment of Stream Near the Daechung Dam in South Korea

Leonid N. Ten¹, Hae-Min Jung², Wan-Taek Im^{2*}, Soon-Ae Yoo¹, and Sung-Taik Lee²

¹Department of Biology and Medicinal Science, Pai Chai University, Daejeon 302-735, Republic of Korea

²Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea

(Received February 15, 2008 / Accepted August 12, 2008)

A Gram-negative, aerobic, rod shaped, non-spore-forming bacterial strain, designated Dae08^T, was isolated from sediment of the stream near Daechung dam in South Korea, and was characterized in order to determine its taxonomic position, using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that strain Dae08^T belongs to the family *Xanthomonadaceae* of the *Gammaproteobacteria*, and is related to *Lysobacter brunescens* ATCC 29482^T (97.3%). The phylogenetic distances from any other species with validly published names within the genus *Lysobacter* were greater than 3.7%. The G+C contents of the genomic DNA of strain Dae08^T was 69.3 mol%. The detection of a quinone system with Q-8 as the predominant compound and a fatty acid profile with iso-C_{15:0}, iso-C_{17:1} ω9c, iso-C_{17:0}, iso-C_{16:0}, and iso-C_{11:0} 3-OH as the major acids supported the affiliation of strain Dae08^T to the genus *Lysobacter*. DNA-DNA relatedness between strain Dae08^T and its phylogenetically closest neighbour was 28%. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Dae08^T (= KCTC 12600^T) should be classified in the genus *Lysobacter* as the novel species, for which the name *Lysobacter daecheongensis* sp. nov. is proposed.

Keywords: 16S rRNA gene, *Xanthomonadaceae*, *Lysobacter daecheongensis*

The genus *Lysobacter*, belonging to the family *Xanthomonadaceae* of the *Gammaproteobacteria*, was established by Christensen and Cook (1978) for non-fruiting body gliding bacteria with high G+C content, and at present comprises thirteen species with validly published names, namely *Lysobacter antibioticus* (Christensen and Cook, 1978), *Lysobacter brunescens* (Christensen and Cook, 1978), *Lysobacter capsici* (Park *et al.*, 2008), *Lysobacter concretionis* (Bae *et al.*, 2005), *Lysobacter daejeonensis* (Weon *et al.*, 2006), *Lysobacter defluvii* (Yassin *et al.*, 2007), *Lysobacter enzymogenes* (Christensen and Cook, 1978), *Lysobacter gummosus* (Christensen and Cook, 1978), *Lysobacter korensis* (Lee *et al.*, 2006), *Lysobacter niabensis* (Weon *et al.*, 2007), *Lysobacter niastensis* (Weon *et al.*, 2007), *Lysobacter spongiicola* (Romanenko *et al.*, 2007), and *Lysobacter yangpyeongensis* (Weon *et al.*, 2006).

During the course of a study on the culturable aerobic and facultative anaerobic bacterial community living in the sediment from Daechung dam (South Korea), a large number of bacterial strains were isolated. In this study, we characterized one of these isolates, namely strain Dae08^T. The phenotypic, chemotaxonomic, and phylogenetic analyses established the affiliation of this isolate with the genus *Lysobacter*. The data obtained also suggest that the isolate represents a novel species of the genus *Lysobacter*.

Materials and Methods

Isolation of bacterial strain and culture condition

Strain Dae08^T was isolated from a sediment sample of stream collected in near Daechung dam (South Korea). The sample was suspended in 50 mM phosphate buffer (pH 7.0) and serial decimal dilutions of the suspension were spread on modified-R2A agar plates (0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g Casamino acid, 0.25 g soytone, 0.5 g dextrose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K₂HPO₄, 0.05 g MgSO₄, 0.05 g CaCl₂, 15 g agar per liter). The plates were incubated at 25°C for two weeks. Single colonies on the plates were purified by transferring them onto fresh plates of modified R2A agar and incubating again. One isolate, Dae08^T, was cultured routinely on R2A agar (Difco) at 25°C and preserved in a glycerol solution (20%, w/v) at -70°C. This organism was then submitted to the Korean Collection for Type Cultures (= KCTC 12600^T).

Phenotypic and biochemical characteristics

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology and motility was observed under a Nikon light microscope at ×1,000, with cells grown on R2A agar for 6 days at 25°C. Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman (2002). Tests for anaerobic growth, assimilation of most amino acids and some carbohydrates, nitrate- and nitrite-reduction were performed as described previously (Ten *et al.*, 2006). In addition, biochemical test

* To whom correspondence should be addressed.
(Tel) 82-42-869-5617; (Fax) 82-42-863-5617
(E-mail) wandra@kaist.ac.kr

were carried out using API 20NE, API ID 32GN, and API 20E test 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 M HCl), casein, chitin, starch (Atlas, 1993), lipid (Kouker and Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 7 days. Growth

at different temperatures (4, 10, 15, 20, 25, 30, 37, and 42°C) and various pH values (pH 4.5~10.0 at intervals of 0.5 pH units) was assessed after 7 days of incubation. Salt tolerance was tested on R2A medium supplemented with 1~10 % (w/v) NaCl after 7 days of incubation. Growth on nutrient agar, trypticase soy agar (TSA), and MacConkey agar was also evaluated at 25°C.

Table 1. Differential phenotypic characteristics of strain Dae08^T and recognized *Lysobacter* species

Taxa: 1, strain Dae08^T (present study); 2, *L. brunescens* ATCC 29482^T [data in columns 2, 3, 5, 8, and 14 are from Christensen and Cook (1978) and Weon *et al.* (2007)]; 3, *L. gummosus* ATCC 29489^T; 4, *L. daejeonensis* KACC 11406^T (Weon *et al.*, 2006); 5, *L. antibioticus* DSM 2044^T; 6, *L. capsici* KCTC 22007^T (Park *et al.*, 2008); 7, *L. niastensis* DSM 18481^T; 8, *L. enzymogenes* DSM 2043^T; 9, *L. concretionis* DSM 16239^T (Bae *et al.*, 2005); 10, *L. spongicola* JCM 14760^T (Romanenko *et al.*, 2007); 11, *L. yangpyeongensis* KACC 11407^T; 12, *L. koreensis* KCTC 12204^T (Lee *et al.*, 2006); 13, *L. defluvii* DSM 18482^T (Yassin *et al.*, 2007); 14, *L. niabensis* DSM 18244^T (Weon *et al.*, 2007). +, Positive; (+), weakly positive; -, negative; ND, no data available. All strains are positive for gelatin hydrolysis but negative for glucose acidification, urease, indole production and assimilation of inositol.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Growth at/on														
15°C	-	+	+	+	+	+	+	+	+	+	+	+	-	+
37°C	-	+	+	+	+	+	+	+	+	+	+	-	+	+
MacConkey agar	-	-	-	-	-	-	-	+	ND	-	-	+	-	-
Gliding motility	+	+	+	-	+	+	+	+	+	-	-	ND	ND	-
Nitrate reduction	-	-	-	+	+	ND	+	-	+	-	-	-	-	-
Aesculin hydrolysis	(+)	+	+	+	+	+	+	+	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	+	-	-	-	+	-	-	+
Catalase	-	+	+	(+)	+	+	+	+	+	+	-	+	+	+
Oxidase	+	+	ND	+	+	+	+	+	+	+	+	-	+	+
β-Galactosidase	-	-	+	-	+	-	+	+	-	-	-	-	-	-
NaCl tolerance (range, %)	0~7	0~1	0~2	0~3	0~2	0~2	0~1	0~2	ND	0~6	0~0.5	0~1	0~6	0~1
API 20NE and API ID 32GN tests for assimilation of														
Acetate	-	-	(+)	+	+	ND	-	(+)	+	-	-	-	ND	-
N-Acetyl-D-glucosamine	-	-	+	-	+	-	+	+	-	-	-	-	-	-
Glycogen	-	-	+	+	+	ND	-	+	(+)	-	+	-	ND	-
D-Maltose	-	-	+	+	+	-	+	+	-	-	-	-	-	-
D-Melibiose	-	-	+	-	-	ND	-	+	-	-	-	-	ND	-
L-Rhamnose	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Sucrose	-	-	+	-	-	-	-	+	-	-	-	-	ND	-
L-Arabinose	-	-	-	-	-	-	-	-	-	-	-	+	-	-
L-Serine	-	-	+	-	+	ND	-	+	-	-	-	+	ND	-
D-Sorbitol	-	-	-	-	-	-	-	-	-	-	-	+	-	-
D-Mannose	-	-	+	-	+	ND	-	+	-	-	-	-	-	-
D-Mannitol	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Valerate	(+)	-	(+)	+	+	ND	-	+	+	-	-	+	ND	-
D-Glucose	-	-	+	+	+	-	(+)	+	-	-	-	-	-	-
L-Histidine	-	-	-	-	+	ND	+	(+)	-	-	-	-	ND	-
Citrate	-	-	-	-	-	+	-	+	-	-	-	+	+	-
3-Hydroxybutyrate	-	-	+	+	+	ND	-	+	+	-	+	-	ND	-
5-Ketogluconate	-	-	-	-	-	-	-	-	-	+	-	-	ND	-
Malate	(+)	-	+	-	+	ND	-	+	-	-	-	-	-	-
L-Proline	-	-	+	-	+	ND	-	+	+	-	-	-	ND	-
DNA G+C content (mol%)	69.3	67.7	65.7	61.7	69.2	65.4	66.6	69.0	63.8	69.0	67.3	68.9	67.1	62.5

Table 2. Cellular fatty acid profiles of strain Dae08^T and recognized *Lysobacter* species

Taxa: 1, strain Dae08^T (the present study); 2, *L. brunescens* ATCC 29482^T; 3, *L. gummosus* ATCC 29489^T; 4, *L. daejeonensis* KACC 11406^T; 5, *L. antibioticus* DSM 2044^T; 6, *L. capsici* KCTC 22007^T (Park *et al.*, 2008); 7, *L. niastensis* DSM 18481^T; 8, *L. enzymogenes* DSM 2043^T; 9, *L. concretionis* DSM 16239^T; 10, *L. spongiicola* JCM 14760^T (Romanenko *et al.*, 2007); 11, *L. yangpyeongensis* KACC 11407^T; 12, *L. korensis* KCTC 12204^T; 13, *L. defluvii* DSM 18482^T (Yassin *et al.*, 2007); 14, *L. niabensis* DSM 18244^T. Data are from Weon *et al.* (2007) unless indicated. Except taxa 6, 10, and 13, all strains were grown on R2A agar for 48 h at 28°C. Other cultured conditions: TSA, 48 h, 28°C for *L. capsici*; R2A, 72 h, 28°C for *L. spongiicola*; BHI broth, 1 week, 37°C for *L. defluvii*. Results are presented as a percentage of the total fatty acids. -, <1% or not detected; ECL, equivalent chain length.

Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14
C _{14:0}	-	-	-	-	1.1	1.9	-	1.0	-	-	-	-	-	-
C _{16:0}	3.7	1.5	6.0	1.4	8.0	10.8	-	8.6	1.6	-	3.1	-	2.9	1.1
C _{17:0} cyclo	-	-	1.0	-	7.2	-	-	6.2	1.9	-	-	-	3.2	-
iso-C _{10:0}	-	-	-	-	-	-	-	-	-	-	-	1.1	-	1.0
iso-C _{11:0}	1.1	5.9	3.8	3.7	3.1	2.3	4.1	3.4	5.7	9.5	4.3	5.3	1.8	6.4
iso-C _{12:0}	-	-	-	2.0	-	-	-	-	-	-	1.1	1.1	-	1.3
iso-C _{14:0}	-	3.7	-	11.2	1.3	-	4.2	1.4	2.3	3.3	4.5	4.0	-	8.7
iso-C _{15:0}	43.4	19.6	25.2	13.1	24.9	23.3	21.9	20.5	33.6	23.0	14.5	12.5	40.9	12.7
iso-C _{16:0}	7.5	23.5	5.7	33.7	10.3	-	23.3	13.8	20.4	32.5	27.5	26.3	19.3	23.7
iso-C _{17:0}	10.3	2.3	7.8	-	3.4	3.7	1.3	2.9	4.1	2.8	1.9	1.8	11.1	1.6
anteiso-C _{15:0}	2.1	2.6	5.5	3.2	3.8	-	3.8	3.8	1.2	-	5.1	-	-	5.9
anteiso-C _{17:0}	-	-	1.4	-	-	-	-	-	-	-	1.1	-	-	-
C _{10:0} 3-OH	-	-	-	-	-	-	-	1.1	-	-	-	-	-	-
iso-C _{11:0} 3-OH	3.5	7.2	9.7	6.0	8.0	3.8	8.0	6.6	6.9	15.5	5.5	9.0	7.2	9.3
iso-C _{12:0} 3-OH	-	-	-	-	-	-	-	-	-	-	1.0	-	-	-
iso-C _{15:1} AT5	-	-	1.7	-	1.0	-	1.6	-	-	-	3.1	4.4	-	3.4
iso-C _{15:1} F	1.5	1.7	-	3.2	-	-	-	-	3.2	-	-	-	-	-
iso-C _{16:1} H	-	1.5	-	2.6	-	-	1.3	-	-	-	1.1	2.1	-	1.0
C _{16:1} ω11c	-	-	4.5	-	4.1	2.2	-	-	-	-	2.2	-	-	1.0
C _{16:1} ω7c alcohol	-	-	1.7	-	1.6	-	4.5	-	-	-	8.8	10.8	-	7.8
iso-C _{17:1} ω9c	22.0	15.5	12.2	6.7	6.4	-	10.9	4.7	15.1	13.2	6.7	16.7	5.8	10.0
C _{18:1} ω7c	-	-	2.5	-	1.7	6.5	-	3.3	-	-	-	-	-	-
Summed feature 4 ^a	4.8	9.5	6.4	6.1	8.3	20.4	6.5	15.8	-	-	3.3	1.4	-	2.0
ECL 11.799 ^b	-	-	1.8	-	2.0	-	1.4	1.5	-	-	-	-	-	-

^a Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained iso-C_{15:0} 2-OH and/or C_{16:1} ω7c.

^b The unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified.

PCR amplification, 16S rRNA gene sequencing and phylogenetic analysis

For phylogenetic analysis of the isolated strains, genomic DNA was extracted using a commercial genomic DNA extraction kit (Core Biosystem, Korea) 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). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed by CLUSTAL X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic trees were constructed by using the neighbour-joining (Saitou and Nei, 1987) and the maximum-parsimony (Fitch, 1971) methods with the MEGA3 Program

(Kumar *et al.*, 2004) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Determination of DNA G+C content

For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain Dae08^T was extracted and purified as described by Moore and Dowhan (1995) and enzymatically degraded into nucleosides. The G+C content was then determined as described by Mesbah *et al.* (1989), using reverse-phase HPLC.

Isoprenoid quinones, cellular fatty acids, and polar lipids

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum and re-extracted in *n*-hexane-water (1:1, v/v). The crude quinone in *n*-hexane solution was purified using Sep-Pak Vac Cartridges Silica

(Waters) and subsequently analyzed by HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for strains grown on R2A agar (Difco) for 48 h at 28°C. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analyzed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). The value range was obtained by duplicate experiments. Polar lipids were extracted and examined by two-dimensional TLC according to Minnikin *et al.* (1984).

DNA-DNA hybridization

DNA-DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes (Sigma) and microdilution wells (Greiner), with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as DNA-DNA hybridization values.

Results and Discussion

Morphological and phenotypic characteristics

Cells of strain Dae08^T were aerobic, Gram-negative, rod-shaped, oxidase-positive, and catalase-negative. The isolate grew on nutrient agar and TSA (Difco) but not on MacConkey agar. Strain Dae08^T was able to grow at 20–30°C. The isolate hydrolyzes gelatin, indicating proteolytic activity; this was also observed for all the type strains of the recognized *Lysobacter* species. The capability to degrade chitin and starch that is present in some *Lysobacter* species (Christensen and Cook, 1978; Weon *et al.*, 2006, 2007) was not observed in our strain. As some other recognized *Lysobacter* species such as *L. brunescens*, *L. niastensis*, *L. niabensis*, *L. spongicola*, and *L. yangpyeongensis*, strain Dae08^T was unable to utilize a number of organic acids, sugars and amino acids. Phenotypic and chemotaxonomic characteristics that differentiate strain Dae08^T from other *Lysobacter* species are listed in Table 1. In particular, it can be readily differentiated from most *Lysobacter* species by the absence of growth at 15 and 37°C and negative catalase activity.

Cellular fatty acid, quinone, and polar lipid compositions

The fatty acid profiles of strain Dae08^T is shown in Table 2 and was compared with those of the type strains of recognized *Lysobacter* species. It was characterized by the predominance of branched fatty acids iso-C_{15:0}, iso-C_{17:1} ω9c, iso-C_{17:0}, iso-C_{16:0}, and iso-C_{11:0} 3-OH, which is 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 Dae08^T and the phylogenetically closest relatives. In particular, our strain differed from other *Lysobacter* species by higher content of iso-C_{15:0}, iso-C_{17:1} ω9c, and iso-C_{17:0} and lower amount of iso-C_{11:0} 3-OH and iso-C_{11:0}. Strain Dae08^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; Romanenko *et al.*, 2007; Yassin *et al.*, 2007; Park *et al.*, 2008). Strain Dae08^T produced large amounts of diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol, and smaller amounts of phosphatidyl-N-methylethanolamine and unknown aminolipid showing the same chromatographic behaviour as one of aminolipid AL1 detected in other *Lysobacter* species (Park *et al.*, 2008). Aminolipid profile of strain Dae08^T further supported its affiliation to the genus *Lysobacter*.

DNA G+C content

The genomic DNA G+C content of strain Dae08^T was 69.3 mol%, which lies near the range observed for members of the genus *Lysobacter* (61.7–69.2 mol%).

Phylogenetic analysis

The 16S rRNA gene sequence of strain Dae08^T was continuous stretches of 1,452 bp. Comparative 16S rRNA gene sequence analyses showed that strain Dae08^T is phylogenetically affiliated to *Lysobacter* species. The phylogenetic tree (Fig. 1) showed that strain Dae08^T appeared within the genus *Lysobacter* of the *Gammaproteobacteria*, joining *Lysobacter brunescens* ATCC 29482^T with a bootstrap re-sampling value of 97%, which was supported by the neighbour-joining and maximum-parsimony methods employed. On the basis of 16S rRNA gene sequence similarity data, strain Dae08^T was closely related to *Lysobacter brunescens* ATCC 29482^T (97.3%), *Lysobacter gummosus* ATCC 29489^T (96.2%), and *Lysobacter daejeonensis* KACC 11406^T (96.1%). The phylogenetic distances from other species of the genus *Lysobacter* with validly published names were greater than 4.0% (i.e. sequence similarities were less than 96.0%). Generally recommended and accepted criteria for delineating bacterial species state that strains with a DNA-DNA relatedness below 70%, as measured by hybridization, or with 16S rDNA gene sequence dissimilarity above 3% are considered as belonging to separate species (Wayne *et al.*, 1987; Stackebrandt and Goebel, 1994). Taking into account this definition, the above mentioned data indicate that strain Dae08^T has high enough probability to be novel species of the genus *Lysobacter*. To further verify the taxonomic position of strain Dae08^T, DNA-DNA hybridization was performed with the closest member of the genus *Lysobacter*.

DNA-DNA hybridization

DNA-DNA relatedness value of strain Dae08^T to *Lysobacter brunescens* ATCC 29482^T was 28%, which is low enough (Wayne *et al.*, 1987; Stackebrandt and Goebel, 1994) to assign strain Dae08^T as a novel species of genus *Lysobacter*.

Taxonomic conclusions

The phenotypic and phylogenetic characterizations indicated that strain Dae08^T belongs to the genus *Lysobacter*. The phylogenetic distinctiveness, together with DNA-DNA hybridization data, confirmed that our strain represent the species that is distinct from recognized *Lysobacter* species. There are some phenotypic differences between strain Dae08^T and phylogenetically related *Lysobacter* species

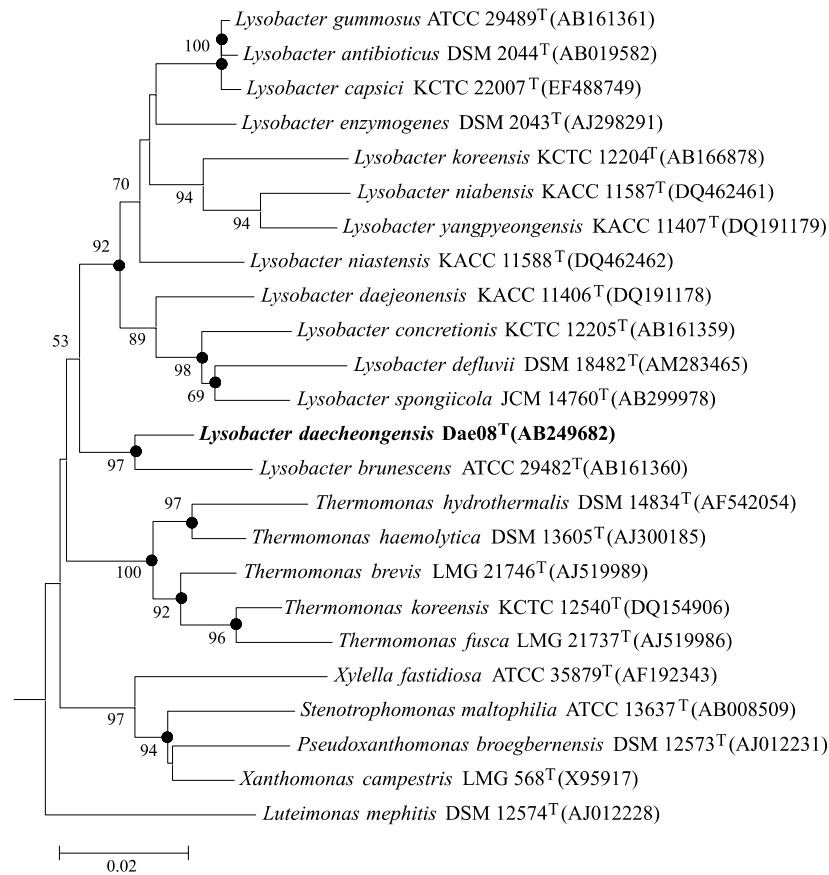


Fig. 1. Neighbor-joining tree showing the phylogenetic position of strain Dae08^T among neighboring species selected from the class *Gammaproteobacteria* on the basis of 16S rDNA gene sequences. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are shown at branch points. *Escherichia coli* ATCC 11775^T was used as an outgroup (not shown). Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithms. Bar, 0.02 substitutions per nucleotide position.

(Table 1). Therefore, on the basis of the data presented, strain Dae08^T should be classified within the genus *Lysobacter* as the type strains of novel species, for which the name *Lysobacter daecheongensis* sp. nov., is proposed.

Description of *Lysobacter daecheongensis* sp. nov.

Lysobacter daecheongensis (*dae.che.ong.en'sis*. N.L. msc. adj. *daecheongensis* referring to Lake Daecheong, from where the type strain was recovered)

Cells are Gram-negative, aerobic, rod-shaped, non-spore forming, and non-motile but having gliding activity, with various sizes (0.7~1.0 by 1.0~5.0 µm) after grown on R2A agar plate (Difco) at 25°C for 6 days. The colonies grown on R2A agar plate (Difco) for three days are 2~4 mm in diameter, smooth, circular, non-glossy, and cream colored. Nitrate is not reduced to nitrite and nitrite is not reduced to nitrogen gas. Growth occurs at 20~30°C. The bacterium grows within pH values of between 5.0 and 8.5; the optimum pH is 6.5~7.0. Growth occurs on nutrient agar and TSA but not on MacConkey agar. Strain hydrolyzes casein, but not chitin, starch, cellulose, xylan, lipids, and DNA. The isolate assimilates D-galactose, L-tyrosine, malate (w), and valerate (w). The following substrates are not utilized for

growth as sole carbon source: D-glucose, D-mannose, D-fructose, D-arabinose, L-arabinose, D-fucose, L-rhamnose, L-sorbose, D-xylose, D-ribose, D-xylose, L-xylose, *p*-nitrophenyl-β-D-galactopyranoside, *N*-acetyl-D-glucosamine, salicin, D-cellobiose, D-lactose, D-maltose, D-melibiose, D-sucrose, D-trehalose, D-raffinose, amygdalin, inulin, dextran, pyruvate, formate, acetate, propionate, DL-3-hydroxybutyrate, caprate, maleic acid, fumarate, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, citrate, lactate, malonate, succinate, glutarate, tartrate, itaconate, adipate, suberate, oxalate, gluconate, dulcitol, inositol, D-adonitol, D-mannitol, D-sorbitol, xylitol, methanol, ethanol, glycerol, glycogen, urea, L-alanine, L-arginine, L-asparagine, L-aspartate, L-cysteine, L-glutamate, L-glutamine, L-histidine, glycine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophane, and L-valine. Gelatinase and tryptophane deaminase are positive; the Voges-Proskauer test, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, β-galactosidase, hydrogen sulphide, and indole production are all negative. Acid is not produced from D-glucose, D-melibiose, amygdalin, L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose, and D-sucrose. Q-8 is the predominant quinone. The

major fatty acids are iso-C_{15:0}, iso-C_{17:1} ω_{9c}, iso-C_{17:0}, and iso-C_{16:0}. The G+C content of genomic DNA is 69.3 mol%. The polar lipids detected are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl-*N*-methylethanolamine, and unknown aminolipid.

The type strain, Dae08^T (= KCTC 12600^T) was isolated from a sediment of stream near the Daechung dam, South Korea

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Applications Center Program, Ministry of Science and Technology (Grant MG05-0101-4-0), Republic of Korea.

References

- Atlas, R.M. 1993. Handbook of Microbiological Media. CRC Press, Boca Raton, Florida, USA.
- Bae, H.S., W.T. Im, and S.T. Lee. 2005. *Lysobacter concretionis* sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. *Int. J. Syst. Bacteriol.* 55, 1155-1161.
- Buck, J.D. 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* 44, 992-993.
- Cappuccino, J.G. and N. Sherman. 2002. Microbiology: a laboratory manual, 6th ed. Pearson Education, Inc., California, USA.
- Christensen, P. and F.D. Cook. 1978. *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int. J. Syst. Bacteriol.* 28, 367-393.
- Ezaki, T., Y. Hashimoto, and E. Yabuuchi. 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution 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 limit 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.
- Hiraishi, A., Y. Ueda, J. Ishihara, and T. Mori. 1996. Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J. Gen. Appl. Microbiol.* 42, 457-469.
- Kim, M.K., W.T. Im, H. Ohta, M. Lee, and S.T. Lee. 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, Cambridge, New York, N.Y., USA.
- Kouker, G. and K.E. Jaeger. 1987. Specific and sensitive plate assay for bacterial lipases. *Appl. Environ. Microbiol.* 53, 211-213.
- Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform.* 5, 150-163.
- Lee, J.W., W.T. Im, M.K. Kim, and D.C. Yang. 2006. *Lysobacter ko-reensis* sp. nov., isolated from a ginseng field. *Int. J. Syst. Evol. Microbiol.* 56, 231-235.
- Mesbah, M., U. Premachandran, and W. Whitman. 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., A.G. O'Donnell, M. Goodfellow, G. Alderson, M. Athalye, K. Schaal, and J.H. Parlett. 1984. An integrated procedure for the extraction of isoprenoid quinones and polar lipids. *J. Microbiol. Methods* 2, 233-241.
- Moore, D.D. and D. Dowhan. 1995. Preparation and analysis of DNA, p. 2-11. In F.W. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl (eds.), Current Protocols in Molecular Biology. Wiley, New York, N.Y., USA.
- Park, J.H., R. Kim, Z. Aslam, C.O. Jeon, and Y.R. Chung. 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.
- Romanenko, L.A., M. Uchino, N. Tanaka, G.M. Frolova, and V.V. Mikhailov. 2007. *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int. J. Syst. Evol. Microbiol.* 58, 370-374.
- Saitou, N. and M. Nei. 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. DE: MIDI Inc., Newark, USA.
- Stackebrandt, E. and B.M. Goebel. 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.
- Ten, L.N., W.T. Im, M.K. Kim, M.S. Kang, and S.T. Lee. 2004. Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J. Microbiol. Methods* 56, 375-382.
- Ten, L.N., Q.M. Liu, W.T. Im, M. Lee, D.C. Yang, and S.T. Lee. 2006. *Pedobacter ginsengisoli* sp. nov., a DNase producing bacterium isolated from soil of a ginseng field in South Korea. *Int. J. Syst. Evol. Microbiol.* 56, 2565-2570.
- Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876-4882.
- Wayne, L.G., D.J. Brenner, R.R. Colwell, P.A.D. Grimont, O. Kandler, M.I. Krichevsky, L.H. Moore, W.E.C. Moore, R.G.E. Murray, E. Stackebrandt, M.P. Starr, and H.G. Truper. 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., B.Y. Kim, Y.K. Baek, S.H. Yoo, S.W. Kwon, E. Stackebrandt, and S.J. Go. 2006. Two novel species, *Lysobacter daejeonensis* sp. nov. and *Lysobacter yangpyeongensis* sp. nov., isolated from Korean greenhouse soils. *Int. J. Syst. Evol. Microbiol.* 56, 947-951.
- Weon, H.Y., B.Y. Kim, M.K. Kim, S.H. Yoo, S.W. Kwon, S.J. Go, and E. Stackebrandt. 2007. *Lysobacter niabensis* sp. nov. and *Lysobacter niastensis* sp. nov., isolated from greenhouse soils in Korea. *Int. J. Syst. Evol. Microbiol.* 57, 548-551.
- Yassin, A.F., W.M. Chen, H. Hupfer, C. Siering, R.M. Kroppenstedt, A.B. Arun, W.A. Lai, F.T. Shen, P.D. Rekha, and C.C. Young. 2007. *Lysobacter defluvii* sp. nov., isolated from municipal solid waste. *Int. J. Syst. Evol. Microbiol.* 57, 1131-1136.