Lysobacter koreensis sp. nov., isolated from a ginseng field

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

DOI: 10.1099/ijs.0.63955-0 · Source: PubMed

4 authors, including:

Wan Taek Im
Hankyong National University
292 PUBLICATIONS **6,399** CITATIONS

SEE PROFILE

Deok-Chun Yang
Kyung Hee University
755 PUBLICATIONS **17,582** CITATIONS

SEE PROFILE

Lysobacter koreensis sp. nov., isolated from a ginseng field

Jun Won Lee, Wan-Taek Im, Myung Kyum Kim and Deok-Chun Yang

Department of Oriental Medicinal Material and Processing, College of Life Science, Kyung Hee University, 1 Seocheon, Kihung Yongin, Kyunggi 449-701, South Korea

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

Strain Dae16^T, a Gram-negative, non-spore-forming, rod-shaped bacterium, was isolated from the soil of a ginseng field in South Korea and characterized in order to determine its taxonomic position. 16S rRNA gene sequence analysis revealed that strain Dae16^T belongs to the *Gammaproteobacteria* and had the highest degree of sequence similarity to *Lysobacter gummosus* ATCC 29489^T (97·1 %), *Lysobacter antibioticus* DSM 2044^T (96·6 %), *Lysobacter enzymogenes* DSM 2043^T (96·2 %), *Lysobacter concretionis* KCTC 12205^T (94·7 %) and *Lysobacter brunescens* ATCC 29482^T (93·7 %). Chemotaxonomic data revealed that strain Dae16^T possesses a quinone system with Q-8 as the predominant compound and $C_{15:0}$ iso, $C_{16:0}$ iso and $C_{17:1}$ iso ω9*c* as the predominant iso-branched fatty acids, all of which corroborated the assignment of the strain to the genus *Lysobacter*. Results of DNA–DNA hybridization and physiological and biochemical tests clearly demonstrated that strain Dae16^T represents a distinct species. Based on these data, it is proposed that Dae16^T (=KCTC 12204^T=NBRC 101156^T) should be classified as the type strain of a novel *Lysobacter* species, *Lysobacter koreensis* sp. nov.

The genus *Lysobacter* was established by Christensen & Cook (1978) for gliding bacteria with high G+C contents that do not produce fruiting bodies, with *Lysobacter enzymogenes* as the type species. At the time of writing, the genus *Lysobacter* comprises *Lysobacter antibioticus*, *L. brunescens*, *L. concretionis*, *L. enzymogenes* and *L. gummosus*. Except for *L. concretionis* (Bae *et al.*, 2005), these species were proposed by Christensen & Cook (1978) on the basis of phenotypic characteristics; their taxonomic positions were confirmed by phylogenetic and chemotaxonomic features (Bae *et al.*, 2005). The names of two subspecies of *L. enzymogenes* proposed by Christensen & Cook (1978), *L. enzymogenes* subsp. *cookii* and *L. enzymogenes* subsp. *enzymogenes*, were inadvertently omitted from the Approved Lists and these names are therefore not validly published.

In a series of studies, micro-organisms have been isolated from a ginseng field in order to investigate the community structure based on a culture-dependent method. In this study, one strain was isolated from the soil of a ginseng field in Daejeon city, South Korea, and characterized by a polyphasic approach. The polyphasic approach, including phylogenetic analysis based on 16S rRNA gene sequences, genomic relatedness, and chemotaxonomic and phenotypic properties, was conducted to determine the precise taxonomic position of strain Dae16^T. The results obtained in this study indicated that Dae16^T is a member of the genus *Lysobacter*, but it is clearly distinguishable from all *Lysobacter* species. Here, it is proposed that Dae16^T should be classified as the type strain of a novel species.

Strain Dae16^T was isolated from soil of a ginseng field near Daechung lake via direct plating onto R2A agar (Difco). Single colonies on these plates were purified by transferring them onto new plates and subjecting them to an additional incubation for 3 days at 30 °C. Purified colonies were tentatively identified using partial 16S rRNA gene sequences.

Cell morphology and motility were observed with a Nikon light microscope (1000× magnification), with the cells being allowed to grow for 3 days at 30 °C on R2A agar. Gram reactions were determined according to the non-staining method, as described by Buck (1982). Oxidase activity was evaluated via the oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined by measurements of bubble production after the application of 3 % (v/v) hydrogen peroxide solution. Acid production from carbohydrates was assessed using procedures outlined by Cappuccino & Sherman (2002). Growth at various temperatures (4, 15, 25, 30, 37 and 42 °C) was assessed on R2A agar and growth at various pH values was assessed on R2A broth. Growth on nutrient agar, trypticase soy agar (TSA) and MacConkey agar was also evaluated at 30 °C. The API 20NE and API ID32 GN microtest systems were

Published online ahead of print on 4 November 2005 as DOI 10.1099/ijs.0.63955-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Dae16^T is AB166878.

employed in these tests, according to the recommendations of the manufacturer (bioMérieux).

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

The genomic DNA of strain Dae16^T was extracted and purified with the QIAGEN Genomic-tip system 100/G; it was then enzymically degraded into nucleosides, as described previously (Tamaoka & Komagata, 1984; 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. Hybridization was conducted 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 relatedness values.

Genomic DNA was extracted and purified with the Genomic DNA Isolation kit (Core Bio System). The 16S rRNA gene was amplified from chromosomal DNA of strain Dae16^T using the universal bacterial primer set 9F and 1512R (Weisburg *et al.*, 1991) and the purified PCR products were sequenced by Genotec (Daejeon, Korea) (Kim *et al.*, 2005). The full sequence of the 16S rRNA gene was compiled with SeqMan software and the 16S rRNA gene sequences of the test strain were edited using the program BioEdit (Hall, 1999). The 16S rRNA gene sequences of related taxa were obtained from GenBank/EMBL. Multiple alignments were performed with the program CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed via the neighbour-joining method (Saitou & Nei, 1987) in the program MEGA2 (Kumar *et al.*, 2001). Bootstrap analysis with 1000 replicates was also conducted in order to obtain confidence levels for the

Table 1. Differential phenotypic characteristics between strain Dae16^T and related *Lysobacter* species

Strains: 1, *L. koreensis* Dae16^T; 2, *L. antibioticus* DSM 2044^T; 3, *L. brunescens* ATCC 29482^T; 4, *L. concretionis* KCTC 12205^T; 5, *L. enzymogenes* DSM 2043^T; 6, *L. gummosus* ATCC 29489^T. Data for related taxa were taken from Bae *et al.* (2005) except results in parentheses, which were taken from Christensen & Cook (1978). All strains could liquefy gelatin. +, Positive; −, negative.

Characteristic	1	2	3	4	5	6
Cell size (μm)	0·5–0·8 × 1·5–2·0	0·4 × 6·5	0·3 × 11·0	0·7 × 1·0–13·5	0·5 × 38·0	0·4 × 2·0
$NO_3^- \rightarrow NO_2^-$/$NO_2^- \rightarrow N_2$	−	−/− (+/−)	−/−	−/−	−/−	−/−
Aesculin hydrolysis	−	+	+	−	+	+
Indole production	−	−	+	−	−	−
Glucose acidification	−	+	−	−	+	−
Arginine dihydrolase	−	−	+	−	−	−
Urease	−	−	+	−	−	−
β-Galactosidase	−	+	−	−	+	+
Growth on:						
3-Hydroxybutyrate	−	+	−	+	+	+
Acetate	−	−	+	+	−	+
Arabinose	+	−	−	−	−	+
Citrate	+	−	−	−	+	+
D-Glucose	−	−	−	−	+	+
D-Melibiose	−	−	−	−	+	+
Glycogen	−	+	−	+	+	+
Histidine	−	−	−	−	−	+
L-Proline	−	+	−	+	+	+
L-Serine	+	−	−	−	+	−
Malate	−	−	−	−	+	−
Maltose	−	+	−	−	+	+
Mannose	−	+	−	−	+	−
N-Acetylglucosamine	−	−	−	−	+	+
Salicin	−	−	−	−	+	−
Valerate	+	+	−	+	−	+
DNA G+C content (mol%)	68·9	69·2	67·7	63·8	69	65·7

branches (Felsenstein, 1985). All of the species in the genus *Lysobacter* were included in the phylogenetic tree.

Strain Dae16^T was cultured on R2A agar (Difco) at 30 °C, yielding colonies that were yellow-coloured, circular and glossy in appearance. Strain Dae16^T was an aerobic, Gram-negative, non-motile, rod-shaped bacterium. Strain Dae16^T was also able to grow at 20–30 °C, but did not grow at 4 or 37 °C. Growth at 30 °C was not observed on nutrient agar or TSA. The physiological characteristics of strain Dae16^T are summarized in the species description and a comparison of selective characteristics with related type strains is shown in Table 1.

The cellular fatty acid profiles of strain Dae16^T and related *Lysobacter* type strains are shown in Table 2. The major cellular fatty acids in strain Dae16^T included iso-hexadecanoic acid ($C_{16:0}$ iso, 33·0 %), iso-pentadecanoic acid ($C_{15:0}$ iso, 17·0 %) and iso-heptadecenoic acid ($C_{17:1}$ iso ω9*c*, 19·9 %). Minor amounts of the iso-branched fatty acids $C_{11:0}$ iso (3·5 %), $C_{14:0}$ iso (2·7 %), $C_{15:0}$ iso AT5 (2·7 %) and $C_{17:0}$ iso (2·5 %) were present and minor amounts of the hydroxy fatty acids $C_{11:0}$ iso 3-OH (5·6 %) and $C_{16:1}$ ω7*c* alcohol (4·1 %) were also found. The presence of $C_{15:0}$ iso, $C_{16:0}$ iso and $C_{17:1}$ iso ω9*c* as the major fatty acids is a characteristic composition of genera in the *Xanthomonas* branch containing the genera *Xanthomonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Xylella* and *Luteimonas* (Assih *et al.*, 2002; Roumagnac *et al.*, 2004; Yang *et al.*, 2005). Significant differences in fatty acid profiles were found between different species in the genus *Lysobacter*.

Q-8 was the predominant ubiquinone of strain Dae16^T. The quinone system supported our assignment of strain Dae16^T to the *Xanthomonas* branch, in which the majority of species (including *Lysobacter* species) also have Q-8 as the predominant quinone.

The 16S rRNA gene sequence of strain Dae16^T was a continuous stretch of 1474 nt. The 16S rRNA gene sequences of related taxa were obtained from GenBank/EMBL. Strain Dae16^T belonged to the *Gammaproteobacteria* and had the highest degree of sequence similarity to *L. gummosus* ATCC 29489^T (97·1 %), *L. antibioticus* DSM 2044^T (96·6 %), *L. enzymogenes* DSM 2043^T (96·2 %), *L. concretionis* KCTC 12205^T (94·7 %) and *L. brunescens* ATCC 29482^T (93·7 %). In the phylogenetic tree (Fig. 1), strain Dae16^T clearly belonged to the *Lysobacter* lineage, as shown by the high bootstrap value.

Table 2. Cellular fatty acid composition (%) of strain Dae16^T and related *Lysobacter* species

Strains: 1, *L. koreensis* Dae16^T; 2, *L. antibioticus* DSM 2044^T; 3, *L. brunescens* ATCC 29482^T; 4, *L. concretionis* KCTC 12205^T; 5, *L. enzymogenes* DSM 2043^T; 6, *L. gummosus* ATCC 29489^T. –, Not detected. Data for related taxa were taken from Bae *et al.* (2005). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. Summed feature 4 contains $C_{15:0}$ iso 2-OH and/or $C_{16:1}\omega7c$ and summed feature 7 contains $C_{18:1}\omega7c/\omega9t/\omega12t$ and/or $C_{18:1}\omega7c/\omega9c/\omega12t$, which could not be separated by GLC with the Microbial Identification System (MIDI).

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

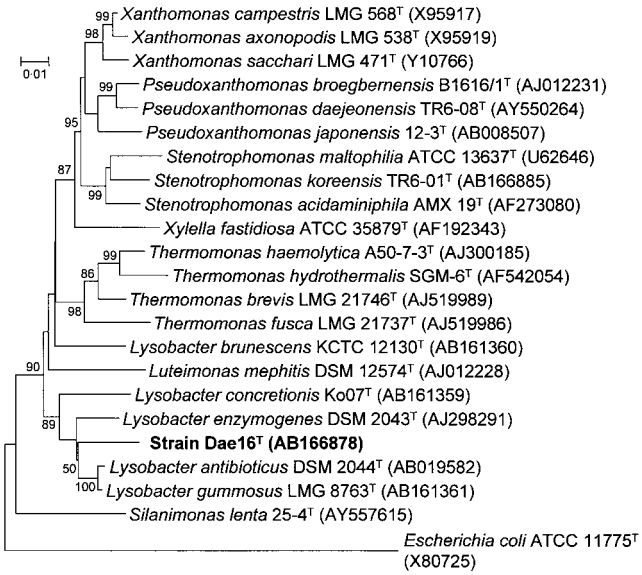

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain Dae16^T and related species (all *Lysobacter* species and related genera in the *Xanthomonas* branch). The neighbour-joining method was used. Bar, 0·01 substitutions per nucleotide position.

The G+C content of the genomic DNA of strain Dae16^T was 68·9±0·3 mol%. Strain Dae16^T exhibited relatively low levels of DNA–DNA relatedness to the type strains *L. gummosus* ATCC 29489^T (55·5 %), *L. antibioticus* DSM 2044^T (45·9 %), *L. enzymogenes* DSM 2043^T (49·0 %) and *L. concretionis* KCTC 12205^T (16·6 %). The DNA–DNA hybridization level was less than 70 % (Stackebrandt & Goebel, 1994), which is the threshold value that delineates a genomic species. Our results support the designation of strain Dae16^T as a representative of a separate, novel species within the genus *Lysobacter*, for which the name *Lysobacter koreensis* sp. nov. is proposed.

Description of *Lysobacter koreensis* sp. nov.

Lysobacter koreensis sp. nov. (ko.re.en′sis. N.L. masc. adj. *koreensis* pertaining to Korea, the location of the soil sample from which the type strain was isolated).

Gram-negative, aerobic rods (1·5–2·0×0·5–0·8 μm) after growth on R2A agar (Difco) at 25 °C for 10 days. Does not move by flagella. Colonies grown on R2A agar for 2 days are yellow-coloured, glossy circles. Optimal temperature and pH for growth are 30 °C and pH 6·8–8·0. Growth can occur in a salt concentration of 1 %, but not above 2 %. Catalase-positive and oxidase-negative. Produces protease, but does not produce arginine dihydrolase, urease, β-glucosidase or β-galactosidase. Assimilates 3-hydroxybenzoate, citrate, D-mannitol, D-sorbitol, L-arabinose, L-rhamnose, L-serine, propionate and valerate. Does not assimilate 2-ketogluconate, 3-hydroxybutyrate, 4-hydroxybenzoate, 5-ketogluconate, acetate, adipate, caprate, D-glucose, D-maltose, D-mannose, D-melibiose, D-ribose, sucrose, gluconate, glycogen, itaconate, L-alanine, L-fucose, L-histidine, L-proline, lactate, malate, malonate, *myo*-inositol, *N*-acetylglucosamine, phenylacetate, salicin or suberate. Does not produce any biopolymer-hydrolysing enzymes, e.g. amylase, cellulase, chitinase, DNase, lipase, protease or xylanase. DNA G+C content of the type strain is 68·9 mol%, as determined by HPLC. Q-8 is the predominant quinone. The major cellular fatty acids are $C_{16:0}$ iso (33·0 %), $C_{15:0}$ iso, (17·0 %) and $C_{17:1}$ iso $\omega 9c$ (19·9 %). Minor fatty acids are $C_{11:0}$ iso (3·5 %) and $C_{11:0}$ iso 3-OH. Does not reduce nitrate. Can liquefy gelatin. Other phenotypic characteristics, such as substrate utilization and enzyme production, are summarized in Table 1.

The type strain is Dae16^T (=KCTC 12204^T=NBRC 101156^T), isolated from soil of a ginseng field in Daejeon, South Korea.

Acknowledgements

This study was carried out with the support of 'Specific Joint Agricultural Research-promoting Projects', RDA, Republic of Korea.

References

Assih, E. A., Ouattara, A. S., Thierry, S., Cayol, J.-L., Labat, M. & Macarie, H. (2002). *Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. *Int J Syst Evol Microbiol* **52**, 559–568.

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.

Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual*, 6th edn. San Francisco: Benjamin Cummings.

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 implication. *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.

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., Jakobsen, I.-B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.

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.

Roumagnac, P., Gagnevin, L., Gardan, L., Sutra, L., Manceau, C., Dickstein, E. R., Jones, J. B., Rott, P. & Pruvost, O. (2004). Polyphasic characterization of xanthomonads isolated from onion, garlic and Welsh onion (*Allium* spp.) and their relatedness to different *Xanthomonas* species. *Int J Syst Evol Microbiol* **54**, 15–24.

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*. Technical Note 101. Newark, DE: MIDI.

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.

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.

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

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.

Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.

Yang, D.-C., Im, W.-T., Kim, M. K. & Lee, S.-T. (2005). *Pseudoxanthomonas koreensis* sp. nov. and *Pseudoxanthomonas daejeonensis* sp. nov. *Int J Syst Evol Microbiol* **55**, 787–791.