Lysobacter dokdonensis sp. nov., isolated from soil

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A Gram-negative, non-motile, rod-shaped bacterial strain, DS-58^T, was isolated from a soil sample from Dokdo, an island of Korea, and its taxonomic position was investigated. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-58^T fell within the family *Xanthomonadaceae*. The isolate showed 96.9 % 16S rRNA gene sequence similarity with its closest phylogenetic neighbour, *Lysobacter niastensis* GH41-7^T, and 93.4–95.7 % 16S rRNA gene sequence similarity with other members of the genus *Lysobacter*. Strain DS-58^T contained Q-8 as the predominant ubiquinone and iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega 9c$ as the major fatty acids. The DNA G+C content was 68.1 mol%. Strain DS-58^T could be distinguished phenotypically from type strains of closely related species of the genus *Lysobacter* and phylogenetically from all members of the genus *Lysobacter*. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain DS-58^T is considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter dokdonensis* sp. nov. is proposed. The type strain is DS-58^T (=KCTC 12822^T =DSM 17958^T).

The genus *Lysobacter* was first proposed by Christensen & Cook (1978). At the time of writing, the genus comprises at least 16 species with validly published names (Euzéby, 1997): *Lysobacter enzymogenes*, *L. antibioticus*, *L. brunescens* and *L. gummosus* (Christensen & Cook, 1978), *L. concretionis* (Bae *et al.*, 2005), *L. koreensis* (Lee *et al.*, 2006), *L. daejeonensis* and *L. yangpyeongensis* (Weon *et al.*, 2006), *L. niabensis* and *L. niastensis* (Weon *et al.*, 2007), *L. defluvii* (Yassin *et al.*, 2007), *L. spongiicola* (Romanenko *et al.*, 2008), *L. capsici* (Park *et al.*, 2008), *L. oryzae* (Aslam *et al.*, 2009), *L. ximonensis* (Wang *et al.*, 2009) and *L. panaciterrae* (Ten *et al.*, 2009). Phylogenetic analyses based on 16S rRNA gene sequences have shown that the genus *Lysobacter* forms an evolutionary lineage within the *Gammaproteobacteria* (Bae *et al.*, 2005; Lee *et al.*, 2006; Yassin *et al.*, 2007; Park *et al.*, 2008).

Strain DS-58^T was isolated from a soil sample collected from Dokdo, an island of Korea. The organism was isolated on 10-fold-diluted nutrient agar (NA; Difco) after incubation at 25 °C for 7 days. The type strains of seven *Lysobacter* species were obtained from the Korean Agricultural Culture Collection (KACC), Suwon, Korea, and used as reference strains for substrate assimilation tests and fatty acid analysis: *L. antibioticus* KACC 11383^T, *L. brunescens* KACC 11385^T, *L. capsici* KACC 14554^T, *L. enzymogenes* KACC 11382^T, *L. gummosus* KACC 11386^T, *L. niastensis* KACC 11588^T and *L. ximonensis* KACC 14084^T. The morphological, physiological and biochemical characteristics of strain DS-58^T were investigated using routine cultivation on NA at 30 °C. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). Flagellation was determined with cells from exponentially growing cultures. For this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and air dried before examination. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at 4, 10, 20, 25, 28, 30, 35, 37, 38, 39 and 40 °C was measured on NA. Growth at pH 4.5–9.5 (at intervals of 0.5 pH units) was determined in nutrient broth (NB; Difco) with the pH adjusted using sodium acetate/acetic acid or Na_2CO_3 buffers. Growth with 0, 0.5 and 1.0–4.0 % (at intervals of 1 %) (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. Growth under anaerobic conditions was determined by incubation in a Forma 1029 anaerobic chamber on NA and on NA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities and hydrolysis of casein, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Aesculin hydrolysis and nitrate reduction were investigated as described by Lányí (1987). Sensitivity to various antibiotics was tested on NA using discs impregnated with the following antibiotics (μg per disc unless otherwise stated): polymyxin B (100 U), streptomycin (50), penicillin

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-58^T is EF100698.

A supplementary table is available with the online version of this paper.

Table 1. Differential phenotypic characteristics of strain DS-58^T and some type strains of species of the genus *Lysobacter*

Strains: 1, *Lysobacter dokdonensis* sp. nov. DS-58^T; 2, *L. antibioticus* KACC 11383^T; 3, *L. brunescens* KACC 11385^T; 4, *L. capsici* KACC 14554^T (data from Park *et al.*, 2008); 5, *L. gummosus* KACC 11386^T; 6, *L. niastensis* KACC 11588^T; 7, *L. ximonensis* KACC 14084^T. Data were obtained in this study unless otherwise stated. All strains are positive for catalase and hydrolysis of casein and gelatin. +, Positive; W, weakly positive; −, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Colony colour on R2A agar (5 days)*	LY	MY	MY	LC	YW	LB	CY
Oxidase	+	+[a]†	+[a]	+	ND	+[b]	−[c]
Nitrate reduction	−	+[a]	−[a]	ND	−[a]	+[b]	−[c]
Hydrolysis of:							
Aesculin	−	+[a]	+[a]	+	+[a]	+[b]	+[c]
Starch	−	−[a]	+[a]	−	−[a]	+[b]	+[c]
β-Galactosidase	−	+[a]	+[a]	−	+[a]	−[b]	W[c]
Assimilation of:							
α-Cyclodextrin	−	−	+	−	−	−	+
Dextrin	+	−	+	+	+	+	+
Glycogen	−	−	W	−	−	−	+
Tween 40	+	−	+	W	−	+	+
Tween 80	−	+	+	−	−	−	+
N-Acetyl-D-galactosamine	−	+	−	−	+	−	−
N-Acetyl-D-glucosamine	−	+	−	+	+	−	−
Cellobiose	−	+	−	W	+	−	+
D-Fructose	−	+	−	+	+	−	+
D-Galactose	−	+	−	+	+	−	−
Gentiobiose	−	+	−	+	+	−	−
D-Glucose	−	+	+	+	+	−	+
Lactose	−	W	−	+	−	−	−
Lactulose	−	−	−	+	+	−	−
Maltose	W	+	−	+	+	−	+
D-Mannose	−	+	−	+	+	−	+
Melibiose	−	−	−	+	+	−	−
D-Psicose	−	−	−	−	+	−	−
D-Sorbitol	−	+	−	−	−	−	−
Trehalose	−	+	−	+	+	−	−
Turanose	−	+	−	+	+	−	−
Succinic acid monomethyl ester	−	−	−	+	−	−	−
Acetic acid	−	+	−	+	−	−	−
cis-Aconitic acid	−	+	−	+	+	−	−
Citric acid	−	+	−	+	−	−	−
Formic acid	−	+	−	−	+	−	−
D-Galacturonic acid	−	−	+	−	−	−	−
β-Hydroxybutyric acid	−	+	+	+	+	+	+
p-Hydroxyphenylacetic acid	−	−	+	−	−	−	−
α-Ketobutyric acid	+	+	−	+	+	−	−
α-Ketoglutaric acid	−	+	−	+	+	−	−
α-Ketovaleric acid	−	+	−	W	+	+	−
DL-Lactic acid	−	−	+	−	−	−	−
Propionic acid	−	+	−	W	−	−	−
Quinic acid	−	−	−	−	+	−	−
Succinic acid	−	+	−	+	+	+	−
Bromosuccinic acid	−	−	−	W	+	−	−
Alaninamide	+	+	+	W	−	+	+
L-Alanine	+	+	+	W	−	−	−
L-Alanyl glycine	+	+	W	−	+	+	+
L-Asparagine	−	+	−	−	−	+	−
L-Aspartic acid	−	+	−	W	+	−	+
L-Glutamic acid	+	+	−	+	−	−	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
L-Histidine	−	+	−	−	−	−	−
Hydroxy-L-proline	−	+	−	−	+	−	−
L-Ornithine	−	+	−	−	−	+	−
L-Proline	−	+	+	W	+	+	W
L-Serine	−	−	−	+	+	−	−
L-Threonine	−	+	−	+	+	−	−
Urocanic acid	−	+	−	−	−	−	−
Putrescine	−	−	+	−	−	−	−
α-D-Glucose 1-phosphate	−	+	−	W	+	−	+
D-Glucose 6-phosphate	−	+	−	W	−	−	+
DNA G+C content (mol%)	68.1	69.2[a]	67.7[a]	65.4	65.7[a]	66.6[b]	63.5[c]

*LY, Light yellow; MY, moderate yellow; LC, light cream; YW, yellowish white; LB, light brown; CY, chrome yellow.
†Data taken from: *a*, Christensen & Cook (1978) and Weon *et al.* (2007); *b*, Weon *et al.* (2007); *c*, Wang *et al.* (2009).

G (20 U), chloramphenicol (100), ampicillin (10), cephalothin (30), gentamicin (30), novobiocin (5), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), neomycin (30) and carbenicillin (100). The biochemical properties and enzymes of strain DS-58^T were determined using the API 20 E and API ZYM systems (bioMérieux), according to the manufacturer's instructions. Utilization of carbohydrates and organic acids was determined using GN2 MicroPlates (Biolog), according to the protocol provided by the manufacturer. The morphological, cultural, physiological and biochemical characteristics of strain DS-58^T are given in the species description and Table 1.

Cell biomass for DNA extraction and isoprenoid quinone analysis was obtained from cultures grown in NB for 7 days at 30 °C. Chromosomal DNA was extracted and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The almost-complete 16S rRNA gene sequence of strain DS-58^T, comprising 1500 nt (approximately 97 % of the *Escherichia coli* 16S rRNA gene sequence), was determined in this study. In the neighbour-joining tree (Fig. 1), strain DS-58^T fell within the group comprising type strains of species of the genus *Lysobacter* and clustered with *L. niastensis* GH41-7^T, with which it showed the highest 16S rRNA gene sequence similarity (96.9 %). The relationship between strain DS-58^T and *L. niastensis* GH41-7^T was also maintained in trees constructed using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain DS-58^T exhibited 93.4–95.7 % 16S rRNA gene sequence similarity with the type strains of other species of the genus *Lysobacter*.

Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. Strain DS-58^T contained ubiquinone 8 (Q-8) as the predominant isoprenoid quinone, with a peak area ratio of approximately 95 %, which is the same as that reported earlier for other members of the genus *Lysobacter* (Bae *et al.*, 2005; Park *et al.*, 2008). For fatty acid methyl ester analysis, cell mass of strain DS-58^T and the reference strains was harvested from R2A agar (Difco) after incubation for 5 days at 30 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The cellular fatty acid profile of strain DS-58^T is shown in Table 2 together with those of the reference strains. The fatty acid profiles were essentially similar to each other, although

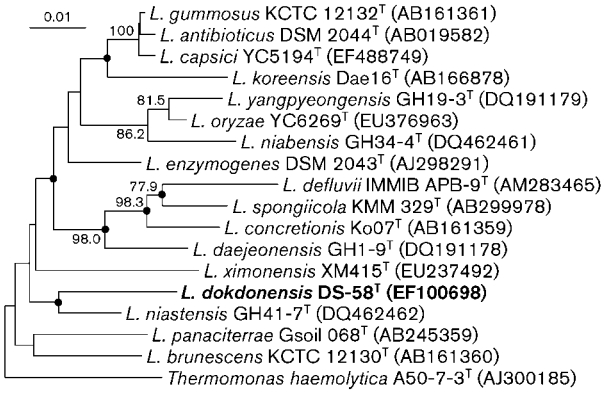

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain DS-58^T in the genus *Lysobacter*. Bootstrap values (>70 %) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. The sequence of *Thermomonas haemolytica* A50-7-3^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions of strain DS-58^T and the type strains of some species of the genus *Lysobacter*

Strains: 1, *Lysobacter dokdonensis* sp. nov. DS-58^T; 2, *L. antibioticus* KACC 11383^T; 3, *L. brunescens* KACC 11385^T; 4, *L. capsici* KACC 14554^T; 5, *L. enzymogenes* KACC 11382^T; 6, *L. gummosus* KACC 11386^T; 7, *L. niastensis* KACC 11588^T; 8, *L. ximonensis* KACC 14084^T. All data were obtained in this study; values are percentages of total fatty acids. Fatty acids that represented <0.5 % in all strains are omitted. ECL, Equivalent chain-length; tr, trace (<0.5 %); −, not detected.

Fatty acid	1	2	3	4	5	6	7	8
$C_{10:0}$	tr	tr	1.2	1.0	−	−	tr	tr
$C_{14:0}$	tr	tr	tr	0.9	0.8	0.7	0.6	0.7
$C_{15:0}$	tr	−	−	tr	tr	0.6	tr	tr
$C_{16:0}$	1.9	2.1	2.0	12.8	3.5	3.5	3.9	2.5
iso-$C_{10:0}$	0.6	−	0.8	tr	−	−	tr	0.8
iso-$C_{11:0}$	5.9	5.9	10.3	3.8	−	6.6	5.8	4.3
iso-$C_{11:0}$ 3-OH	7.8	11.6	11.5	8.0	−	10.6	7.5	5.5
iso-$C_{12:0}$ 3-OH	tr	tr	1.0	tr	−	−	tr	tr
iso-$C_{14:0}$	4.6	0.9	6.1	0.7	2.6	0.8	3.1	8.9
iso-$C_{15:1}$ F*	tr	1.0	1.1	tr	−	1.9	tr	0.9
iso-$C_{15:0}$	18.7	39.5	22.8	22.2	29.4	39.3	35.0	24.3
anteiso-$C_{15:0}$	4.9	2.2	1.9	3.9	5.5	4.2	3.4	4.2
iso-$C_{16:1}$ H*	1.1	−	2.2	−	tr	−	tr	tr
iso-$C_{16:0}$	30.5	7.3	19.7	8.7	26.1	5.8	17.4	33.1
iso-$C_{17:0}$	2.5	2.4	1.4	5.6	2.7	4.3	6.9	2.2
anteiso-$C_{17:0}$	0.6	tr	−	0.8	1.0	tr	0.6	tr
$C_{16:1}\omega7c$ alcohol	−	1.2	tr	0.6	−	1.2	tr	−
$C_{16:1}\omega11c$	−	1.0	−	2.0	−	1.3	−	−
iso-$C_{17:1}\omega9c$	14.0	10.1	9.4	5.1	5.9	10.1	6.9	8.0
$C_{18:1}\omega7c$	tr	0.6	−	1.3	1.9	−	−	−
$C_{10:0}$ 2-OH	−	−	0.8	tr	−	−	−	−
$C_{10:0}$ 3-OH	tr	tr	tr	3.7	−	0.6	tr	tr
$C_{17:0}$	−	1.9	−	10.8	10.7	2.9	−	−
Summed feature 1†	tr	tr	1.1	−	tr	−	tr	tr
Summed feature 3†	2.8	6.4	4.9	2.5	7.0	2.4	5.2	1.4
Unknown ECL 11.799	−	3.4	−	2.4	−	2.7	0.6	−

*Double-bond positions indicated by capital letters are unknown.
†Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 1 consisted of $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$. Summed feature 3 consisted of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

there were differences in the proportions of some fatty acids, particularly $C_{17:0}$ in *L. capsici* KACC 14554^T and *L. enzymogenes* KACC 11382^T. The fatty acid profiles of the reference strains were similar to those reported by Weon *et al.* (2007) and Wang *et al.* (2009). The major fatty acids (>10 % of the total) in strain DS-58^T were iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega9c$. The DNA G+C content of strain DS-58^T was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The DNA G+C content was 68.1 mol%. The chemotaxonomic analysis showed that strain DS-58^T exhibited properties of the genus *Lysobacter* and was in agreement with the result of the phylogenetic analysis, i.e. strain DS-58^T is a member of the genus *Lysobacter*.

Strain DS-58^T could be differentiated phenotypically from closely related type strains of species of the genus *Lysobacter*. This and the phylogenetic distinctiveness of strain DS-58^T were sufficient to categorize the isolate as a member of a species that is distinct from recognized *Lysobacter* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-58^T is considered to represent a novel species of the genus *Lysobacter*, for which the name *Lysobacter dokdonensis* sp. nov. is proposed.

Description of *Lysobacter dokdonensis* sp. nov.

Lysobacter dokdonensis (dok.do.nen′sis. N.L. masc. adj. *dokdonensis* of Dokdo, an island of Korea, from where the type strain was isolated).

Cells are Gram-negative, non-spore-forming and non-flagellated rods (0.4–0.8 × 1.0–5.0 μm). Colonies on NA are circular, convex, smooth, glistening, yellow and 1.0–2.0 mm in diameter after incubation for 7 days at 30 °C. Good growth is observed on R2A agar, but growth is poor on trypticase soy agar. Growth occurs at 4–38 °C (optimum 30 °C), but not at 39 °C, at pH 6.0–8.0 (optimum pH 6.5–7.5), but not at pH 5.5 or 8.5, and with 0–0.5 % (w/v) NaCl, but not with 1.0 % NaCl. Growth does not occur under anaerobic conditions on NA or NA supplemented with nitrate. Catalase- and oxidase-positive. Nitrate is not reduced. Casein, gelatin, L-tyrosine and Tweens 20, 40, 60 and 80 are hydrolysed, but aesculin, hypoxanthine, starch and xanthine are not. With API ZYM, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and acid phosphatase are present, but trypsin, α- and β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase are absent. Sensitive to (μg per disc unless otherwise stated) chloramphenicol (100), gentamicin (30), kanamycin (30), neomycin (30), polymyxin B (100 U), streptomycin (50) and tetracycline (30), but not to ampicillin (10), carbenicillin (100), cephalothin (30), lincomycin (15), novobiocin (5), oleandomycin (15) and penicillin G (20 U). Substrate assimilation data are given in Table 1 and Supplementary Table S1 (available in IJSEM Online). The predominant ubiquinone is Q-8. The major fatty acids (>10 %) are iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega9c$. The DNA G+C content of the type strain is 68.1 mol% (HPLC).

The type strain, DS-58^T (=KCTC 12822^T =DSM 17958^T), was isolated from a soil of Dokdo, an island in Korea.

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