Dokdonella koreensis gen. nov., sp. nov., isolated from soil

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Two Gram-negative, motile, non-spore-forming and rod-shaped bacterial strains, DS-123^T and DS-140, were isolated from soil and their taxonomic positions were investigated by a polyphasic study. Strains DS-123^T and DS-140 grew optimally at 30 °C and pH 6.5 without NaCl. They contained Q-8 as the predominant ubiquione and iso-C_{17:1}ω9c, iso-C_{17:0} and iso-C_{15:0} as the major fatty acids. Major polar lipids detected in the two strains were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an amino-group-containing lipid that was ninhydrin-positive. Their DNA G + C contents were 71.0 mol%. Strains DS-123^T and DS-140 exhibited no difference in their 16S rRNA gene sequences and possessed a mean DNA–DNA relatedness level of 92%. Phylogenetic trees based on 16S rRNA gene sequences showed that strains DS-123^T and DS-140 formed a distinct evolutionary lineage within the *Gammaproteobacteria*. The 16S rRNA gene sequences of strains DS-123^T and DS-140 exhibited similarity values of less than 94.1% to members of the *Gammaproteobacteria*. Strains DS-123^T and DS-140 were distinguished from phylogenetically related genera by differences in some phenotypic properties. On the basis of phenotypic, phylogenetic and genetic data, it is proposed that strains DS-123^T (=KCTC 12396^T=DSM 17203^T) and DS-140 be classified in a novel genus and species, *Dokdonella koreensis* gen. nov., sp. nov.

Dokdo is an island located at the edge of the East Sea, Korea. Civilians have been restricted from entering the island for a long time, which may have resulted in stable preservation of microbial diversity. Recently, in an attempt to investigate the microbial community of Dokdo, many bacterial strains have been isolated and characterized taxonomically. In this study, we report the detailed taxonomic characterization of two bacterial strains, DS-123^T and DS-140, which were considered to belong to the class *Proteobacteria*.

Soil samples collected in Dokdo (37° 14' 12" N 131° 52' 07" E) provided the source for isolation of bacterial strains. Strains DS-123^T and DS-140 were isolated by the standard dilution plating technique on twofold diluted nutrient agar (Difco) at 30 °C. *Frateruria aurantia* LMG 1558^T and *Fulvimonas soli* LMG 19981^T, which were used as reference strains, were obtained from the BCCM/LMG (the Belgian Co-ordinated Collections of Microorganisms/Laboratorium voor Microbiologie, Universiteit Gent), Gent, Belgium. To investigate

their morphological and physiological characteristics, strains DS-123^T and DS-140 were routinely cultivated at 30 °C on trypticase soy agar (TSA; Difco) under aerobic conditions. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. Presence of flagella was determined by transmission electron microscopy using cells from exponentially growing cultures. Gram-reaction was determined by using the bioMérieux Gram Stain kit according to the manufacturer's instructions. The pH range for growth was determined in nutrient broth (Difco) that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization to various levels by the addition of HCl or Na₂CO₃. Growth in the absence of NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that no NaCl was used. Growth at various NaCl concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0%, w/v) was investigated in trypticase soy broth (TSB; Difco). Growth at various temperatures (4–45 °C) was measured on TSA. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, gelatin, starch, urea, hypoxanthine, tyrosine, xanthine and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains DS-123^T and DS-140 are AY987368 and AY987369, respectively.

A transmission electron micrograph of strain DS-123^T is available as supplementary material in IJSEM Online.

were determined as described previously (Lanyi, 1987). Utilization of various substrates as sole carbon and energy sources was determined as described by Shirling & Gottlieb (1966). Enzyme activity was determined by using the API ZYM system (bioMérieux). Susceptibility to antibiotics was tested on TSA plates using antibiotic discs containing the following concentrations: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; carbenicillin,

100 µg. Other physiological and biochemical tests were performed with the API 20E system (bioMérieux).

Cell biomass of strains DS-123^T and DS-140 for DNA extraction and for respiratory lipoquinone and polar lipid analyses was obtained from cultivation in TSB at 30 °C. Cell mass of *Frateuria aurantia* LMG 1558^T and *Fulvimonas soli* LMG 19981^T for respiratory lipoquinone analysis was obtained by cultivation in TSB at 30 °C. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception

Table 1. Differential phenotypic characteristics of *Dokdonella koreensis* gen. nov., sp. nov. and five related genera

Genus: 1, *Dokdonella koreensis* gen. nov.; 2, *Frateuria*, data from Swings *et al.* (1980) and An *et al.* (2005); 3, *Rhodanobacter*, data from Nalin *et al.* (1999) and Im *et al.* (2004); 4, *Fulvimonas*, from Mergaert *et al.* (2002) and An *et al.* (2005); 5, *Dyella*, data from Xie & Yokota (2005) and An *et al.* (2005); 6, *Aquimonas*, data from Saha *et al.* (2005). +, Positive reaction; –, negative reaction; ND, not determined; w, weakly positive reaction; v, variable reaction. Data in parentheses are for the type strain.

Characteristic	1	2	3	4	5	6
Catalase	+	+	+	+	+* or w†	+
Oxidase	+	–	+	+	+‡	+
Colony colour	Yellow	Gold-yellow	Yellow	Deep yellow	Yellow	Yellowish-brown
Motility	+	v (+)	v	+	v	+
Nitrate reduction	v (+)	–	–	–	+	–
Growth at 4% (w/v) NaCl	–	(–)	ND	(+)	–* or w†	–
Growth at pH 4.5	–	+	ND	ND	–*	–
H ₂ S production	–	+	–§	ND	–*	–
β-Glucosidase	–	(–)	–	+	w* or +†	ND
β-Galactosidase	–	(–)	v	+	v	ND
Hydrolysis of:						
Aesculin	–	(–)	+	+	+†	–
Casein	+	(–)	–	(+)	v	+
Gelatin	+	–	–§	–	–*	+
Starch	–	(–)	–	+	–	+
Utilization of:						
L-Arabinose	–	(+)	–	–	–†	–
Maltose	–	(+)	v	+	+	–
D-Mannose	–	(+)	v	v (–)	+	–
Sucrose	–	(–)	+§	(–)	v	ND
Acetate	–	(–)	–§	(+)	v	–
Citrate	–	(–)	v	–	–	–
D-Malate	–	(–)	v	v (–)	v	–
Presence of 2-hydroxy fatty acid	–	+	–§	–	–	–
Presence of cyclo fatty acid	–	+	+§	–	–	–
Major fatty acids (>10% of total fatty acids)	iso-C _{17:1} ω9c, iso-C _{17:0} , iso-C _{15:0}	iso-C _{15:0}	iso-C _{17:1} ω9c, iso-C _{15:0} , iso-C _{17:0} §	iso-C _{15:0} , iso-C _{17:0} , iso-C _{16:0} , iso-C _{17:1} ω9c	iso-C _{17:1} ω9c, iso-C _{15:0} , iso-C _{17:0}	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{16:0}
DNA G+C mol%	71.0	62–64	63–65	71.5–71.9¶	63.4–64.0	75

*Data for *Dyella japonica* from Xie & Yokota (2005).

†Data for *Dyella koreensis* from An *et al.* (2005).

‡Data taken from An *et al.* (2005); a different result for *Dyella japonica* was obtained from Xie & Yokota (2005).

§Data for *Rhodanobacter fulvus* from Im *et al.* (2004).

||Data for *Rhodanobacter lindaniclasticus* from Nalin *et al.* (1999).

¶Data for two strains (LMG 19981^T and LMG 19982).

that ribonuclease T1 was treated in combination with ribonuclease 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). Respiratory lipoquinones 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. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). For fatty acid methyl ester analysis, cell mass of strains DS-123^T and DS-140, *Frateruia aurantia* LMG 1558^T and *Fulvimonas soli* LMG 19981^T was harvested from agar plates after incubation for 6 days at 30 °C on TSA. 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 DNA G+C content 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. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the means of the remaining three values were quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strains DS-123^T and DS-140 are given in the genus and species descriptions (see later) or are shown in Table 1, together with those of the four phylogenetically related genera *Frateruia*, *Rhodanobacter*, *Fulvimonas* and *Dyella*. Strains DS-123^T and DS-140 were motile by means of polar peritrichous flagella (supplementary figure in IJSEM Online). Almost complete 16S rRNA gene sequences of strains DS-123^T and DS-140, comprising 1494 nucleotides (approx. 96% of the *Escherichia coli* 16S rRNA

sequence), were determined in this study. The 16S rRNA gene sequences of strains DS-123^T and DS-140 were identical. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, the two strains joined the genus *Aquimonas* by a bootstrap resampling value of 62.3%, and this cluster joined the clade comprising the genera *Dyella*, *Frateruia*, *Fulvimonas* and *Rhodanobacter* by a bootstrap resampling value of 98.7% (Fig. 1). The relationship between this cluster and the clade comprising the genus *Xanthomonas* and some other genera was supported by a bootstrap resampling value of 100% (Fig. 1). Strains DS-123^T and DS-140 exhibited 16S rRNA gene sequence similarity levels of 94.1% to *Aquimonas voraii* and of less than 92.2% to other species used in the phylogenetic analysis. Strains DS-123^T and DS-140 exhibited a mean level of DNA–DNA relatedness of 92%, when their DNA was used individually as labelled DNA probes, indicating that the two strains are members of the same genomic species (Wayne *et al.*, 1987).

The predominant respiratory lipoquinone detected in strains DS-123^T and DS-140 was ubiquinone-8 (Q-8) at a peak area ratio of approximately 92–95%. In this study, *Frateruia aurantia* LMG 1558^T and *Fulvimonas soli* LMG 19981^T were analysed to contain Q-8 as the predominant ubiquinone at peak area ratios of 93 and 97%, respectively. Strains DS-123^T and DS-140 had cellular fatty acid profiles that contained large amounts of branched, straight-chain and hydroxy fatty acids; the major fatty acids (>10% of total fatty acids) were iso-C_{17:1}ω9c, iso-C_{17:0} and iso-C_{15:0} (Table 2). Major polar lipids detected in strains DS-123^T and DS-140 were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an amino-group-containing lipid that was ninhydrin-positive. The DNA G+C contents of strains DS-123^T and DS-140 were 71.0 mol%.

The phylogenetic analyses based on 16S rRNA gene sequences indicated that strains DS-123^T and DS-140 did not fall within the radiation encompassed by a recognized genus but form a distinct lineage within the

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strains DS-123^T and DS-140 and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. *Escherichia coli* ATCC 11775^T was used as an outgroup. Dots indicate that the corresponding nodes are also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Scale bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid content (%) of *Dokdonella koreensis* gen. nov., sp. nov. and five related genera

Strain: 1, *Dokdonella koreensis* DS-123^T; 2, *Dokdonella koreensis* DS-140; 3, *Frateriuria aurantia* LMG 1558^T, data from this study; 4, *Rhodanobacter fulvus* Jip2^T, data from Im *et al.* (2004); 5, *Fulvimonas soli* LMG 19981^T, from this study; 6, *Dyella japonica* XD53^T, data from Xie & Yokota (2005); 7, *Dyella koreensis* BB4^T, data from An *et al.* (2005); 8, *Aquimonas voraii* GPTSA 20^T, data from Saha *et al.* (2005). –, Not detected or not described. Fatty acids that represented <1.0% in all strains were omitted.

Fatty acid	1	2	3	4	5	6	7	8
Straight-chain fatty acid								
C _{14:0}	0.4	0.3	3.2	—	0.1	<0.5	—	1.1
C _{16:0}	4.6	4.0	2.7	6.3	0.51	1.5	3.2–6.4	4.5
C _{18:0}	0.3	—	0.3	—	—	<0.5	1.1–1.5	—
Branched fatty acid								
iso-C _{11:0}	4.7	6.2	0.1	3.5	5.5	3.8	4.3–10.4	7.4
iso-C _{13:0}	0.1	—	1.2	—	0.1	<0.5	— or <0.5	<1.0
iso-C _{14:0}	—	—	—	—	0.8	<0.5	— or <0.5	1.4
iso-C _{15:0}	10.1	11.8	60.4	21.3	24.4	23.6	18.2–22.6	25.0
iso-C _{15:1}	0.3	0.3	—	1.2	0.2	—	— or <0.5	2.3
anteiso-C _{15:0}	3.7	3.6	—	6.7	3.0	1.6	0.9–1.0	<1.0
iso-C _{16:0}	6.2	3.8	—	2.2	14.3	4.9	2.1–2.8	17.7
iso-C _{17:0}	19.8	17.8	1.8	10.9	16.8	20.0	15.6–26.0	2.8
anteiso-C _{17:0}	7.7	5.9	—	—	0.8	0.7	—	—
iso-C _{17:1ω9c}	31.2	32.9	0.7	24.6	13.2	25.6	21.3–28.1	19.3
iso-C _{19:0}	—	—	—	—	—	0.7	1.6–1.8	—
Unsaturated fatty acid								
C _{17:1ω6c}	0.4	0.4	—	—	2.0	—	—	—
Hydroxy fatty acid								
C _{12:0} 2-OH	—	—	2.3	—	—	—	—	—
C _{12:0} 3-OH	—	—	4.7	—	—	—	—	—
iso-C _{11:0} 3-OH	6.8	9.6	—	4.4	6.5	3.7	3.4–9.9	9.3
iso-C _{12:0} 3-OH	0.1	—	—	—	1.0	<0.5	—	—
iso-C _{13:0} 3-OH	—	—	1.7	3.0	3.8	3.9	2.3–7.1	—
iso-C _{17:0} 3-OH	—	—	—	—	0.7	1.1	0.8–1.1	—
Cyclo C _{17:0}	—	—	8.7	2.0	—	—	—	—
Summed feature*								
1	—	—	1.5	—	0.2	—	— or <0.5	—
3	1.0	0.9	4.5	5.9	1.8	4.0	4.9–7.0	4.2
Unknown fatty acid (ECL 11.799)	0.8	1.2	0.8	2.2†	2.9	1.9	1.4–4.4†	—

*Summed feature represents groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 1 contained C_{13:0} 3-OH and/or iso-C_{15:1}. Summed feature 3 contained C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

†Unknown fatty acid (ECL 11.798).

Gammaproteobacteria. The predominant ubiquinone type (Q-8) of strains DS-123^T and DS-140 was the same as those of some phylogenetically related genera, *Frateriuria*, *Fulvimonas* and *Dyella*, and *Rhodanobacter fulvus*, from the data compiled by Swings *et al.* (1980), Im *et al.* (2004), Xie & Yokota (2005) and An *et al.* (2005) and those generated in this study. However, strains DS-123^T and DS-140 were differentiated from phylogenetically related genera by differences in some phenotypic characteristics, including DNA G+C contents, fatty acid profiles and others (Tables 1 and 2). The DNA G+C contents of strains DS-123^T and DS-140 were higher than those of the genera *Frateriuria*, *Rhodanobacter* and *Dyella* (Table 1). The fatty acid profiles,

particularly proportions of the major fatty acid(s), distinguish strains DS-123^T and DS-140 from phylogenetically related genera *Frateriuria*, *Fulvimonas*, *Dyella* and *Aquimonas* and *Rhodanobacter fulvus* (Table 2). In particular, the genus *Frateriuria* was clearly differentiated from the genera *Fulvimonas* and *Dyella* as well as from strains DS-123^T and DS-140 by the proportion of iso-C_{15:0} and the presence of hydroxy (2- and 3-) C_{12:0} and cyclo C_{17:0} (Table 2). Unfortunately, the ubiquinone type and fatty acid profile of the type species of the genus *Rhodanobacter*, *Rhodanobacter lindaniclasticus*, were not reported previously and could not be analysed in this study. It was confirmed by the original author and LMG that *R. lindaniclasticus* is not extant

because the culture they held was contaminated and the original author's backup culture was lost. Therefore, on the basis of the phenotypic, phylogenetic and genetic data, strains DS-123^T and DS-140 should be classified in a novel genus and species, for which the name *Dokdonella koreensis* gen. nov., sp. nov. is proposed.

Description of *Dokdonella* gen. nov.

Dokdonella (Dok.do.nel'la. N.L. fem. dim. n. *Dokdonella* of Dokdo, an island located on the East Sea in Korea, from where the organisms were isolated).

Cells are Gram-negative, non-spore-forming and rod-shaped. Strictly aerobic. Motile by means of polar peritrichous flagella. Catalase- and oxidase-positive. Urease-negative. The predominant ubiquinone is Q-8. The major fatty acids are iso-C_{17:1}ω9c, iso-C_{17:0} and iso-C_{15:0}. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an amino-group-containing lipid that is ninhydrin-positive. The DNA G + C content is 71.0 mol%. The type species is *Dokdonella koreensis*.

Description of *Dokdonella koreensis* sp. nov.

Dokdonella koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* pertaining to Korea, where Dokdo is located).

Exhibits the following properties in addition to those given in the genus description. Cells are Gram-negative, strictly aerobic rods, 0.3–0.5 × 1.0–2.0 μm. Colonies on TSA are circular, convex, glistening, smooth, moderate yellow in colour and 1.0–2.0 mm in diameter after incubation for 6 days at 30 °C. Growth occurs at 10 and 39 °C with an optimum temperature of 30 °C; growth does not occur at 4 and 40 °C. Optimal growth occurs without NaCl; growth does not occur in the presence of >3 % (w/v) NaCl. Optimal pH for growth is 6.5; good growth occurs at pH 6.5–7.5. Growth is observed at pH 5.0 and 9.0, but not at pH 4.5 and 9.5. Urease-negative. Tyrosine and Tweens 20, 40, 60 and 80 are hydrolysed. Hypoxanthine and xanthine are not hydrolysed. Indole is not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Susceptible to polymyxin B, chloramphenicol, gentamicin and tetracycline, but not to streptomycin, penicillin G, ampicillin, cephalothin, novobiocin, kanamycin, lincomycin, oleandomycin, neomycin or carbenicillin. Pyruvate is utilized for growth, but D-glucose, D-fructose, D-galactose, D-cellobiose, D-trehalose, D-xylose, succinate, benzoate, formate, L-glutamate and salicin are not utilized. The predominant ubiquinone is Q-8. The major fatty acids are iso-C_{17:1}ω9c, iso-C_{17:0} and iso-C_{15:0}. Major

polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an amino-group-containing lipid that is ninhydrin-positive. The DNA G + C content is 71.0 mol%. Other phenotypic characteristics are given in Table 1.

The type strain, DS-123^T (= KCTC 12396^T = DSM 17203^T), was isolated from soil. The reference strain is DS-140.

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