Stenotrophomonas dokdonensis sp. nov., isolated from soil

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A Gram-negative, rod-shaped, *Stenotrophomonas*-like bacterial strain, DS-16^T, was isolated from soil from Dokdo, Korea, and subjected to a polyphasic taxonomic study. Strain DS-16^T grew optimally at pH 6.0–7.0 and 30 °C in the presence of 0.5 % (w/v) NaCl. It contained Q-8 as the predominant ubiquinone and iso-C_{16:0}, iso-C_{15:0} and iso-C_{17:1}ω9c as the major fatty acids. The DNA G + C content was 65.1 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain DS-16^T joined the cluster comprising *Stenotrophomonas* species. The levels of 16S rRNA gene sequence similarity between strain DS-16^T and the type strains of *Stenotrophomonas* species ranged from 95.5 to 97.5 %. DNA–DNA relatedness data and differential phenotypic properties, together with the phylogenetic distinctiveness of strain DS-16^T, demonstrated that this novel strain differs from *Stenotrophomonas* species with validly published names. On the basis of phenotypic, phylogenetic and genetic data, strain DS-16^T (=KCTC 12543^T = CIP 108839^T) should be classified in the genus *Stenotrophomonas* as a member of a novel species, for which the name *Stenotrophomonas dokdonensis* sp. nov. is proposed.

The genus *Stenotrophomonas* was proposed through the reclassification of *Xanthomonas maltophilia* as *Stenotrophomonas maltophilia* (Palleroni & Bradbury, 1993). At the time of writing, the genus comprises four species with validly published names: *Stenotrophomonas maltophilia* (Palleroni & Bradbury, 1993), *Stenotrophomonas nitritireducens* (Finkmann *et al.*, 2000), *Stenotrophomonas acidaminiphila* (Assih *et al.*, 2002) and *Stenotrophomonas rhizophila* (Wolf *et al.*, 2002). In this study, we report on the taxonomic characterization of a *Stenotrophomonas*-like bacterial strain, DS-16^T, which was isolated from a soil sample from Dokdo island (37° 14' 12" N, 131° 52' 07" E), Korea.

Strain DS-16^T was isolated by means of the standard dilution plating technique at 25 °C on 10 × diluted nutrient agar (Difco). The type strains of four *Stenotrophomonas* species were used as reference strains: *S. maltophilia* KCTC 1773^T, *S. nitritireducens* KCTC 12168^T, *S. acidaminiphila* KCTC 12167^T and *S. rhizophila* KCTC 12169^T were obtained from the Korean Collection for Type Cultures, Taejeon, Korea. The morphological, physiological and biochemical characteristics of strain DS-16^T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 30 °C. The cell morphology was examined by using light

microscopy (E600; Nikon) and transmission electron microscopy. The presence of flagella was determined with a transmission electron microscope (CM-20; Philips), using cells from exponentially growing cultures. For transmission electron microscopic observation, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were air-dried. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–45 °C) was measured on TSA. Growth in the absence of NaCl and at various NaCl concentrations [0.5 % (w/v) and 1.0–5.0 % (w/v) using 1.0 % increments] was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that no NaCl was included. The pH range for growth was determined in nutrient broth (Difco) adjusted to various pH values (pH 4.5–10.5, using increments of 0.5 pH units) prior to sterilization by the addition of HCl or Na₂CO₃. 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 the hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were investigated as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were studied as described previously (Lanyi, 1987). Sensitivity 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;

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-16^T is DQ178977.

Phenotypic characteristics of *Stenotrophomonas dokdonensis* sp. nov. and the type strains of other *Stenotrophomonas* species are provided in a supplementary table available in IJSEM Online.

Table 1. Differential phenotypic characteristics of *S. dokdonensis* sp. nov. and *Stenotrophomonas* species

Species: 1, *S. dokdonensis* sp. nov.; 2, *S. maltophila*, data from Palleroni (1984), Palleroni & Bradbury (1993), Finkmann *et al.* (2000), Wolf *et al.* (2002) and this study; 3, *S. nitritireducens*, data from Finkmann *et al.* (2000), Assih *et al.* (2002), Wolf *et al.* (2002) and this study; 4, *S. acidaminiphila*, data from Assih *et al.* (2002) and this study; 5, *S. rhizophila*, data from Wolf *et al.* (2002) and this study. Symbols: +, positive reaction; –, negative reaction; ND, not determined or not described; v, variable reaction; w, weakly positive. Data for the type strain are shown in parentheses. All species are straight or slightly curved rods, positive for assimilation (data for type strains of *S. maltophila* and *S. nitritireducens*) of *N*-acetylglucosamine, and negative for assimilation (data for type strains of *S. maltophila*, *S. nitritireducens* and *S. rhizophila*) of arabinose, mannitol, gluconate, caprate, adipate and phenylacetate.

Characteristic	1	2	3	4	5
Oxidase	+	+*	(–)	+	(+)†
Growth at:					
4 °C	–	–	(–)	–	+
37 °C	+	+	v (+)	+	+
41 °C	–	–	(–)†	+	–
Growth in the presence of 5% (w/v) NaCl	+	+	(–)†	(–)†	–
Nitrate reduction	+	+	–	+	+
Hydrolysis of:					
Aesculin	+	(+)	–	+	+
Casein	–	(+)†	(–)†	(w)†	(+)†
Gelatin	+	+	(–)†	(–)†	(+)†
Starch	–	(w)†	(–)†	(–)†	(–)†
Tween 80	+	+	v	+	(+)†
Assimilation of:					
Cellobiose	+	(+)†	(–)†	(–)†	(+)†
Gentiobiose	+	(+)†	(–)†	(–)†	(+)†
Glucose	+	+	(–)†	(+)	+
Lactose	–	(–)†	(–)†	(–)†	(+)†
D-Lyxose	+	(–)†	(–)†	(–)†	(–)†
Mannose	+	+	(–)	(+)	+
Maltose	+	+	(–)	(+)	+
Melibiose	–	(–)†	(–)†	(–)†	(+)†
Sucrose	–	(+)†	(–)†	(–)†	(+)†
Trehalose	+	(+)†	(–)†	(–)†	(+)†
D-Turanose	–	(+)†	(–)†	(–)†	(+)†
D-Xylose	+	(–)†	(+)†	(–)†	(+)†
Amygdalin	+	(+)†	(–)†	(–)†	(+)†
Arbutin	–	(+)†	(–)†	(–)†	(+)†
Aesculin	+	(+)†	(–)†	(–)†	(+)†
Glycogen	+	(–)†	(–)†	(–)†	(–)†
Salicin	–	(+)†	(–)†	(–)†	(+)†
Starch	+	(w)†	(–)†	(–)†	(–)†
Malate	–	+	(–)†	(–)	(+)†
Citrate	–	+	(+)†	(–)	+
API ZYM					
Leucine arylamidase	+	(–)†	(–)†	(–)†	(–)†
Naphthol-AS-BI-phosphohydrolase	+	(w)†	(–)†	(–)†	(+)†
α -Glucosidase	–	(–)†	(–)†	(w)†	(–)†
<i>N</i> -acetyl- β -glucosaminidase	+	(–)†	(+)†	(+)†	(–)†
Susceptibility to:					
Ampicillin	–	(–)	v(–)	(–)†	(–)†
Gentamicin	+	(–)	v(–)	+	–
Kanamycin	+	–‡	+	(+)†	+
Neomycin	+	(–)	v(–)	(+)†	(+)†
Novobiocin	+	–	–	(–)†	(–)†
Penicillin G	+	(–)	v(–)	(–)†	(–)†

Table 1. cont.

Characteristic	1	2	3	4	5
Streptomycin	+	(-)	+	(+) [†]	(+) [†]
Tetracycline	+	- [‡]	-	(-) [†]	+
DNA G+C content (mol%)	65.1	(66.7) [†]	(69.1) [†]	66.4–67.4	(67.9) [†]

*Positive for the majority of strains.

[†]Data from this study for the type strain.

[‡]Data from Palleroni & Bradbury (1993); opposite results were obtained by Finkmann *et al.* (2000) for the type strain.

cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; and carbenicillin, 100 µg. Assimilation of various substrates and enzyme activity were tested by using the API 20E, API 20NE, API 50CH and API ZYM systems (bioMérieux). The cells were suspended in AUX medium according to the manufacturer's instructions to inoculate the API 50CH system.

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultures grown in tryptic soy broth at 30 °C. Chromosomal DNA was isolated 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 using a PCR with 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). 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. For fatty acid methyl ester analysis, cell mass of strain DS-16^T was harvested from TSA plates after incubation for 3 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 DNA G+C content was determined by using the method of Tamaoka & Komagata (1984) with the 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 strain DS-16^T are given in the species description (see later) or are shown in Table 1 and Supplementary Table S1 in IJSEM Online. The almost complete 16S rRNA gene sequence of strain DS-16^T determined in this study comprised 1500 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree based on the neighbour-joining algorithm, strain DS-16^T joined the clade comprising *Stenotrophomonas* species at a bootstrap confidence value of 77.2 % (Fig. 1). The relationships between strain DS-16^T and *Stenotrophomonas* species were also recovered in the

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of *S. dokdonensis* DS-16^T, other *Stenotrophomonas* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) above 50 % are shown at branch points. *E. coli* ATCC 11775^T was used as an outgroup. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

trees based on the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain DS-16^T exhibited a 16S rRNA gene sequence similarity value of 97.5% with respect to *S. rhizophila* e-p10^T and similarities of 95.5–95.9% to the type strains of the other *Stenotrophomonas* species. Sequence similarities to all other species included in the phylogenetic analysis were below 97.0% (Fig. 1). The fatty acid profile of strain DS-16^T comprised the following: branched fatty acids iso-C_{16:0} (27.6%), iso-C_{15:0} (19.6%), iso-C_{17:1}ω9c (11.9%), iso-C_{11:0} (5.6%), anteiso-C_{15:0} (3.4%), iso-C_{17:0} (3.4%), iso-C_{14:0} (2.1%) and anteiso-C_{17:0} (1.5%); hydroxy fatty acids iso-C_{11:0} 3-OH (4.1%) and iso-C_{12:0} 3-OH (2.8%); unsaturated fatty acids C_{18:1}ω7c (1.5%) and C_{16:1}ω7c alcohol (1.0%); straight-chain fatty acid C_{16:0} (1.8%); and summed feature 3, comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (7.8%). This fatty acid profile was similar to those of *Stenotrophomonas* species (Finkmann *et al.*, 2000; Assih *et al.*, 2002; Wolf *et al.*, 2002). The predominant respiratory lipoquinone detected in strain DS-16^T was Q-8, at a peak area ratio of approximately 98%. The DNA G+C content of strain DS-16^T was 65.1 mol%. The mean DNA–DNA relatedness levels between strain DS-16^T and the type strains of the four *Stenotrophomonas* species were in the range 9–21%, indicating that these strains represent different genomic species (Wayne *et al.*, 1987). Strain DS-16^T differed from the recognized *Stenotrophomonas* species in several phenotypic characteristics (Table 1). The phylogenetic distinctiveness and differential phenotypic properties were sufficient to categorize strain DS-16^T as a member of a species that is distinct from previously recognized *Stenotrophomonas* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-16^T should be placed in the genus *Stenotrophomonas* as a member of a novel species, for which the name *Stenotrophomonas dokdonensis* sp. nov. is proposed.

Description of *Stenotrophomonas dokdonensis* sp. nov.

Stenotrophomonas dokdonensis (dok.do.nen'sis. N.L. fem. adj. *dokdonensis* of Dokdo, a Korean island, from where the organism was isolated).

Cells are Gram-negative, non-motile rods (0.2–0.4 × 0.7–2.6 µm). Colonies on TSA are circular, convex, sticky, glistening, cream–yellow in colour and 1.5–2.5 mm in diameter after 3 days incubation at 30 °C. Optimal temperature for growth is 30 °C. Growth occurs at 10 and 40 °C, but not at 4 or 41 °C. Optimal pH for growth is between 6.0 and 7.0; growth occurs at pH 5.5 and 8.5, but not at pH 5.0 or 9.0. Growth occurs in the presence of 0–6% (w/v) NaCl; optimal growth occurs in the presence of 0.5% (w/v) NaCl. Anaerobic growth does not occur on TSA or on TSA supplemented with nitrate. Catalase-positive. Urease-negative. Tweens 20, 40 and 60 are hydrolysed. H₂S and indole are not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. Susceptible to polymyxin B, chloramphenicol,

cephalothin, carbenicillin and oleandomycin, but not to lincomycin. The predominant ubiquinone is Q-8. The major fatty acids (>10% of total fatty acids) are iso-C_{16:0}, iso-C_{15:0} and iso-C_{17:1}ω9c. The DNA G+C content is 65.1 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DS-16^T (= KCTC 12543^T = CIP 108839^T), was isolated from soil.

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