Devosia insulae sp. nov., isolated from soil, and emended description of the genus *Devosia*

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A Gram-negative, motile, aerobic and oval- or rod-shaped bacterial strain, DS-56^T, which is phylogenetically closely related to the genus *Devosia*, was isolated from soil from Dokdo, Korea. Strain DS-56^T grew optimally at pH 6.5–7.5 and 25 °C in the presence of 0.5 % (w/v) NaCl. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain DS-56^T joins the cluster comprising *Devosia* species, at a bootstrap resampling value of 100 %. The levels of 16S rRNA gene sequence similarity between strain DS-56^T and the type strains of recognized *Devosia* species ranged from 95.4 to 96.4 %. Strain DS-56^T contained Q-11 as the predominant ubiquinone, unlike *Devosia* species, which contain Q-10 as the predominant ubiquinone. The novel strain also contained some fatty acids, including branched and hydroxy fatty acids, that are not detected in *Devosia* species. The DNA G + C content of strain DS-56^T was 66.2 mol%. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain DS-56^T represents a novel species of the genus *Devosia*, for which the name *Devosia insulae* sp. nov. is proposed. The type strain is DS-56^T (=KCTC 12821^T = DSM 17955^T).

In the course of the screening of micro-organisms from Dokdo, an island located in the East Sea of Korea, many novel bacterial strains have been isolated (Yoon *et al.*, 2005a, b, 2006a, b). One of these strains, DS-56^T, which is phylogenetically closely related to the genus *Devosia*, is the subject of this study. The genus *Devosia* was created by the reclassification of *Pseudomonas riboflavina* as *Devosia riboflavina* (Nakagawa *et al.*, 1996). Phylogenetic analyses based on 16S rRNA gene sequences have shown that the genus *Devosia* falls within the *Alphaproteobacteria* (Nakagawa *et al.*, 1996; Lee *et al.*, 2005). At the time of writing, the genus *Devosia* comprises four species with validly published names: *Devosia riboflavina* (Nakagawa *et al.*, 1996), *Devosia neptuniae* (Rivas *et al.*, 2003), *Devosia limi* (Vanparrys *et al.*, 2005) and *Devosia soli* (Yoo *et al.*, 2006). The aim of the present study was to determine the exact taxonomic position of strain DS-56^T by means of a polyphasic characterization that included phenotypic and chemotaxonomic determinations and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

Strain DS-56^T was isolated from a soil sample collected from Dokdo (37° 14' 12" N 131° 52' 07" E), Korea, by means of the standard dilution plating technique performed at 25 °C on 10 × diluted nutrient agar (Difco). The type strains of *D. riboflavina*, *D. limi*, *D. neptuniae* and *D. soli* were used

as reference strains for fatty acid analyses; *D. riboflavina* DSM 7230^T and *D. limi* DSM 17137^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), *D. neptuniae* LMG 21357^T was obtained from the Laboratorium voor Microbiologie Universiteit Gent (Ghent, Belgium) and *D. soli* KACC 11509^T was obtained from the Korean Agricultural Culture Collection (Suwon, Korea). The morphological, physiological and biochemical characteristics of strain DS-56^T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 25 °C. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy. The presence of flagella was determined by transmission electron microscopy using cells from exponentially growing cultures. For transmission electron microscopic observations, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after air-drying with a Philips CM-20 transmission electron microscope. The Gram reaction was determined using the bioMérieux Gram-stain kit according to the manufacturer's instructions. Growth at various temperatures (4–40 °C) was measured on TSA. Growth in the absence of NaCl and at various NaCl concentrations (0.5 and 1.0–5.0 %, w/v, in increments of 1.0 %) was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that no NaCl was used. The pH range for growth was determined in nutrient broth (Difco) adjusted to various pH values (pH 4.5–10.5, in increments of 0.5 pH units), prior to sterilization, by the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-56^T is EF012357.

addition of HCl or Na₂CO₃. Growth under anaerobic conditions was determined after incubation of the novel strain 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, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin

and reduction of nitrate were studied as described previously (Lanyi, 1987). Susceptibility to antibiotics was tested on TSA plates, using antibiotic discs containing the following amounts (µg unless otherwise indicated): polymyxin B, 100 U; streptomycin, 50; penicillin G, 20 U; chloramphenicol, 100; ampicillin, 10; cephalothin, 30; gentamicin, 30; novobiocin, 5; tetracycline, 30; kanamycin, 30; lincomycin, 15; oleandomycin, 15; neomycin, 30; carbenicillin, 100. The

Table 1. Differential phenotypic characteristics of strain DS-56^T and *Devosia* species

Taxa: 1, strain DS-56^T; 2, *D. riboflavina* (data from Nakagawa *et al.*, 1996; Rivas *et al.*, 2003; Vanparys *et al.*, 2005; Yoo *et al.*, 2006); 3, *D. neptuniae* (Rivas *et al.*, 2003; Vanparys *et al.*, 2005; Yoo *et al.*, 2006); 4, *D. limi* (Vanparys *et al.*, 2005; Yoo *et al.*, 2006); 5, *D. soli* (Yoo *et al.*, 2006). All of the taxa are Gram-negative, aerobic and non-spore-forming. All are positive for catalase, hydrolysis of aesculin and for the activity of leucine arylamidase, acid phosphatase, β-galactosidase, β-glucosidase and N-acetyl-β-glucosaminidase. All are negative for hydrolysis of gelatin, indole production, for the activity of arginine dihydrolase, lipase (C14), cystine arylamidase, α-chymotrypsin and β-glucuronidase, for the assimilation of gentiobiose, caprate, adipate, malate, citrate and phenylacetate and for susceptibility to polymyxin B and gentamicin. +, Positive; –, negative; W, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5
Cell morphology	Ovals or rods	Rods	Rods	Rods	Rods
Motility	+	+	+	–	ND
Colony colour	Ivory	Cream	Pearl white	Light yellow to light brown	Light beige
Oxidase	–	+	+	+	–
Urease	+	+*	+*	–	+
Nitrate reduction	–	+*	+*	–	–
Hydrolysis of:					
Casein	–	–	+	–	–
Starch	–	–†	–	–	+
Tyrosine	–	–	–	+	–
Assimilation of:					
Glucose	–	+	+	–	–
L-Arabinose	–	+	+	–	–
D-Mannose	–	+	+	–	–
Mannitol	–	+	+	–	–
Maltose	–	+	+	–	–
N-Acetylglucosamine	–	+	–	–	–
API ZYM results					
Alkaline phosphatase	–	+	+	+	+
Esterase (C4)	+	+	–	W	+
Esterase lipase (C8)	+	+	–	W	+
Naphthol-AS-BI-phosphohydrolase	–	+	+	+	+
α-Galactosidase	–	+	+	–	–
α-Glucosidase	+	–	–	–	+
α-Mannosidase	–	+	+	–	+
α-Fucosidase	+	+	+	–	–
Susceptibility to:					
Ampicillin	+	–‡	–‡	+	+
Chloramphenicol	+	–	–	–	+
Kanamycin	–	–	–	–	+
Tetracycline	+	+	–	W	+
DNA G+C content (mol%)	66.2	61.4	62.0–62.4	61.9	59.5

*Reported as positive by Nakagawa *et al.* (1996) and Rivas *et al.* (2003) but negative by Vanparys *et al.* (2005).

†Reported as negative by Nakagawa *et al.* (1996) but positive by Yoo *et al.* (2006).

‡Reported as negative by Rivas *et al.* (2003) but positive by Vanparys *et al.* (2005).

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain DS-56^T with respect to recognized *Devosia* species and some other related taxa. Bootstrap percentages (based on 1000 replications) greater than 50% are shown at branch points. *E. coli* ATCC 11775^T was used as an outgroup (not shown). Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

assimilation of various substrates and the activities of various enzymes were tested by using the API 20E, API 20NE and API ZYM systems (bioMérieux).

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultures grown in trypticase soy broth (Difco) at 25 °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-56^T was harvested from TSA plates after incubation for 7 days at 25 °C, and cell mass of *D. riboflavina* DSM 7230^T, *D. limi* DSM 17137^T, *D. neptuniae* LMG 21357^T and *D. soli* KACC 11509^T was harvested from TSA plates after incubation for 3 days at 28 °C and from TSA plates after incubation for 7 days at 25 °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 the method of Tamaoka & Komagata (1984), with the modification that the DNA was hydrolysed and the resulting nucleotides were analysed by reversed-phase HPLC.

Cells of strain DS-56^T were Gram-negative, non-spore-forming and oval- or rod-shaped. Strain DS-56^T showed optimal growth at pH 6.5–7.5 and at 25 °C. The growth of strain DS-56^T was slower than that of the type strains of the four recognized *Devosia* species. In tests with the API 20NE system, strain DS-56^T did not assimilate any substrates (after incubation for 10 days). Morphological, cultural, physiological and biochemical characteristics of strain DS-56^T are given in the species description (see below) or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain DS-56^T determined in this study comprised 1437 nt, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree constructed using the neighbour-joining algorithm, strain DS-56^T joined '*Devosia ginsengisoli*' Gsoil 326 at a bootstrap resampling value of 100%, and this cluster joined the clade comprising recognized *Devosia* species at a bootstrap confidence value of 100% (Fig. 1). Strain DS-56^T exhibited 16S rRNA gene sequence similarity values of 96.4, 95.8, 95.7, 95.4 and 99.1% with respect to *D. riboflavina* DSM 7230^T, *D. neptuniae* J1^T, *D. limi* LMG 22951^T, *D. soli* GH2-10^T and '*D. ginsengisoli*' Gsoil 326, respectively. The sequence similarities with respect to other species included in the phylogenetic analysis were below 92.4% (Fig. 1). The fatty acid profile of strain DS-56^T comprised major amounts of unsaturated, branched, straight-chain and 11-methyl C_{18:1}ω7c; the major fatty acids (>10% of total fatty acids) were 11-methyl C_{18:1}ω7c (29.6%), C_{18:1}ω7c (18.5%), iso-C_{17:0} (14.5%) and C_{16:0} (13.6%) (Table 2). The predominant isoprenoid quinone detected in strain DS-56^T was Q-11, at a peak area ratio of approximately 85%; a minor amount of Q-10 (<10% approx.) was present. The DNA G+C content of strain DS-56^T was 66.2 mol%.

Strain DS-56^T was distinguishable from recognized *Devosia* species by differences in ubiquinone and fatty acid profiles and in DNA G+C contents (Table 1). *D. riboflavina* and *D. soli* have been reported to have Q-10 as the predominant ubiquinone (Nakagawa *et al.*, 1996; Yoo *et al.*, 2006). It was found in this study that the type strains of *D. riboflavina*, *D. neptuniae* and *D. limi* have Q-10 as the predominant ubiquinone. Strain DS-56^T can be differentiated from recognized *Devosia* species on the basis of the fatty acid profiles, e.g. by the presence of branched fatty acids, by the absence of hydroxy fatty acids and by the presence or absence of some other fatty acids (Table 2). Strain DS-56^T also shows slight differences from recognized *Devosia* species in terms of DNA G+C content (Table 1). However, the many phylogenetic similarities between strain DS-56^T and *Devosia* species preclude the placement of strain DS-56^T in a novel genus. In particular, the closest phylogenetic neighbour, '*D. ginsengisoli*' Gsoil 326, has been shown to contain Q-10 as the predominant menaquinone (W.-T. Im and S.-T. Lee, personal communication). Accordingly, it appears to be appropriate for strain DS-56^T to be classified within the genus *Devosia*. The phylogenetic distinctiveness of DS-56^T, together with the differential phenotypic properties, is sufficient to allocate the strain to a

Table 2. Cellular fatty acid compositions (%) of strain DS-56^T and type strains of *Devosia* species

Strains: 1, DS-56^T; 2, *D. riboflavina* DSM 7230^T; 3, *D. neptuniae* LMG 21357^T; 4, *D. limi* DSM 17137^T; 5, *D. soli* KACC 11509^T. –, Not detected; ECL, equivalent chain length.

Fatty acid	1	2		3		4		5	
	25 °C	28 °C	25 °C	28 °C	25 °C	28 °C	25 °C	28 °C	25 °C
Straight-chain fatty acids									
C _{14:0}	–	0.3	–	0.7	0.6	0.4	0.4	0.3	–
C _{15:0}	0.2	–	–	–	–	–	–	–	–
C _{16:0}	12.7	22.0	15.6	25.6	17.1	13.8	8.5	19.5	12.8
C _{17:0}	2.2	–	–	–	–	0.5	0.5	–	–
C _{17:0} cyclo	–	0.7	1.8	2.6	3.0	0.3	1.0	–	–
C _{18:0}	3.2	3.8	3.4	6.7	3.9	9.3	6.5	4.5	3.3
Branched fatty acids									
iso-C _{11:0} 3-OH	0.6	–	–	–	–	–	–	–	–
iso-C _{13:0} 3-OH	1.0	–	–	–	–	–	–	–	–
iso-C _{15:0}	0.3	0.2	–	–	–	–	–	–	–
iso-C _{17:0}	14.0	–	–	–	–	–	–	–	–
iso-C _{17:1} ω9c	0.3	–	–	–	–	–	–	–	–
iso-C _{19:0}	0.3	–	–	–	–	–	–	–	–
Unsaturated fatty acids									
C _{17:1} ω6c	0.5	–	–	–	–	–	–	–	–
C _{17:1} ω8c	1.1	–	–	–	–	–	–	–	–
C _{18:1} ω7c	22.5	44.7	38.9	21.6	30.6	39.4	39.5	55.2	65.6
C _{18:1} ω9c	2.5	–	–	–	–	–	–	–	–
C _{20:1} ω7c	–	0.5	0.7	–	–	0.5	0.5	–	–
Hydroxy fatty acids									
C _{10:0} 3-OH	–	1.7	1.2	0.7	0.5	1.7	1.2	1.1	1.2
C _{16:0} 3-OH	–	–	–	–	–	–	–	0.3	–
C _{18:0} 3-OH	–	0.4	–	5.5	4.3	5.3	4.4	3.3	4.0
11-Methyl C _{18:1} ω7c	30.2	21.8	30.8	27.0	30.3	25.1	30.4	13.5	11.4
C _{19:0} ω8c cyclo	1.1	1.6	5.3	7.8	5.7	1.7	2.6	0.7	–
Unknown ECL 18.814	6.2	–	–	–	–	–	–	–	–
Summed features*									
3	0.6	2.4	2.0	1.9	4.0	2.1	4.5	1.7	1.8
7	0.6	–	0.3	–	–	–	–	–	–

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 7 contained one or more of C_{19:1}ω6c, C_{17:0} cyclo and/or an unknown fatty acid of ECL 18.846.

species that is distinct from recognized *Devosia* species (Stackebrandt & Goebel, 1994). On the basis of the data presented, strain DS-56^T represents a novel species of the genus *Devosia*, for which the name *Devosia insulae* sp. nov. is proposed.

Description of *Devosia insulae* sp. nov.

Devosia insulae (in.su'lae. L. fem. gen. n. *insulae* of an island, from where the type strain was isolated).

Cells are Gram-negative, non-spore-forming and oval- or rod-shaped (0.3–0.5 × 0.5–2.0 µm). Motile by means of a single flagellum. Colonies on TSA are circular, convex, smooth, glistening, ivory in colour and 0.3–0.5 mm in

diameter after 7 days incubation at 25 °C. Optimal temperature for growth is 25 °C. Growth occurs at 10 and 31 °C, but not at 4 or 32 °C. Optimal pH for growth is between 6.5 and 7.5. Growth does not occur in the absence of NaCl or in the presence of > 2.0 % (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. Hypoxanthine, xanthine and Tweens 20, 40, 60 and 80 are not hydrolysed. H₂S is not produced. Lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, valine arylamidase and trypsin are absent. Susceptible to penicillin G, cephalothin, novobiocin and carbenicillin, but not to lincomycin, neomycin, oleandomycin or streptomycin. The predominant ubiquinone is Q-11. The major fatty acids

(>10% of total fatty acids) are 11-methyl $C_{18:1\omega7c}$, $C_{18:1\omega7c}$, iso- $C_{17:0}$ and $C_{16:0}$. The DNA G+C content is 66.2 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DS-56^T (=KCTC 12821^T=DSM 17955^T), was isolated from soil from Dokdo, Korea.

Emended description of the genus *Devosia* Nakagawa *et al.* 1996

The description of the genus *Devosia* is as given by Nakagawa *et al.* (1996) and emended by Rivas *et al.* (2003), Vanparrys *et al.* (2005) and Yoo *et al.* (2006), with the following further amendments. The predominant ubiquinone is Q-10 or Q-11. The fatty acid profile consists of unsaturated and straight-chain fatty acids, 11-methyl $C_{18:1\omega7c}$ and hydroxy or branched fatty acids. The DNA G+C contents are in the range 59.5–66.2 mol%.

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