Roseomonas terrae sp. nov.

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A Gram-negative, non-motile, coccobacilli-shaped bacterium, DS-48^T, was isolated from soil from Dokdo, Korea, and its taxonomic position was investigated by means of a polyphasic study. Strain DS-48^T grew optimally at 25 °C and pH 7.0–8.0 in the presence of 0.5 % (w/v) NaCl. It contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c and C_{18:1} 2-OH as the major fatty acids. The DNA G + C content was 69.3 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-48^T fell within the genus *Roseomonas*, clustering with *Roseomonas lacus* TH-G33^T (at a bootstrap confidence level of 100 %). The levels of similarity between the 16S rRNA gene sequence of strain DS-48^T and those of the type strains of recognized *Roseomonas* species were in the range 93.2–98.0 %. DNA–DNA relatedness data and differential phenotypic properties, together with the phylogenetic distinctiveness of DS-48^T, revealed that this strain differs from recognized *Roseomonas* species. On the basis of phenotypic, phylogenetic and genetic data, therefore, strain DS-48^T represents a novel species within the genus *Roseomonas*, for which the name *Roseomonas terrae* sp. nov. is proposed. The type strain is DS-48^T (=KCTC 12874^T=JCM 14592^T).

The genus *Roseomonas* was erected by Rihs *et al.* (1993) with the description of three novel species, *Roseomonas gilardii*, *Roseomonas cervicalis* and *Roseomonas fauriae*, and three unnamed genomospecies. Recently, *R. fauriae* was reclassified as a later heterotypic synonym of *Azospirillum brasilense* (Helsel *et al.*, 2006). At the time of writing, the genus comprises five *Roseomonas* species with validly published names: *R. gilardii* (Rihs *et al.*, 1993; Han *et al.*, 2003), *R. cervicalis* (Rihs *et al.*, 1993), *Roseomonas mucosa* (Han *et al.*, 2003), *Roseomonas lacus* (Jiang *et al.*, 2006) and *Roseomonas aquatica* (Gallego *et al.*, 2006). A phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Roseomonas* falls within the *Alphaproteobacteria* (Gallego *et al.*, 2006). Here, we report on the taxonomic characterization of a *Roseomonas*-like bacterial strain, DS-48^T, which was isolated from soil from Dokdo, Korea.

Strain DS-48^T was isolated from a soil sample collected from Dokdo (37° 14' 12" N, 131° 52' 07" E), an island of Korea, using the standard dilution plating technique at 25 °C on 10 × diluted nutrient agar (NA; Difco). *R. aquatica* JCM 13556^T and *R. lacus* JCM 13283^T, which were used as reference strains for phenotypic characterization, were obtained from the Japan Collection of Microorganisms, Saitama, Japan. The morphological, physiological and biochemical characteristics of strain DS-48^T were

investigated using routine cultivation on 2 × diluted NA at 25 °C. The cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy. Flagellation was determined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–40 °C) was determined on 2 × diluted NA. Growth in the absence of NaCl and at various NaCl concentrations [0.5 and 1.0–5.0 %, (w/v) using increments of 1.0 %] was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. The pH range for growth was determined in 2 × diluted nutrient broth (Difco) that had been 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 2 × diluted NA and on 2 × diluted NA 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 determined as described by Cowan & Steel (1965). Aesculin hydrolysis and nitrate reduction were studied as described previously (Lanyi, 1987). Antibiotic susceptibility was tested on 2 × diluted NA plates using antibiotic discs containing the following

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-48^T is EF363716.

A table showing phenotypic characteristics of strain DS-48^T and *Roseomonas* species is available with the online version of this paper.

concentrations (μg unless indicated otherwise); 100 U polymyxin B, 50 streptomycin, 20 U penicillin G, 100 chloramphenicol, 10 ampicillin, 30 cephalothin, 30 gentamicin, 5 novobiocin, 30 tetracycline, 30 kanamycin, 15 lincomycin, 15 oleandomycin, 30 neomycin, 100 carbenicillin. Substrate utilization, enzyme activities and other physiological and biochemical properties were tested by using the API 50CH, API ZYM, API 20E and API 20NE systems (bioMérieux); the utilization of various substrates was determined by inoculating the API 50CH strip with cells suspended in AUX medium (bioMérieux).

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in $2\times$ diluted nutrient broth at 25°C . Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), except that RNase T1 was used in combination with RNase A to minimize contamination from 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 with a YMC ODS-A ($250\times 4.6\text{ mm}$) column. For fatty acid methyl ester analysis, cell mass of strain DS-48^T was harvested from $2\times$ diluted NA plates after incubation for 7 days at 25°C . The fatty acids were extracted and fatty acid methyl esters prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined using the method of Tamaoka & Komagata (1984), with the modification that the DNA was hydrolysed and the resultant nucleotides analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically according to 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 for each sample were excluded and the means of the remaining three values were quoted as the DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain DS-48^T are given in the species description (see below) or are shown in Table 1 (see also Supplementary Table S1 available in IJSEM Online). The almost-complete 16S rRNA gene sequence of strain DS-48^T determined in this study comprised 1442 nt (representing approx. 96% of the *Escherichia coli* 16S rRNA gene sequence). Comparative 16S rRNA gene sequence analyses showed that strain DS-48^T was most closely related phylogenetically to the genus *Roseomonas* within the *Alphaproteobacteria* (Fig. 1). In the neighbour-joining tree based on 16S rRNA gene sequences, strain DS-48^T fell within the cluster comprising *Roseomonas* species, clustering with *R. lacus* TH-G33^T at a bootstrap confidence level of 100% (Fig. 1). Strain DS-48^T exhibited 98.0% 16S

rRNA gene sequence similarity with respect to *R. lacus* TH-G33^T and 93.2–94.3% similarity with respect to the type strains of the other *Roseomonas* species. The sequence similarity values with respect to other species used in the phylogenetic analysis were below 93.7%.

Strain DS-48^T contained Q-10, at a peak area ratio of approximately 90%, as the predominant isoprenoid quinone; minor amounts of Q-7 and Q-11 were present. *R. lacus* and *R. aquatica* were reported to have Q-10 as predominant ubiquinone (Jiang *et al.*, 2006; Gallego *et al.*, 2006). The fatty acid profile of strain DS-48^T was composed of the following (each constituting $>0.5\%$ of total fatty acids): unsaturated fatty acids $\text{C}_{18:1}\omega 7c$ (63.9%), $\text{C}_{16:1}\omega 5c$ (3.9%), $\text{C}_{17:1}\omega 7c$ (1.2%) and $\text{C}_{18:1}\omega 5c$ (0.6%); hydroxyl fatty acids $\text{C}_{18:1}$ 2-OH (10.3%), $\text{C}_{16:0}$ 3-OH (1.4%), $\text{C}_{16:0}$ 2-OH (1.1%) and $\text{C}_{18:0}$ 3-OH (0.5%); straight-chain fatty acid $\text{C}_{16:0}$ (8.8%); cyclo fatty acid $\text{C}_{19:0}$ cyclo $\omega 8c$ (5.2%); summed feature 3 ($\text{C}_{16:1}\omega 7c$ and/or iso- $\text{C}_{15:0}$ 2-OH, 1.3%); branched fatty acid iso- $\text{C}_{17:0}$ 3-OH (0.9%); and summed feature 2 (iso- $\text{C}_{16:1}$, $\text{C}_{14:0}$ 3-OH and/or an unknown fatty acid with an equivalent chain length of 10.928, 0.6%). This fatty acid profile was similar to those of *Roseomonas* species, although there were differences with respect to *R. lacus*, perhaps resulting from differences in the cultivation conditions (Han *et al.*, 2003; Jiang *et al.*, 2006; Gallego *et al.*, 2006). The DNA G+C content of strain DS-48^T was 69.3 mol%. These chemotaxonomic properties were in agreement with the result of the phylogenetic classification, i.e. that strain DS-48^T belongs to the genus *Roseomonas* (Han *et al.*, 2003; Jiang *et al.*, 2006; Gallego *et al.*, 2006).

Strain DS-48^T exhibited a mean DNA–DNA relatedness value of 17% with respect to *R. lacus* TH-G33^T, showing a 16S rRNA gene sequence similarity value of greater than 97%. Strain DS-48^T is distinguishable from *Roseomonas* species through differences in several phenotypic characteristics (Table 1 and Supplementary Table S1). The phylogenetic distinctiveness of DS-48^T, the DNA–DNA relatedness data and the differential phenotypic properties are sufficient to show that this strain is distinct from the recognized *Roseomonas* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-48^T represents a novel species of the genus *Roseomonas*, for which the name *Roseomonas terrae* sp. nov. is proposed.

Description of *Roseomonas terrae* sp. nov.

Roseomonas terrae (ter'rae. L. gen. n. *terrae* of the soil).

Cells are Gram-negative, non-spore-forming coccobacilli ($0.6\text{--}0.9\times 0.6\text{--}3.0\text{ }\mu\text{m}$). Colonies on $2\times$ diluted NA are circular, convex, smooth, glistening, pale yellow to pale pink in colour and $0.5\text{--}0.8\text{ mm}$ in diameter after incubation for 7 days at 25°C . Optimal growth occurs at 25°C ; growth occurs weakly at 10 and 36°C , but not at 4 or 37°C . Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 5.5 and 10.5, but not at pH 5.0 or

Table 1. Differential phenotypic characteristics (determined in this study) for strain DS-48^T and phylogenetically related *Roseomonas* species

Strains: 1, DS-48^T; 2, *R. lacus*; 3, *R. aquatica*. All of the strains are positive for catalase, oxidase, alkaline phosphatase, esterase (C4) and esterase lipase (C8) and for susceptibility to chloramphenicol, gentamicin, kanamycin, streptomycin and tetracycline. All of the strains are negative for the following: motility [data for *R. lacus* and *R. aquatica* from Jiang *et al.* (2006) and Gallego *et al.* (2006), respectively]; hydrolysis of aesculin, casein, hypoxanthine, xanthine, starch, tyrosine, gelatin and Tween 80; utilization of glycerol, erythritol, adonitol, methyl β -D-xyloside, glucose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-glucoside, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-tagatose, D-arabitol and L-arabitol; arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase; and susceptibility to ampicillin, cephalothin, lincomycin, novobiocin, oleandomycin, penicillin G and polymyxin B. +, Positive reaction; –, negative reaction; w, weakly positive reaction.

Characteristic	1	2	3
Optimal growth temperature (°C)	25	30*	25–28†
Maximum growth temperature (°C)	36	40*	35†
Maximum NaCl concentration for growth (%)	1.0	6.5*	<2.0†
Nitrate reduction	–	+	–
Hydrolysis of:			
Tween 20	+	–	+
Tween 40	–	–	+
Tween 60	–	–	+
Urea	+	+	–
Utilization of:			
D-Arabinose	–	–	+
L-Arabinose	–	–	+
Ribose	–	–	+
D-Xylose	–	–	+
L-Xylose	–	–	+
Galactose	–	–	+
Methyl α -D-mannoside	–	+	–
D-Lyxose	–	–	+
D-Fucose	–	–	+
L-Fucose	–	–	+
Gluconate	–	–	+
2-Ketogluconate	–	–	+
5-Ketogluconate	–	–	+
Adipate	–	+	–
Malate	–	+	+
Enzyme activity (API ZYM)			
Naphthol-AS-BI-phosphohydrolase	+	–	–
Acid phosphatase	+	+	–
Susceptibility to:			
Carbenicillin	–	w	–
Neomycin	+	+	w
Isolation source	Soil	Freshwater-lake sediment	Drinking water
DNA G + C content (mol%)	69.3	71.9*	68.6†

*Data for *R. lacus* from Jiang *et al.* (2006).

†Data for *R. aquatica* from Gallego *et al.* (2006).

11.0. Growth occurs in the presence of 0–1.0 % (w/v), with an optimum in the presence of 0.5 % (w/v) NaCl. Growth does not occur under anaerobic conditions on 2× diluted

NA or on 2× diluted NA supplemented with nitrate. H₂S and indole are not produced. None of the substrates in the API 20NE and API 50CH strips is utilized as a sole carbon

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain DS-48^T and some related taxa. Bootstrap percentages (based on 1000 replications) $\geq 50\%$ are shown at the nodes. *Methylobacterium organophilum* JCM 2833^T (GenBank accession number, D32226) was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

source. The predominant ubiquinone is Q-10. The major fatty acids ($>10\%$ of total fatty acids) are C_{18:1} ω 7c (63.9%) and C_{18:1} 2-OH (10.3%). The DNA G+C content is 69.3 mol% (HPLC). Other phenotypic properties are shown in Table 1 (see also Supplementary Table S1 available in IJSEM Online).

The type strain, DS-48^T (=KCTC 12874^T=JCM 14592^T), was isolated from soil from Dokdo in Korea.

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