Devosia soli sp. nov., isolated from greenhouse soil in Korea

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A Gram-negative, obligately aerobic, rod-shaped bacterium was isolated from greenhouse soil used to cultivate lettuce. The strain, GH2-10^T, was characterized on the basis of phenotypic and genotypic data. 16S rRNA gene sequence analysis revealed that the isolate belonged to the genus *Devosia*, with highest sequence similarity (98.5 %) to *Devosia riboflavina* IFO 13584^T. Sequence similarities with other strains tested were below 97.0 %. Strain GH2-10^T had Q-10 as the predominant ubiquinone and C_{18:1}ω7c and C_{16:0} as the major fatty acids. The G + C content of the genomic DNA was 59.5 mol%. The results of DNA–DNA hybridization experiments (47 % relatedness between *D. riboflavina* DSM 7230^T and strain GH2-10^T) and physiological and biochemical tests suggested that strain GH2-10^T represents a novel species of the genus *Devosia*, for which the name *Devosia soli* sp. nov. is proposed. The type strain is GH2-10^T (=KACC 11509^T=DSM 17780^T).

Nakagawa *et al.* (1996) erected the genus *Devosia* with the transfer of '*Pseudomonas riboflavina*' (Foster, 1944) to *Devosia riboflavina*. Since then, *Devosia neptuniae* (Rivas *et al.*, 2003), '*Candidatus Devosia euplotis*' (Vannini *et al.*, 2004) and *Devosia limi* (Vanparys *et al.*, 2005) have been described within the genus.

In the present study, a soil sample was collected from a greenhouse planted with lettuce (*Lactuca sativa* L.) in Daejeon City, Korea. The soil sample was diluted and spread on R2A medium (Reasoner & Geldreich, 1985). Strain GH2-10^T was isolated after incubation for 5 days at 28 °C.

The morphological, physiological and biochemical characteristics of strain GH2-10^T were tested using routine cultivation on R2A medium at 28 °C. Gram staining, presence of oxidase and catalase and hydrolysis of agar, casein, DNA, gelatin and starch were determined as described by Smibert & Krieg (1994). Motility testing was performed on one-tenth strength R2A broth supplemented with 0.2 % agar. Growth was assessed at 5, 10, 20, 25, 30, 37, 40 and 45 °C, at pH 4, 5, 6, 7, 8, 9 and 10 and at 0, 1, 3, 5 and 7 % NaCl. Anaerobic growth was checked by using a BBL

anaerobic jar (Becton Dickinson), with incubation for up to 21 days. Carboxymethylcellulose (CM-cellulose; Sigma) (0.1 %, w/v) and tyrosine (0.5 %, w/v) were also used. Strain GH2-10^T was additionally characterized by using the whole test spectra of the API 20NE, API 50CH and API ZYM (bioMérieux) systems according to the manufacturer's instructions. For tests of antimicrobial susceptibility, discs containing the following antibiotics were used: amikacin (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), methicillin (5 µg), polymyxin B (300 U), rifampicin (5 µg), tetracycline (30 µg) and vancomycin (30 µg).

The strain grew rapidly (2 days) on R2A and nutrient agar (NA; Difco) and rather slowly (5 days) on trypticase soy agar (TSA; Difco) and did not grow on MacConkey agar (Difco). The pH, NaCl concentration and temperature ranges for growth were pH 4–8, 0–5 % and 10–37 °C, respectively. Strain GH2-10^T was catalase-positive and oxidase-negative. The isolate hydrolysed starch, CM-cellulose and urea, but not casein, DNA, gelatin or tyrosine. Hydrolysis of urea was negative after incubation for 2 days, but positive after 7 days. According to the API 20NE test strips, strain GH2-10^T did not assimilate any substrates (up to 7 days incubation). API 50CH strips did not give reproducible results even with prolonged incubation (up to

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GH2-10^T is DQ303125.

Table 1. Differential physiological characteristics of strain GH2-10^T and the type strains of recognized members of the genus *Devosia*

Strains: 1, strain GH2-10^T; 2, *D. riboflavina* DSM 7230^T; 3, *D. neptuniae* LMG 21357^T; 4, *D. limi* LMG 22951^T. All strains were positive for catalase and negative for hydrolysis of CM-cellulose. In the API 20NE and API ZYM test strips, all strains were positive for aesculin hydrolysis, alkaline and acid phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, β -glucosidase and *N*-acetyl- β -glucosaminidase. All strains were negative for lipase (C14), cystine arylamidase, α -chymotrypsin, β -glucuronidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and gelatinase. Data are from Vanparys *et al.* (2005) and this study. +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3	4
Hydrolysis of:				
Casein	–	–	+	–
Starch	+	+	–	–
Tyrosine	–	–	–	+
Oxidase	–	+	+	+
Enzyme activities:				
Esterase lipase (C4 and C8)	+	+	–	(+)
α -Galactosidase, α -fucosidase	–	+	+	–
α -Glucosidase	+	–	–	–
α -Mannosidase	+	+	+	–
Assimilation of:				
Glucose, arabinose, mannose, mannitol, maltose	–	+	+	–
<i>N</i> -Acetylglucosamine	–	+	–	–
Presence of <i>nifH</i> and <i>nodC</i> genes	–	–	+	–
DNA G + C content (mol%)	59.5	61.4	62.0	61.9

7 days). Strain GH2-10^T was sensitive to all the antibiotics tested except gentamicin and polymyxin B. Details of the physiological and biochemical properties of strain GH2-10^T are given in Table 1 and in the species description below.

The DNA G + C content of the isolate was determined with an HPLC method as described by Mesbah *et al.* (1989) by using a reversed-phase column (Supelcosil LC-18-S; Supelco). The cellular fatty acid profile was determined by using the standard protocol of the Microbial Identification System (MIDI; Microbial ID) after cells were grown on TSA for 48 h at 28 °C. Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). The DNA G + C content was 59.5 mol%. The predominant fatty acids of strain GH2-10^T were C_{18:1} ω 7c (67.2 %), C_{16:0} (12.7 %), 11-methyl C_{18:1} ω 7c (5.8 %) and C_{18:0} 3-OH (5.2 %) (Table 2). The major respiratory lipoquinone of strain GH2-10^T was ubiquinone 10 (Q-10).

The 16S rRNA gene sequence of strain GH2-10^T was amplified by PCR using conserved primers, as described by Kwon *et al.* (2003), and direct sequencing (Hiraishi, 1992). Multiple alignments with sequences of isolate GH2-10^T and those of members of the genus *Devosia* and *Rhizobium leguminosarum* USDA 2370^T were carried out with the CLUSTAL W program (Thompson *et al.*, 1994). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) on MEGA version 2.1 (Kumar *et al.*, 2001) (Fig. 1). The stability of relationships was assessed by performing bootstrap analyses of the neighbour-joining data based on 1000 resamplings.

The nearly complete 16S rRNA gene sequence (1417 bp) of strain GH2-10^T was subjected to comparative analysis. Phylogenetically, strain GH2-10^T was most closely related to *D. riboflavina* IFO 13584^T, with a sequence similarity of 98.5 %. The sequences of other *Devosia* strains were shown to have similarities of <97 % to strain GH2-10^T.

Levels of DNA–DNA hybridization were determined based on a membrane filter technique using a DIG High Prime

Table 2. Cellular fatty acid profiles of strain GH2-10^T and the type strains of recognized members of the genus *Devosia*

Strains: 1, strain GH2-10^T; 2, *D. riboflavina* DSM 7230^T; 3, *D. neptuniae* LMG 21357^T; 4, *D. limi* LMG 22951^T. Only fatty acids that represent more than 1 % of the total are included.

Fatty acid	1	2	3	4
C _{10:0} 3-OH	3.5	2.7	–	1.3
C _{16:0}	12.7	14.7	19.0	9.7
C _{18:1} ω 7c	67.2	68.3	43.9	49.2
C _{18:0}	3.0	2.6	5.2	10.0
11-Methyl C _{18:1} ω 7c	5.8	6.8	18.8	19.4
C _{18:0} 3-OH	5.2	–	2.0	2.4
Cyclo-C _{19:0} ω 8c	–	–	1.6	–
Summed feature 3*	2.8	3.0	4.7	2.8
Unknown 14.959	–	–	2.4	2.0

*Summed feature 3 comprises C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain GH2-10^T. Bootstrap values are shown as percentages of 1000 replicates. GenBank accession numbers are given in parentheses. Bar, 1 substitution per 100 nucleotides.

DNA labelling and detection starter kit II (Roche Molecular Biochemicals) (Kwon *et al.*, 2003). Relatedness between strain GH2-10^T and *D. riboflavina* DSM 7230^T was 47%. This value was lower than the 70% value considered to be the threshold for the delineation of genomic species (Stackebrandt & Goebel, 1994), and clearly indicated that strain GH2-10^T represented a different species from *D. riboflavina*.

To test the presence of the nitrogen-fixing genes *nodC* and *nifH*, PCR was conducted according to the method of Rivas *et al.* (2002). The primer pairs for the amplification of the *nodC* and *nifH* genes were *nodCF* (5'-AYGTHGTGAYGACGGTTC-3') and *nodCI* (5'-CGYGACAGCCANTCKCTATTG-3') (Laguerre *et al.*, 2001), and 5'-GTCTCCTATGACGTGCTCGG-3' and 5'-GCTTCCATGGTGATCGGGT-3' (Rivas *et al.*, 2002), respectively. Whereas *R. leguminosarum* LMG 14904^T, *Ensifer fredii* LMG 6217^T, *Mesorhizobium loti* LMG 6125^T and *Bradyrhizobium japonicum* LMG 6138^T formed PCR fragments of the expected size, strain GH2-10^T and *D. riboflavina* DSM 7230^T did not.

On the basis of its phenotypic characteristics, which differ from those of recognized species of the genus *Devosia*, 16S rRNA gene sequence analysis and levels of DNA–DNA relatedness, strain GH2-10^T is considered to represent a novel species of the genus *Devosia*, for which we propose the name *Devosia soli* sp. nov.

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

The description is as given by Rivas *et al.* (2003), with the following amendments. Oxidase-positive or oxidase-negative. The G + C content of the DNA is 59–63 mol%.

Description of *Devosia soli* sp. nov.

Devosia soli (so'li. L. gen. n. *soli* of soil).

Cells are Gram-negative, obligately aerobic, non-spore-forming and rod-shaped (about 0.4–0.6 µm wide and 1.5–3.5 µm long). Colonies are light beige and round with clear margins after 2 days on R2A medium. Tolerates

up to 5% NaCl and grows at temperatures between 10 and 37 °C. Growth occurs at initial pH values between 4 and 8. Catalase-positive and oxidase-negative. Hydrolyses starch, CM-cellulose and urea, but not casein, DNA, gelatin or tyrosine. Contains C_{18:1ω7c} (67.2%), C_{16:0} (12.7%), 11-methyl C_{18:1ω7c} (5.8%) and C_{18:0} 3-OH (5.2%) as major fatty acids and Q-10 as the predominant ubiquinone. The DNA G + C content is 59.5 mol%. The closest relative on the basis of 16S rRNA gene sequence similarity is *Devosia riboflavina*.

The type strain, GH2-10^T (=KACC 11509^T=DSM 17780^T), was isolated from greenhouse soil planted with lettuce (*Lactuca sativa* L.) in Daejeon City, Korea.

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