Devosia geojensis sp. nov., isolated from diesel-contaminated soil in Korea

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A Gram-negative, strictly aerobic, non-spore-forming bacterium, designated strain BD-c194^T, was isolated from diesel-contaminated soil in Geoje, Korea. The cells were short, motile rods with single polar flagella. Growth of strain BD-c194^T was observed between 15 and 45 °C (optimally at 30–35 °C) and between pH 6.0 and 9.5 (optimally at pH 7.5–9.0). The predominant fatty acids were 11-methyl C_{18:1}ω7c, C_{16:0}, C_{18:1}ω7c, C_{18:0} and an unknown fatty acid (equivalent chain-length 18.814); a large amount of phosphatidylglycerol and a small amount of diphosphatidylglycerol were present as polar lipids. The G+C content of the genomic DNA was 60.8 mol% and the major isoprenoid quinone was Q-10. A comparative 16S rRNA gene sequence analysis showed that strain BD-c194^T formed a well-defined phyletic lineage within the genus *Devosia* (with 100 % bootstrap support). The levels of 16S rRNA gene sequence similarity with respect to the type strains of other *Devosia* species ranged from 95.0 to 96.1 %. On the basis of chemotaxonomic data and molecular properties, strain BD-c194^T represents a novel species within the genus *Devosia*, for which the name *Devosia geojensis* sp. nov. is proposed. The type strain is BD-c194^T (=KCTC 22082^T =DSM 19414^T).

The genus *Devosia* (family *Hyphomicrobiaceae*, order *Rhizobiales*, class *Alphaproteobacteria*) was first described by Nakagawa *et al.* (1996) with the transfer of '*Pseudomonas riboflavina*' (Foster, 1944) to *Devosia riboflavina*. At the time of writing, the genus comprises six species: *Devosia riboflavina* (Nakagawa *et al.*, 1996), *D. neptuniae* (Rivas *et al.*, 2003), *D. limi* (Vanparys *et al.*, 2005), *D. soli* (Yoo *et al.*, 2006), *D. insulae* (Yoon *et al.*, 2007) and *D. subaequoris* (Lee, 2007). In the course of the screening of micro-organisms from contaminated soil, we isolated a novel bacterium, designated strain BD-c194^T, belonging to the genus *Devosia*. Here we describe the polyphasic analysis used to determine its taxonomic position.

Diesel-contaminated soil was collected from the diesel-contaminated soil surface of a gas (petrol) station on Geoje Island, Korea. The total petroleum hydrocarbon content of the contaminated soil was about 2000 mg kg⁻¹. The soil

sample was serially diluted with a 1 % (w/v) saline solution and spread on R2A agar (Difco). After 5 days incubation at 25 °C, strain BD-c194^T was selected from an agar plate and analysed. Although strain BD-c194^T was isolated from diesel-contaminated soil, it did not grow on minimal agar medium (Stanier *et al.*, 1966) containing diesel as the sole carbon and energy source, showing that the strain is not a diesel degrader.

The morphological, physiological and biochemical characteristics of strain BD-c194^T were examined using routine cultivation on R2A medium at 30 °C for 3 days (except where indicated otherwise). The type strains of some related taxa (see Table 1) were used as reference strains for the biochemical tests.

The temperatures and pH for growth of strain BD-c194^T were investigated by growing the isolate on R2A agar at different temperatures (5–50 °C, in 5 °C increments) and in R2A broth adjusted to different pH values (pH 5.0–10.0, in increments of 0.5 pH units) (Gomori, 1955). Gram staining was performed using a bioMérieux Gram Stain kit according to the instructions of the manufacturer. Oxidase activity was tested by assessing the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck); catalase activity

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BD-c194^T is EF575560.

A transmission electron micrograph of negatively stained cells of strain BD-c194^T and a table comparing the cellular fatty acid compositions of strain BD-c194^T and type strains of related *Devosia* species are available as supplementary material with the online version of this paper.

Table 1. Differential physiological and biochemical characteristics of strain BD-c194^T and type strains of related *Devosia* species

Strains: 1, BD-c194^T; 2, *D. insulae* DS-56^T; 3, *D. riboflavina* IFO 13584^T; 4, *D. neptuniae* LMG 21357^T; 5, *D. limi* LMG 22951^T; 6, *D. soli* GH2-10^T. Data were obtained in this study unless indicated. All strains were Gram-negative, aerobic, non-spore-forming and positive for catalase. In the API 20NE and API ZYM test strips, all strains were positive for aesculin hydrolysis and for leucine arylamidase and β -glucosidase activities. All strains were negative for lipase (C14), cystine arylamidase, α -chymotrypsin and β -glucuronidase activities, indole production, nitrate reduction and the assimilation of caprate, adipate, malate, citrate and phenyl acetate. +, Positive; w, weakly positive; –, negative; NA, data not available.

Characteristic	1	2	3	4	5	6
Cell morphology	Rods	Ovals or rods ^{a*}	Rods ^b	Rods ^c	Rods ^d	Rods ^e
Motility	+	+ ^a	+ ^b	+ ^c	– ^d	NA
Colony colour†	WH	I ^a	C ^b	PWH ^c	LY to LBR ^d	LBE ^e
Oxidase	+	–	+	+	+	–
Urease	+	+	–	–	–	+
Hydrolysis of:						
Casein	+	–	–	+	–	–
Starch	–	–	+	–	–	+
L-Tyrosine	+	–	–	–	+	–
Enzyme activities						
Alkaline phosphatase	w	–	+	+	+	+
Esterase (C4)	+	+	+	–	+	+
Esterase lipase (C8)	w	+	+	–	+	+
Naphthol-AS-BI-phosphohydrolase	w	–	+	+	+	+
α -Galactosidase	–	–	+	+	–	–
α -Glucosidase	w	+	–	–	–	+
α -Mannosidase	–	–	+	+	–	+
α -Fucosidase	–	+	+	+	–	–
Assimilation of:						
D-Glucose, D-mannose, D-mannitol, maltose	+	–	+	+	–	–
L-Arabinose	–	–	+	+	–	–
N-Acetylglucosamine	+	–	+	–	–	–
DNA G + C content (mol%)	60.8	66.2 ^a	61.4 ^b	62.0 ^c	61.9 ^d	59.5 ^e

*Data taken from: a, Yoon *et al.*, 2007; b, Nakagawa *et al.*, 1996; c, Rivas *et al.*, 2003; d, Vanparys *et al.*, 2005; e, Yoo *et al.*, 2006.

†C, Cream; I, ivory; LBE, light beige; LBR, light brown; LY, light yellow; PWH, pearl white; WH, white.

was evaluated by determining the production of oxygen bubbles in a 3% (v/v) aqueous hydrogen peroxide solution. Cell morphology and motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) at different growth stages, as described by Jeon *et al.* (2005). Hydrolysis of casein, gelatin, Tween 80, Tween 20, aesculin, urea, tyrosine and starch was investigated on R2A agar after 7 days incubation, as described previously (Lanyi, 1987; Smibert & Krieg, 1994). Nitrate reduction was investigated according to the method of Lanyi (1987) and acid production from carbohydrates was tested as described by Leifson (1963). Additional enzyme activities and biochemical features were determined using API ZYM and API 20NE kits at 30 °C as recommended by the manufacturer (bioMérieux). Antibiotic-susceptibility tests were performed in duplicate using filter-paper discs (diameter, 8 mm) on R2A agar containing the following antibiotics: ampicillin (10 µg), polymyxin B (100 U), streptomycin (50 µg), penicillin G (10 IU), chloramphenicol (100 µg), gentamicin (30 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin

(15 µg), oleandomycin (15 µg), neomycin (30 µg), carbenicillin (100 µg) and novobiocin (50 µg).

Growth of strain BD-c194^T was observed at temperatures between 15 and 45 °C, with an optimum at 30–35 °C. The strain grew at pH 6.0–9.5, with an optimum at pH 7.5–9.0. All of the cells that were observed were rods (0.5–0.8 µm wide and 1.0–2.0 µm long), motile by means of single polar flagella (Supplementary Fig. S1, available in IJSEM Online). Anaerobic growth was not observed after 10 days incubation at 30 °C on R2A agar. Other phenotypic features of strain BD-c194^T are listed in the description of the novel species.

For the analysis of fatty acid methyl esters, cells of strain BD-c194^T and reference strains were harvested from agar plates after 3 days incubation on trypticase soy agar (TSA; Difco) at 25 °C. Analysis of the fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Analysis of isoprenoid quinones and polar lipids was carried out using the methods described by Komagata & Suzuki (1987). The

DNA G+C content of strain BD-c194^T was determined using an HPLC apparatus fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). The major cellular fatty acids of strain BD-c194^T were C_{16:0} (32.02 %), 11-methyl C_{18:1ω7c} (26.47 %) and C_{18:0} (14.42 %); this profile is similar to those of related type strains of members of the genus *Devosia* (Supplementary Table S1). The predominant polar lipid was phosphatidylglycerol, but a small amount of diphosphatidylglycerol was also present. The major respiratory lipoquinone of strain BD-c194^T was Q-10. The DNA G+C content of strain BD-c194^T was 60.8 mol%. The major fatty acids, major lipoquinone and polar lipids and DNA G+C content are in accordance with those of members of the genus *Devosia* (Nakagawa *et al.*, 1996; Rivas *et al.*, 2003; Vanparys *et al.*, 2005; Yoo *et al.*, 2006). A comparison of the typical phenotypic and chemotaxonomic properties of strain BD-c194^T with those of phylogenetically related relatives is shown in Table 1. Some of properties are similar to those of members of the genus *Devosia*, whereas others serve to differentiate strain BD-c194^T from closely related *Devosia* species.

Sequencing and assembly of the 16S rRNA gene were carried out as described previously (Lane, 1991). The resultant 16S rRNA gene sequence (1449 nt) of strain BD-c194^T was compared with 16S rRNA gene sequences available from GenBank by using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) to determine an approximate phylogenetic affiliation, and the gene sequence was then aligned with those of closely related species by using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees were constructed using three different methods, the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms, available in PHYLIP version

3.6 (Felsenstein, 2002). Values for sequence similarity between the isolate and related members of the genus *Devosia* were computed using SIMILARITY MATRIX version 1.1 (Ribosomal Database Project II; <http://35.8.164.52/html/>) (Cole *et al.*, 2003). A bootstrap analysis was performed according to the algorithm of Kimura's two-parameter model (Kimura, 1980) of the neighbour-joining method in the PHYLIP package. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BD-c194^T formed a well-defined phyletic lineage within the genus *Devosia* (with 100 % bootstrap support) (Fig. 1). The overall topology of the neighbour-joining tree was supported by the topologies of the trees built using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). A comparative 16S rRNA gene sequence analysis showed that the isolate was most closely related to *D. insulae* DS-56^T, *D. neptuniae* LMG 21357^T, *D. riboflavina* IFO 13584^T, *D. limi* LMG 22951^T and *D. soli* KACC 11509^T, with similarities of 96.1, 95.7, 95.4, 95.1 and 95.0 %, respectively; these levels of similarity conform to the criteria for classification as different species in the same genus (Rosselló-Mora & Amann, 2001; Stackebrandt *et al.*, 2002). Therefore, the physiological, biochemical and phylogenetic properties of strain BD-c194^T support its description as a novel species within the genus *Devosia*, for which the name *Devosia geojensis* sp. nov. is proposed.

Description of *Devosia geojensis* sp. nov.

Devosia geojensis (geo.jen'sis. N.L. fem. adj. *geojensis* pertaining to Geoje in Korea, the location of the soil sample from which the type strain was isolated).

Colonies are white, raised and circular with entire margins after 3 days on R2A agar. Cells are strictly aerobic, Gram-negative, short, motile rods (about 0.5–0.8 µm wide and

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain BD-c194^T and related taxa. Bootstrap percentages (based on 1000 replicates) greater than 50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Escherichia coli* ATCC 11775^T (GenBank accession no. X80725) was used as an outgroup (not shown). Bar, 0.1 changes per nucleotide position.

1.0–2.0 µm long) with single polar flagella. Growth occurs optimally at 30–35 °C and pH 7.5–9.0. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Aesculin, urea and L-tyrosine are hydrolysed, but casein, Tween 80, Tween 20, gelatin and starch are not. Produces acid from raffinose, *myo*-inositol, D-mannose, D-glucose, D-fructose, D-mannitol, L-arabinose, melibiose, lactose, arbutin and salicin, but not from D-galactose. Negative for indole production, D-glucose fermentation and arginine dihydrolase activity. Positive for assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetylglucosamine and maltose, but negative for assimilation of L-arabinose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid (API 20NE). Produces esterase (C4) and leucine arylamidase, but not lipase (C14), cystine arylamidase, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase. Weak enzyme activities are observed for alkaline phosphatase, esterase lipase (C8), α -glucosidase, β -glucosidase and *N*-acetyl- β -glucosaminidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β -galactosidase. Resistant to chloramphenicol, polymyxin B, streptomycin, gentamicin, kanamycin, novobiocin, oleandomycin, neomycin and lincomycin, but sensitive to ampicillin, penicillin G, tetracycline and carbenicillin. Contains a large amount of phosphatidylglycerol and a small amount of diphosphatidylglycerol as the polar lipids. The major isoprenoid quinone is Q-10. The major cellular fatty acids are 11-methyl C_{18:1}ω7c, C_{16:0} and C_{18:0}. The DNA G+C content of the type strain is 60.8 mol% (HPLC).

The type strain, BD-c194^T (=KCTC 22082^T =DSM 19414^T), was isolated from diesel-contaminated soil from Geoje, Korea.

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References

Cole, J. R., Chai, B., Marsh, T. L., Farris, R. J., Wang, Q., Kulam, S. A., Chandra, S., McGarrell, D. M., Schmidt, T. M. & other authors (2003). The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.

Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Foster, J. W. (1944). Microbiological aspects of riboflavin. I. Introduction. II. Bacterial oxidation of riboflavin to lumochrome. *J Bacteriol* **47**, 27–41.

Gomori, G. (1955). Preparation of buffers for use in enzyme studies. *Methods Enzymol* **1**, 138–146.

Jeon, C. O., Lim, J. M., Lee, J. M., Xu, L. H., Jiang, C. L. & Kim, C. J. (2005). Reclassification of *Bacillus haloalkaliphilus* Fritze 1996 as *Alkalibacillus haloalkaliphilus* gen. nov., comb. nov. and the description of *Alkalibacillus salilacus* sp. nov., a novel halophilic bacterium isolated from a salt lake in China. *Int J Syst Evol Microbiol* **55**, 1891–1896.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Lee, S. D. (2007). *Devosia subaequoris* sp. nov., isolated from beach sediment. *Int J Syst Evol Microbiol* **57**, 2212–2215.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.

Nakagawa, Y., Sakane, T. & Yokota, A. (1996). Transfer of “*Pseudomonas riboflavina*” (Foster 1944), a gram-negative, motile rod with long-chain 3-hydroxy fatty acids, to *Devosia riboflavina* gen. nov., sp. nov., nom. rev. *Int J Syst Bacteriol* **46**, 16–22.

Rivas, R., Willems, A., Subba-Rao, N. S., Mateos, P. F., Dazzo, F. B., Kroppenstedt, R. M., Martínez-Molina, E., Gillis, M. & Velázquez, E. (2003). Description of *Devosia neptuniae* sp. nov. that nodulates and fixes nitrogen in symbiosis with *Neptunia natans*, an aquatic legume from India. *Syst Appl Microbiol* **26**, 47–53.

Rosselló-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.

Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966). The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**, 159–271.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Vanparys, B., Heylen, K., Lebbe, L. & De Vos, P. (2005). *Devosia limi* sp. nov., isolated from a nitrifying inoculum. *Int J Syst Evol Microbiol* **55**, 1997–2000.

Yoo, S.-H., Weon, H. Y., Kim, B. Y., Hong, S.-B., Kwon, S.-W., Cho, Y.-H., Go, S.-J. & Stackebrandt, E. (2006). *Devosia soli* sp. nov., isolated from greenhouse soil in Korea. *Int J Syst Evol Microbiol* **56**, 2689–2692.

Yoon, J.-H., Kang, S.-J., Park, S. & Oh, T.-K. (2007). *Devosia insulae* sp. nov., isolated from soil, and emended description of the genus *Devosia*. *Int J Syst Evol Microbiol* **57**, 1310–1314.