Devosia pacifica sp. nov., isolated from deep-sea sediment

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A novel bacterial strain, NH131^T, was isolated from deep-sea sediment of South China Sea. Cells were strictly aerobic, Gram-stain negative, short rod-shaped and motile with a single lateral flagellum. Strain NH131^T grew optimally at pH 6.5–7.0 and 25–30 °C. 16S rRNA gene sequence analysis revealed that strain NH131^T belonged to the genus *Devosia*, sharing the highest sequence similarity with the type strain, *Devosia geojensis* BD-c194^T (96.2 %). The predominant fatty acids were $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, $C_{18:0}$ and $C_{16:0}$. Ubiquinone 10 was the predominant ubiquinone. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phospholipid, three glycolipids and two unknown lipids. The DNA G+C content of strain NH131^T was 63.0 mol%. On the basis of the results of polyphasic identification, it is suggested that strain NH131^T represents a novel species of the genus *Devosia* for which the name *Devosia pacifica* sp. nov. is proposed. The type strain is NH131^T (=JCM 19305^T=KCTC 32437^T).

The genus *Devosia* was first described by Nakagawa *et al.* (1996) as a result of the reclassification of '*Pseudomonas riboflavina*' (Foster, 1944). The genus accommodates Gram-stain negative, rod-shaped, aerobic, oxidase-positive bacteria containing ubiquinone 10 (Q-10) or Q-11 as the predominant respiratory quinone. The DNA G+C contents of bacteria of the genus *Devosia* range from 59.5 to 66.2 mol% (Yoon *et al.*, 2007). At the time of writing, the genus *Devosia* comprises 15 species with validly published names, which can be found in the LPSN (Parte, 2014), and the species '*Devosia lucknowensis*' (Dua *et al.*, 2013) with a non-validly published name. In our attempts to study bacterial diversity of deep-sea sediments in the South China Sea, the marine strain NH131^T was isolated. In this paper, the classification of strain NH131^T by a polyphasic approach based on physiological, chemotaxonomic and phylogenetic analyses is described.

Strain NH131^T was isolated from deep-sea sediment in the South China Sea. The sample of sediment was diluted with a tenfold dilution series method, spread on medium 702 (Nakagawa *et al.*, 1996), which contained (per litre) 10.0 g peptone, 2.0 g yeast extract, 1.0 g $MgSO_4.7H_2O$ and 15 g agar (if needed) (pH 7.0), and incubated at 28 °C. After 3 days of aerobic incubation, one white colony, designated NH131^T, was picked. The colony was slightly convex, shiny and ranged from 0.5 to 1.0 mm in diameter with a complete rim.

Cell morphology was examined by optical (BX40, Olympus) and transmission electron (JEM-1230, JEOL) microscopy using exponentially growing cells which were incubated in medium 702 for 24 h. The temperature range for growth of strain NH131^T was investigated on medium 702 at different temperatures (5–50 °C, in 5 °C increments) and the pH range was determined by adjusting the medium to pH 5.0–10.0 (in increments of 0.5 pH units) (Gomori, 1955). Growth under anaerobic conditions was determined after incubation of the novel strain in an anaerobic chamber on tryptic soy agar (TSA) supplemented with nitrate, both of which had been prepared anaerobically using nitrogen.

All the following biochemical and nutritional tests were performed on strains NH131^T, *Devosia geojensis* BD-c194^T and *Devosia riboflavina* DSM 7230^T. Single carbon source assimilation tests were performed using basal medium (Baumann *et al.*, 1984) supplemented with 0.1 g yeast extract l^{-1} and the corresponding filter-sterilized sugar solution (0.2 %, w/v, sugar final concentration), alcohol (0.2 %, v/v), organic acid (0.1 %, v/v) or amino acid solution (0.1 %, w/v). The basal medium contained (per litre distilled

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NH131^T is KF111722.

Two supplementary figures and one supplementary table are available with the online version of this paper.

water): 1.0 g NH_4Cl, 0.075 g $K_2HPO_4 . 3H_2O$, 0.028 g $FeSO_4 . 7H_2O$, 50 ml Tris/HCl (1 M, pH 7.5). Oxidase activity was tested by assessing the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck). Catalase activity was determined by bubble production in 3 % (v/v) H_2O_2 solution. Hydrolysis of casein, gelatin, Tween 80, Tween 60, Tween 40, Tween 20, aesculin, urea, tyrosine and starch was investigated on medium 702 after 7 days of incubation according to the methods of Lányí (1987) and Smibert & Krieg (1994). Acid production was tested by using API 50CH strips (bioMérieux). Leifson modified O/F medium (Leifson, 1963) was used to suspend the cells for inoculation of API 50CH tests. The strips were read after 24 h and 48 h. Additional physiological characteristics and enzyme activities were tested by using API ZYM and API 20NE kits at 30 °C as recommended by the manufacturer (bioMérieux). Antibiotic-susceptibility tests were performed on medium 702 with discs containing the following antibiotics: ampicillin (10 μg), carbenicillin (100 μg), chloramphenicol (100 μg), gentamicin (30 μg), kanamycin (30 μg), neomycin (30 μg), novobiocin (50 μg), penicillin G (10 U), polymyxin B (100 U), streptomycin (50 μg), tetracycline (30 μg) and vancomycin (30 μg).

Isoprenoid quinones were analysed by reversed-phase HPLC (Komagata & Suzuki, 1987). Fatty acid methyl esters were extracted from cells after incubating on medium 702 at 28 °C for 24 h and prepared according to the standard protocol of the Microbial Identification System (MIDI). Cellular polar lipids were extracted using a chloroform/methanol system and separated by two-dimensional TLC using silica gel 60 F 254 aluminium-backed thin-layer plates (Merck) (Kates, 1986). The solvent systems chloroform/methanol/water (65 : 24 : 4, by vol.) and chloroform/glacial acetic acid/methanol/water (80 : 12 : 15 : 4, by vol.) were used in the first and second dimensions, respectively. Separated components were visualized by treating the plates with 50 % (w/v) sulfuric acid ethanol solution followed by heating at 120 °C for 10 min. Zinzadze reagent was used to detect phospholipids.

Genomic DNA was obtained using the method described by Marmur (1961). The purified DNA was hydrolysed with P1 nuclease and the nucleotides were dephosphorylated with calf intestine alkaline phosphatase (Mesbah & Whitman, 1989). The DNA G+C content of the resulting deoxyribonucleosides was determined by reversed-phase HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT). Sequencing and assembly of the 16S rRNA gene were carried out as described previously (Xu *et al.*, 2007). The resultant 16S rRNA gene sequence (1445 nt) of strain NH131^T was compared with closely related sequences of reference organisms from the EzTaxon-e service (Kim *et al.*, 2012). 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 reconstructed using three different methods, neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971), with the MEGA 5 software package (Tamura *et al.*, 2011). Evolutionary distances for the three methods were calculated with the MEGA 5 program, on the basis of the algorithm of the Kimura two-parameter model (Kimura, 1980).

The cell morphology of strain NH131^T was similar to that of other strains representing the genus *Devosia*, which were also short rods (1.0–2.0 μm × 0.5–0.7 μm) (Fig. S1, available in the online Supplementary Material). Strain NH131^T possessed a lateral flagellum; however the flagella in other strains representing the genus *Devosia* are located at a polar end. Growth of strain NH131^T was observed at

Table 1. Characteristics that differentiate strain NH131^T from *D. geojensis* BD-c194^T and *D. riboflavina* DSM 7230^T

Strains: 1, NH131^T; 2, *D. geojensis* BD-c194^T; 3, *D. riboflavina* DSM 7230^T. +, Positive; −, negative; W, weakly positive. Unless stated otherwise, data were obtained from this study under identical growth conditions.

Characteristic	1	2	3
Pigment	White	White	Cream
Nitrate reductase	+	−	W
Hydrolysis of:			
Casein	+	+	−
Arginine	+	−	+
L-Tyrosine	−	+	−
Assimilation of:			
Fructose	+	+	−
Sucrose	−	+	−
Asparagine	−	+	+
Arginine	+	−	−
Sodium glutamate	−	+	+
Sodium malate	−	+	+
Rhamnose	−	+	+
Ribose	−	+	+
Arabinose	−	−	+
Salicin	−	+	+
Acid production from:			
D-Xylose	+	+	−
Glucose	+	+	−
Fructose	+	+	−
Sorbose	+	−	W
Inositol	−	+	−
Mannitol	−	+	−
Methyl α-D-mannose	+	W	−
N-acetylglucosamine	+	+	−
Amygdalin	+	−	−
Maltose	+	+	−
Lactose	+	+	−
D-Tagatose	+	−	+
Sucrose	+	+	−
Susceptibility to:			
Polymyxin B	−	+	+
Streptomycin	−	+	+
Gentamicin	−	+	+
Neomycin	+	+	−

15–45 °C, and the optimum growth temperature was 25–30 °C. The strain grew at pH 6.0–9.0, with optimum pH for growth at pH 6.5–7.0. Anaerobic growth was not observed after 10 days of incubation at 28 °C on medium 702. A comparison of the physiological and biochemical characteristics of strains NH131^T, *D. geojensis* BD-c194^T and *D. riboflavina* DSM 7230^T is given in Tables 1 and S1. In contrast to the reference strains, strain NH131^T utilized arginine but not asparagine, rhamnose, ribose, salicin, sodium glutamate or sodium malate, and produced acid from amygdalin, methyl α-D-mannose and sorbose. Strain NH131^T was resistant to gentamicin, polymyxin B and streptomycin whilst the reference strains were not. All strains were sensitive to ampicillin, carbenicillin, penicillin G, tetracycline and vancomycin, and all were resistant to chloramphenicol, kanamycin and novobiocin.

Detailed fatty acid compositions of strain NH131^T and reference strains are shown in Table S1. The fatty acid profile of strain NH131^T was similar to those of the reference strains, as the major components in strain NH131^T were $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, $C_{18:0}$ and $C_{16:0}$. Strain NH131^T could be distinguished from the reference strains by lower levels of $C_{16:0}$ and higher levels of $C_{18:0}$. Unlike *D. geojensis* BD-c194^T and *D. riboflavina* DSM 7230^T, strain NH131^T contained small amounts of $C_{17:1}\omega6c$ but did not contain $C_{17:1}\omega8c$. The polar lipid profiles of strain NH131^T and the two reference strains were similar, and included phosphatidylglycerol, diphosphatidylglycerol, phospholipid (PL1), three uncharacterized glycolipids and two uncharacterized lipids (L3, L4) (Fig. S2). The presence of L2 in strain *D. riboflavina* DSM 7230^T and L1 and PL2 in strain *D. geojensis* BD-c194^T enabled separation of these strains. The major respiratory quinone of strain NH131^T was Q-10 and the DNA G+C content was 63.0 mol%, which was in accordance with other members of the genus *Devosia*.

In the analysis of 16S rRNA gene sequences, based on the EzTaxon-e service, strain NH131^T was most closely related to *D. geojensis* BD-c194^T (96.2 %), and showed sequence similarities of less than than 96 % to other species. Phylogenetic trees reconstructed with all three treeing methods showed that strain NH131^T clustered with members of the genus *Devosia*, as supported by a high bootstrap resampling value (72 % by the neighbour-joining method). Within this cluster, strain NH131^T was found to be closely related to *D. geojensis* BD-c194^T (Fig. 1).

On the basis of the phenotypic, phylogenetic and chemotaxonomic characterization, strain NH131^T is considered to represent a novel species of the genus *Devosia*, for which the name *Devosia pacifica* sp. nov. is proposed.

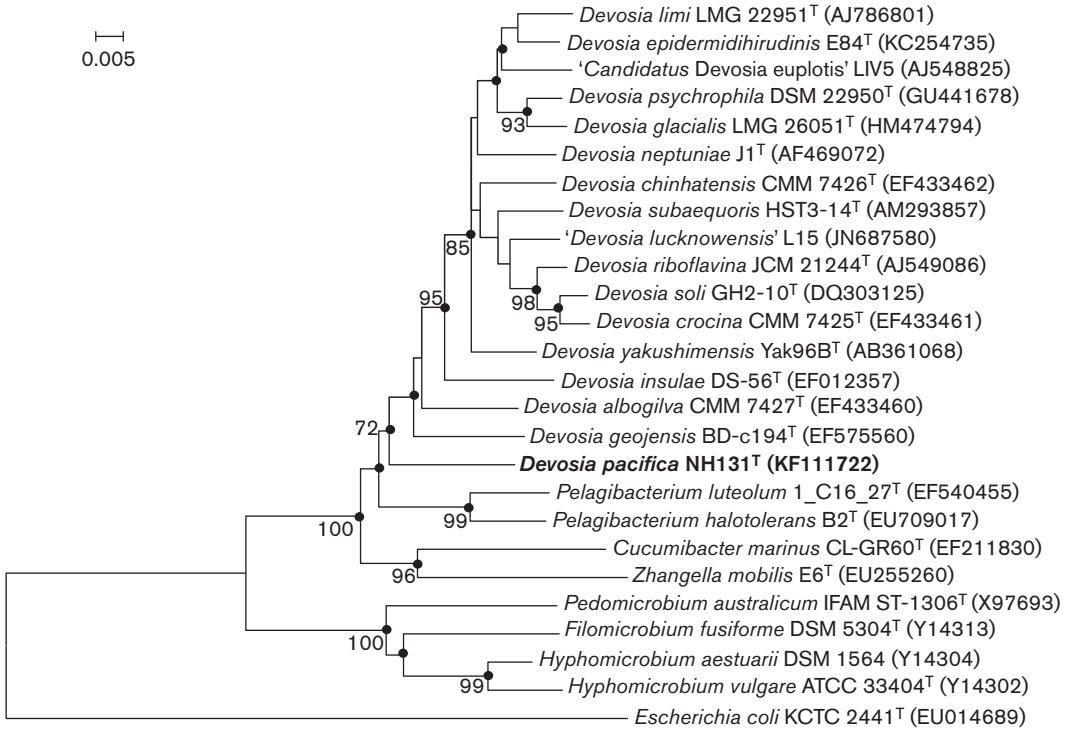

Fig. 1. Neighbour-joining tree using the Kimura two-parameter model, based on 16S rRNA gene sequences, showing the phylogenetic relationships of the novel isolate and related members of the genus *Devosia* and related taxa. Bootstrap values are based on 1000 replicates; values >70 % are shown. Filled circles indicate nodes also recovered in both maximum-likelihood and maximum-parsimony trees. Bar, 0.005 substitutions per nucleotide position.

Description of *Devosia pacifica* sp. nov.

Devosia pacifica (pa.ci′fi.ca. L. fem. adj. *pacifica* peaceful, pertaining to Pacific Ocean from where the organism was isolated).

Cells are aerobic, Gram-stain negative, motile rods, 1.0–2.0 μm long and 0.5–0.7 μm wide, and possess a lateral flagellum. Colonies on medium 702 are convex, shiny, white and circular with an entire margin after 3 days of incubation at 28 °C. Grows at pH 6.0–9.0 and 15–45 °C (optimal growth at pH 6.5–7.0 and 25–30 °C). Nitrate can be reduced to nitrite. Aesculin, gelatin, Tween 40, Tween 60 and urea can be hydrolysed, but casein, starch, Tween 80, Tween 20 and L-tyrosine cannot. Arginine dihydrolase and β-galactosidase activities are positive. Negative for DNA hydrolase, gluconate oxidation, glucose fermentation and indole production. The following substrates are utilized for growth: arginine, cellobiose, fructose, D-glucose, inositol, maltose, D-mannitol, raffinose, sodium acetate, sodium pyruvate, sorbitol, trehalose and xylose. The following compounds are not utilized as sole carbon sources: arabinose, arglycine, asparagine, benzene acid, lactose, D-mannose, melibiose, ribose, rhamnose, salicin, sodium citrate, sodium glutamate, sodium malate and sucrose. Susceptible to ampicillin, carbenicillin, gentamicin, penicillin G, polymyxin B, streptomycin, tetracycline and vancomycin; resistant to chloramphenicol, kanamycin, neomycin and novobiocin. Produces acid from *N*-acetylglucosamine, adonitol, aesculin, amygdalin, D-arabinose, D-arabitol, arbutin, cellobiose, dulcitol, fructose, L-fucose, gentiobiose, glucose, glucoside, glycerin, inulin, lactose, L-arabitol, D-lyxose, maltose, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannose, methyl β-D-xyloside, trehalose, L-arabinose, raffinose, ribose, salicin, sorbitol, sorbose, starch, sucrose, D-tagatose, turanose, D-xylose and L-xylose, but not from erythritol, D-fucose, galactose, gluconate, glycogen, inositol, mannitol, rhamnose or xylitol (API 50CH). Produces catalase and oxidase. According to API ZYM, *N*-acetyl-β-glucosaminidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase lipase (C8), α-galactosidase, α-glycosidase and β-glycosidase, β-glycuronate, naphthol-AS-BI-phosphohydrolase and valine arylamidase activities are present, esterase (C4), β-glucosidase and β-galactosidase activities are weak, whereas acid phosphatase, cystine arylamidase, leucine arylamidase, α-mannosidase and trypsin activities are absent. The predominant respiratory quinone is ubiquinone 10 (Q-10). Polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phospholipid, three unknown glycolipids and two unknown lipids. The major fatty acids are $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, $C_{18:0}$ and $C_{16:0}$.

The type strain, NH131^T (=JCM 19305^T=KCTC 32437^T), was isolated from a deep-sea sediment sample from the South China Sea. The DNA G+C content of the type strain is 63.0 mol% (HPLC).

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