Pseudosphingobacterium domesticum gen. nov., sp. nov., isolated from home-made compost

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A bacterial strain, DC-186^T, isolated from home-made compost, was characterized for its phenotypic and phylogenetic properties. The isolate was a Gram-negative rod that was able to grow at 15–36 °C and pH 5.5–8.0. Strain DC-186^T was positive in tests for catalase, oxidase and β-galactosidase activities and aesculin hydrolysis. The predominant fatty acids were the summed feature $C_{16:1}$/iso-$C_{15:0}$ 2-OH (42 %) and iso-$C_{15:0}$ (26 %), the major respiratory quinone was menaquinone-7 and the genomic DNA G+C content was 42 mol%. 16S rRNA gene sequence analysis and phenetic characterization indicated that this organism belongs to the phylum *Bacteroidetes* and revealed its affiliation to the family *Sphingobacteriaceae*. Of recognized taxa, strain DC-186^T was most closely related to *Sphingobacterium daejeonense* (90 % sequence similarity) based on 16S rRNA gene sequence analysis. The low 16S rRNA gene sequence similarity with other recognized taxa and the identification of distinctive phenetic features for this isolate support the definition of a new genus within the family *Sphingobacteriaceae*. The name *Pseudosphingobacterium domesticum* gen. nov., sp. nov. is proposed, with strain DC-186^T (=CCUG 54353^T=LMG 23837^T) as the type strain.

In a study of culturable heterotrophic bacteria in composts, a Gram-negative bacterium, strain DC-186^T, affiliated to the family *Sphingobacteriaceae*, was isolated from home-made compost. This strain presented a unique genotype (revealed through random amplified polymorphic DNA and repetitive extragenic palindromic-PCR analysis) among the bacterial isolates recovered from the same compost. Those organisms were predominantly members of the genus *Bacillus* and pseudomonads. Strain DC-186^T was further characterized using a polyphasic approach.

The bacterial isolate was recovered on Plate Count Agar (PCA) at 30 °C and purified by subculturing on the same medium. Long-term preservation was made in modified Luria–Bertani medium (MLB) (Tiago *et al.*, 2004), supplemented with 15 % (v/v) glycerol, at −80 °C. PCA was used for culture maintenance and biochemical and physiological tests were performed in MLB incubated at 30 °C. Colony and cell morphology, Gram-staining, cytochrome *c* oxidase and catalase tests, accumulation of poly-β-hydroxybutyrate granules and motility were analysed based on the methodologies of Murray *et al.* (1994) and Smibert & Krieg (1994). Growth and colony morphology on selective/differential agar media were tested on Membrane Faecal Coliform (m-FC), Eosin Methyl Blue (EMB) and MacConkey media. The ability to grow at pH 5.5, 8.0 and 9.0 was examined in culture medium supplemented with commercial buffers (12 mM MES, 12 mM TAPS and 12 mM CAPS, respectively; all from Sigma). Growth was also tested in MLB supplemented with 1, 3, 5 and 7 % (w/v) NaCl, at 15, 22, 30, 36 and 40 °C and under anaerobiosis (N_2-saturated atmosphere) in the presence of 0.1 % (w/v) KNO_3. The production of extracellular amylases, gelatinases and Tweenases (Tween 80) was tested as described by Tiago *et al.* (2004). Antibiotic susceptibility phenotypes were assayed as described previously (Ferreira da Silva *et al.*, 2006), following the interpretation criteria proposed by the Comité de l'Antibiogramme de la Société Française de Microbiologie (1998). Growth in mineral medium was tested in medium B (Barreiros *et al.*, 2003) supplemented with 4 mM $(NH_4)_2SO_4$ and 20 mM mannose in 100 ml screw-capped Erlenmeyer flasks containing 10 ml medium, incubated at 120 r.p.m. Other biochemical and nutritional tests were performed using the API 20E, API 20NE and API 50CH galleries (bioMérieux)

Abbreviation: FAMEs, fatty acid methyl esters.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of DC-186^T is AM407725.

according to the manufacturer's instructions. API 50CH carbon source utilization was examined using both the medium recommended to test acid production (50 CHB/E; bioMérieux) and mineral medium B with $(NH_4)_2SO_4$.

For determination of the genomic DNA G+C content, DNA was extracted based on the method of Cashion *et al.* (1977) and G+C ratios were estimated using the HPLC method as described by Mesbah *et al.* (1989). For analysis of respiratory quinones, freeze-dried cells were extracted according to Tindall (1989) and extracts were analysed by HPLC-UV (Knauer) at 260 nm with a Lichrosphere 5 μm RP-18 column (Merck). A methanol/hexane (75 : 25, v/v) mixture was used as mobile phase at a flow rate of 1 ml min^{-1}. Retention times were compared with known standards. Fatty acid methyl esters (FAMEs) were analysed in 24 h cultures on tryptic casein soy agar. Harvesting of the cells and preparation of FAMEs were performed as described by Kuykendall *et al.* (1988). The separation, identification and quantification of the individual FAMEs were made using the Microbial Identification System, Sherlock version 4.6 (MIS-MIDI). FAMEs were extracted and analysed twice.

The nucleic acid sequence of the 16S rRNA gene was determined after PCR amplification of total DNA extracts as described previously (Rainey *et al.*, 1996). The 16S rRNA gene sequence was compared with others available in GenBank/EMBL/DDBJ using BLASTN from NCBI and aligned with reference sequences. Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis (MEGA) software, version 3.1 (Kumar *et al.*, 2004). Sequence relatedness was estimated based on the model of Jukes & Cantor (1969) and the phylogenetic tree was created using the neighbour-joining method. Other methods, namely maximum-parsimony, minimum-evolution and unweighted pair group analysis with arithmetic mean, were used to assess tree stability. A total of 1193 nt positions in each 16S rRNA gene was included in the analysis. Non-homologous and ambiguous nucleotide positions were excluded from the calculations.

After 24 h incubation at 30 °C on PCA, strain DC-186^T formed yellowish, mucilaginous colonies with a diameter of 2–3 mm. White colonies were produced on EMB after 24 h incubation, whereas colonies were blue/dark on m-FC agar after 3 days. Growth did not occur on MacConkey agar after 7 days incubation. Cells were non-motile, Gram-negative, short rods. Growth occurred at 15–36 °C, with optimal growth at 30 °C. At 5 % NaCl, growth was slow and weak. No growth was observed at 40 °C, pH 9 or in 7 % NaCl. Strain DC-186^T did not require specific organic growth factors, namely vitamins or amino acids, as concluded from the fact that this organism presented strong growth in mineral medium with mannose as sole carbon source. Several sugars, but not organic acids, were assimilated by strain DC-186^T. Nitrate was reduced to nitrite, but not used as an alternative electron acceptor under anaerobic conditions. Strain DC-186^T was able to grow in the presence of cephalothin, ceftazidime, gentamicin, streptomycin, tetracycline and colistin sulphate, but not in the presence of ciprofloxacin, sulfamethoxazole, sulfamethoxazole/trimethoprim or meropenem. The major fatty acids of strain DC-186^T were summed feature 3 ($C_{16:1}\omega7c$/$C_{16:1}\omega6c$/iso-$C_{15:0}$ 2-OH) and iso-$C_{15:0}$, which accounted for about 70 % of the total (Table 1). The major respiratory quinone was menaquinone-7 and the G+C content of the genomic DNA was 42 mol%.

The genera *Pedobacter* and *Sphingobacterium* are members of the family *Sphingobacteriaceae* (Euzéby, 1997). Common phenotypic characteristics of members of the genera *Pedobacter* and *Sphingobacterium* are production of catalase and oxidase, hydrolysis of aesculin, the presence of β-galactosidase and utilization of a large number of carbohydrates and of few organic acids and amino acids (Steyn *et al.*, 1998; Kim *et al.*, 2006). Chemotaxonomic markers of these genera are the presence of MK-7 and sphingolipids, a genomic DNA G+C content of around 40 mol% and a characteristic FAME pattern (Steyn *et al.*, 1998; Kim *et al.*, 2006). They also have iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, iso-$C_{15:0}$ 3-OH, $C_{16:0}$, $C_{16:1}\omega7c$, $C_{16:0}$ 3-OH and iso-$C_{17:0}$ 3-OH as the predominant fatty acids. All these properties were observed in strain DC-186^T, thus confirming the affiliation

Table 1. Fatty acid contents of strain DC-186^T and type strains of related species

Taxa: 1, strain DC-186^T; 2, *Sphingobacterium daejeonense* LMG 23402^T (data from Kim *et al.*, 2006); 3, *Sphingobacterium spiritivorum* NBRC 14948^T (Steyn *et al.*, 1998); 4 *Pedobacter heparinus* LMG 10339^T (Steyn *et al.*, 1998). Values are percentages of total fatty acid methyl esters. Components that made up less than 1 % of the total are summed as 'Other' and included $C_{14:0}$ 2-OH, anteiso-$C_{15:0}$, $C_{15:1}\omega6c$, $C_{14:0}$ 3-OH/iso-$C_{16:1}$, $C_{15:0}$ 3-OH, iso-$C_{16:0}$ 3-OH, $C_{16:1}\omega5c$, $C_{16:1}$ 2-OH, iso-$C_{17:0}$ and $C_{20:0}$. –, Not detected; SF, identified as part of a summed feature.

Fatty acid	1	2	3	4
$C_{14:0}$	1.4	–	1.0	1.1
$C_{15:0}$	–	–	–	1.1
iso-$C_{15:0}$	25.9	45.6	30.1	28.2
iso-$C_{15:0}$ 2-OH	SF*	SF†	21.5	10.4
iso-$C_{15:0}$ 3-OH	2.0	1.5	2.2	2.5
$C_{16:0}$	7.6	3.4	3.5	3.0
$C_{16:0}$ 2-OH	2.8	–	–	–
$C_{16:0}$ 3-OH	3.3	–	2.7	1.5
$C_{16:1}\omega7c$	SF*	SF†	21.1	20.2
iso-$C_{17:0}$ 3-OH	11.1	16.6	12.5	15.2
iso-$C_{17:1}\omega9c$	1.3	2.9	1.7	6.3
Summed feature	42.2*	23.8†	–	–
Other	2.4	6.2	3.7	10.5

*Summed feature corresponds to $C_{16:1}\omega7c$/$C_{16:1}\omega6c$/iso-$C_{15:0}$ 2-OH (Sherlock MIS-MIDI, version 4.6).

†Summed feature corresponds to $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH (Kim *et al.*, 2006).

Table 2. Distinctive characteristics of strain DC-186^T and related species

Taxa: 1, strain DC-186^T; 2, *Sphingobacterium daejeonense* LMG 23402^T (data from Kim *et al.*, 2006); 3, *Sphingobacterium spiritivorum* NBRC 14948^T (Kim *et al.*, 2006; Steyn *et al.*, 1998); 4, *Pedobacter heparinus* LMG 10339^T (Steyn *et al.*, 1998). +, Positive; −, negative; NA, no available data; V, variable.

Characteristic	1	2	3	4
Growth at:				
42 °C	−	+	−	NA
pH 9.0	−	+	NA	NA
Nitrate reduction	+	NA	−	−
Hydrolysis of:				
Starch	+	−	+	NA
Aesculin	+	−	+	+
Gelatin	+	−	−	−
Acetoin production	−	+	NA	+
Acid production from:				
Sucrose	+	−	+	V
L-Arabinose	+	−	−	V
Assimilation of:				
L-Arabinose	+	−	−	+
D-Adonitol	−	+	−	+
L-Rhamnose	−	−	+	+
D-Mannitol	−	−	+	+
D-Raffinose	+	+	+	−
DNA G+C content (mol%)	42	39	40	42–43

of this isolate to the family *Sphingobacteriaceae* (Steyn *et al.*, 1998).

The distinctive phenotypic properties of strain DC-186^T (Table 2) and the low 16S rRNA gene sequence similarity observed between this organism and other genera of the family *Sphingobacteriaceae* support the proposal of a new genus. The closest phylogenetic neighbour of strain DC-186^T was '*Pocheonia soli*' (GenBank/EMBL/DDBJ accession no. AB267715), with 98 % 16S rRNA gene sequence similarity (D. An and S. Lee, unpublished results). Among recognized taxa, *Sphingobacterium daejeonense* (Kim *et al.*, 2006) was the closest relative to strain DC-186^T, with 90 % 16S rRNA gene sequence similarity. As in the present study, *S. daejeonense* TR6-04^T was isolated from a compost sample. Besides the low 16S rRNA gene sequence similarity between strain DC-186^T and its closest phylogenetic neighbours (Fig. 1), several phenotypic features enable this strain to be differentiated from its relatives (Table 2). These results support the description of a novel species, in a new genus, represented by isolate DC-186^T, for which the name *Pseudosphingobacterium domesticum* sp. nov. is proposed.

Description of *Pseudosphingobacterium* gen. nov.

Pseudosphingobacterium (Pseu.do′sphing.o.bac.ter.i.um. Gr. adj. *pseudes* false; N.L. neut. n. *Sphingobacterium* a bacterial generic name; N.L. neut. n. *Pseudosphingobacterium* false *Sphingobacterium*).

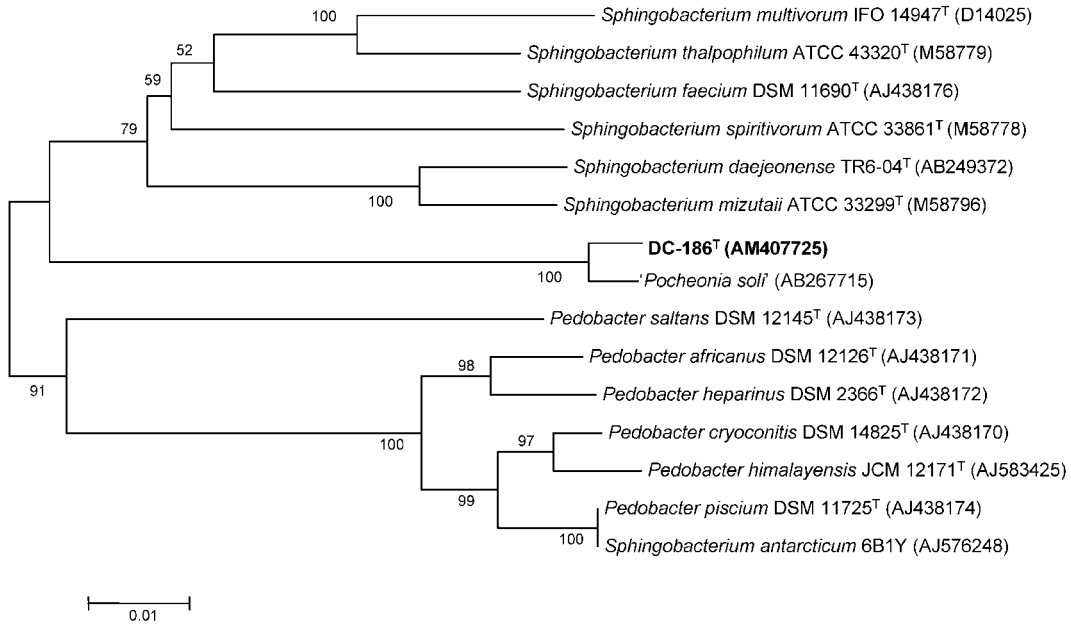

Fig. 1. Phylogenetic tree constructed using the neighbour-joining method based on 16S rRNA gene sequences showing the nearest neighbours of strain DC-186^T. Bootstrap values were generated from 10 000 resamplings. Bar, 1 substitution per 100 nt positions.

Cells are non-spore-forming, Gram-negative, non-motile rods. Poly-β-hydroxybutyrate inclusions are not observed. Positive for catalase, oxidase, aesculin hydrolysis and β-galactosidase. Negative for arginine dihydrolase, lysine and ornithine decarboxylases, urease, tryptophan deaminase, citrate utilization and H_2S, indole and acetoin production. Mesophilic. No specific growth factors are required. Nitrate is reduced to nitrite, but does not support anaerobic growth. Sugars are used as single carbon sources. Chemo-organoheterotrophic and strictly aerobic. Major respiratory quinone is menaquinone-7. Species of the genus have a DNA G+C content of around 40 mol%. The summed feature $C_{16:1}$/iso-$C_{15:0}$ 2-OH, iso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH are the predominant fatty acids. The type species is *Pseudosphingobacterium domesticum*.

Description of *Pseudosphingobacterium domesticum* sp. nov.

Pseudosphingobacterium domesticum (dom.es.ti.cum. L. neut. adj. *domesticum* of or belonging to the house).

Displays the following properties in addition to those given in the genus description. On nutritive non-selective medium, colonies are yellowish, smooth and mucilaginous. Cells are short rods (1.2–1.5 × 0.5–0.6 μm). Growth occurs on EMB and m-FC agar, but not on MacConkey agar. Growth occurs at 15–36 °C and pH 5.5–8.0, but not at 40 °C or pH 9. Growth occurs in the presence of 3 % NaCl, but not in 7 % NaCl. Growth may occur in the presence of the following antibiotics: cephalothin, ceftazidime, gentamicin, streptomycin, tetracycline and colistin sulphate. No growth in the presence of ciprofloxacin, sulfamethoxazole or meropenem. Gelatin and starch are hydrolysed, but Tween 80 is not hydrolysed. No specific nutritional growth factors such as vitamins or amino acids are required. Assimilation and acid production is positive for *N*-acetylglucosamine, amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannose, D-melibiose, methyl α-D-glucopyranoside, D-raffinose, sucrose, D-trehalose and D-xylose. D-Arabinose, arbutin and methyl-α-D-mannopyranoside support acid production, whereas salicin, starch and D-turanose are assimilated. Acid production or assimilation is negative for other API 50 CH and API 20NE carbon sources. The major fatty acid is summed feature $C_{16:1}$/iso-$C_{15:0}$ 2-OH. The G+C content of genomic DNA of strain DC-186^T is 42 mol%.

The type strain, DC-186^T (=CCUG 54353^T=LMG 23837^T), was isolated from home-made compost.

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