Brevundimonas lenta sp. nov., isolated from soil

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A Gram-negative, rod-shaped, *Brevundimonas*-like bacterial strain, DS-18^T, was isolated from soil in Dokdo, Korea, and its exact taxonomic position was investigated by using a polyphasic approach. Strain DS-18^T grew optimally at pH 6.5–7.0 and 25 °C without NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-18^T belonged to the genus *Brevundimonas*. Strain DS-18^T contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c and C_{16:0} as the major fatty acids. The DNA G + C content was 68.7 mol%. Strain DS-18^T exhibited levels of 16S rRNA gene sequence similarity of 96.3–98.7% to the type strains of *Brevundimonas* species and *Mycoplana bullata*. Mean DNA–DNA relatedness values between strain DS-18^T and the type strains of phylogenetically related *Brevundimonas* species and *M. bullata* were in the range 15–32%. Strain DS-18^T differed from *Brevundimonas* species and *M. bullata* in several phenotypic characteristics. On the basis of phenotypic, phylogenetic and genetic data, strain DS-18^T represents a novel species of the genus *Brevundimonas*, for which the name *Brevundimonas lenta* sp. nov. is proposed. The type strain is DS-18^T (=KCTC 12871^T =JCM 14602^T).

The genus *Brevundimonas*, which was proposed by reclassification of two *Pseudomonas* species by Segers *et al.* (1994), currently comprises 12 species with validly published names: *Brevundimonas diminuta* and *B. vesicularis* (Segers *et al.*, 1994), *B. alba*, *B. aurantiaca*, *B. bacterioides*, *B. intermedia*, *B. subvibrioides* and *B. variabilis* (Abraham *et al.*, 1999), *B. nasdae* (Li *et al.*, 2004), *B. mediterranea* (Fritz *et al.*, 2005), *B. kwangchunensis* (Yoon *et al.*, 2006a) and *B. terrae* (Yoon *et al.*, 2006b). Here, the taxonomic characterization is reported of a *Brevundimonas*-like bacterial strain, DS-18^T, which was isolated from a soil sample from Dokdo in Korea.

Strain DS-18^T was isolated by the standard dilution plating technique at 25 °C on 10 × diluted nutrient agar (NA; Difco). The type strains of eight *Brevundimonas* species and *Mycoplana bullata* were used as reference strains for DNA–DNA hybridization: *B. alba* LMG 18360^T, *B. aurantiaca* LMG 18359^T, *B. diminuta* LMG 2089^T, *B. intermedia* LMG 18361^T, *B. subvibrioides* LMG 14903^T and *B. variabilis* LMG 18362^T were obtained from the Laboratorium voor Microbiologie Universiteit Gent (LMG), Gent, Belgium; *B. kwangchunensis* KSL-102^T and *B. terrae* KSL-145^T were obtained from the studies of Yoon *et al.* (2006a, b); and

M. bullata DSM 7126^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The morphological, physiological and biochemical characteristics of strain DS-18^T were investigated using routine cultivation on NA at 25 °C. Cell morphology was examined by light microscopy (Nikon E600) 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, cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–40 °C) was measured on NA. Growth in the absence of NaCl 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 nutrient broth (NB; Difco) that was adjusted to various pH values (initial pH 4.5–10.5 at intervals 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 NA and on NA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were studied as described

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-18^T is EF363713.

Differential phenotypic characteristics of *Brevundimonas lenta* sp. nov. and related species are available as supplementary material with the online version of this paper.

Table 1. Biolog assimilation data for strain DS-18^T, several *Brevundimonas* species and *M. bullata*

Strains: 1, DS-18^T (*B. lenta* sp. nov.); 2, *B. diminuta* LMG 2089^T; 3, *B. vesicularis* LMG 2350^T; 4, *B. aurantiaca* LMG 18359^T; 5, *B. bacteroides* LMG 15096^T; 6, *B. intermedia* LMG 18361^T; 7, *B. subvibrioides* LMG 14903^T; 8, *B. nasdae* DSM 14572^T; 9, *B. mediterranea* LMG 21911^T; 10, *B. kwangchunensis* KSL-102^T (data in columns 2–10 taken from Yoon *et al.*, 2006a); 11, *B. terrae* KSL-145^T (Yoon *et al.*, 2006b); 12, *M. bullata* DSM 7126^T (data from this study). w, Weak. All strains are negative for assimilation of *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *i*-erythritol, L-fucose, *myo*-inositol, lactulose, D-mannitol, methyl β -D-glucoside, D-psicose, D-raffinose, D-sorbitol, sucrose, xylitol, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenyl ethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol and DL- α -glycerol phosphate. All strains are positive for assimilation of L-glutamic acid.

Substrate	1	2	3	4	5	6	7	8	9	10	11	12
α -Cyclodextrin	–	–	+	+	+	+	–	+	+	+	–	–
Dextrin	+	–	+	+	+	+	+	+	+	+	–	+
Glycogen	–	–	–	–	–	+	–	–	+	+	–	–
Tween 40	–	+	+	+	–	–	–	+	+	–	+	–
Tween 80	–	–	+	+	–	–	–	+	+	–	+	–
<i>N</i> -Acetyl-D-glucosamine	–	–	–	–	–	–	–	+	–	–	–	–
Cellobiose	–	–	+	+	+	+	+	+	+	+	–	–
D-Fructose	–	–	–	–	–	–	–	–	–	+	–	–
D-Galactose	–	–	+	–	–	+	–	+	–	–	–	–
Gentiobiose	–	–	–	–	–	+	–	+	+	w	–	–
D-Glucose	–	–	+	+	+	+	+	+	+	+	–	+
D-Lactose	–	–	–	–	–	+	–	–	–	–	–	–
Maltose	–	–	+	+	+	+	–	+	+	+	–	–
D-Mannose	–	–	–	–	–	+	–	–	–	+	–	–
D-Melibiose	–	–	–	–	–	+	–	+	–	–	–	–
L-Rhamnose	–	–	–	–	–	–	–	+	–	–	–	–
Trehalose	–	–	–	–	–	–	–	–	–	+	–	–
Turanose	–	–	–	–	–	–	–	–	–	+	–	w
Methylpyruvate	–	+	+	+	–	–	–	+	+	+	+	+
Monomethyl succinate	–	–	–	–	–	–	–	+	–	–	–	–
Acetic acid	–	+	+	–	–	+	–	+	+	–	–	–
α -Hydroxybutyric acid	–	–	+	–	–	–	–	+	–	–	–	–
β -Hydroxybutyric acid	–	+	+	+	+	+	–	+	+	+	+	+
α -Ketobutyric acid	–	–	–	–	–	–	–	+	–	–	–	+
α -Ketoglutaric acid	+	–	–	–	–	–	–	–	–	–	–	–
α -Ketovaleric acid	–	+	–	–	–	–	–	+	–	–	–	–
DL-Lactic acid	–	–	+	–	–	–	–	+	–	–	–	–
Propionic acid	–	–	–	–	–	–	–	+	–	–	+	–
Succinic acid	–	–	+	–	–	+	–	+	+	+	–	–
Bromosuccinic acid	–	–	+	–	–	–	–	–	–	–	–	–
Alaninamide	–	+	+	–	–	+	+	+	+	+	+	+
D-Alanine	–	+	+	–	–	–	–	+	–	–	+	+
L-Alanine	–	+	+	–	–	+	–	+	+	+	+	+
L-Alanyl glycine	–	+	+	–	–	+	–	+	+	+	+	+
L-Asparagine	–	+	–	–	–	–	–	+	–	+	+	+
L-Aspartic acid	–	+	–	–	–	–	–	+	–	+	+	+
Glycyl L-aspartic acid	–	+	–	–	–	–	–	+	–	–	+	+
Glycyl L-glutamic acid	–	+	+	+	–	+	+	+	+	+	+	+
L-Histidine	–	–	–	–	–	–	–	–	–	–	w	–
Hydroxy L-proline	–	+	–	–	–	–	–	–	–	–	+	–

Table 1. cont.

Substrate	1	2	3	4	5	6	7	8	9	10	11	12
L-Leucine	—	—	—	—	—	—	—	+	—	—	+	—
L-Proline	+	+	+	—	—	—	—	+	—	+	+	+
L-Serine	—	+	+	—	—	—	—	+	+	+	+	+
L-Threonine	—	—	+	—	—	—	—	+	+	+	+	+
Glucose 1-phosphate	—	—	+	—	—	—	—	—	—	+	—	+
Glucose 6-phosphate	—	—	—	—	—	—	—	—	—	+	—	+

previously (Lanyi, 1987). Assimilation of various substrates was determined by using the Biolog GN2 MicroPlate assay as recommended by the manufacturer. Sensitivity to antibiotics was tested on NA plates using antibiotic discs containing: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; and carbenicillin, 100 µg. Other physiological and biochemical properties were investigated using the API 20E and API ZYM systems (bioMérieux).

Cell biomass for DNA extraction and isoprenoid quinone analysis was obtained from cultures grown in NB at 25 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using 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 and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid analysis, cell mass of strain DS-18^T was harvested from NA plates after incubation for 7 days at 25 °C. Fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by 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 in each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain DS-18^T are given in the species description, Table 1 and Supplementary Table S1 (available in IJSEM Online). The almost-complete 16S rRNA gene

sequence of strain DS-18^T determined in this study comprised 1418 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. 16S rRNA gene sequence analyses showed that strain DS-18^T was most closely affiliated with members of the genus *Brevundimonas*. In the phylogenetic tree based on the neighbour-joining algorithm, strain DS-18^T fell within the cluster comprising *Brevundimonas* species and *M. bullata* (Fig. 1). Strain DS-18^T exhibited 16S rRNA gene sequence similarity values of 96.3–98.7 % to the type strains of *Brevundimonas* species and *M. bullata*, and less than 95.1 % to other species included in the phylogenetic analysis (Fig. 1).

Chemotaxonomic properties confirm the phylogenetic affiliation of strain DS-18^T to the genus *Brevundimonas*. The predominant isoprenoid quinone detected in strain DS-18^T was ubiquinone-10 (Q-10), at a peak area ratio of approximately 84 %; a significant amount of Q-8 (approx.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain DS-18^T, *Brevundimonas* species and some other related taxa (GenBank accession numbers are given in parentheses). Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

13%) was also present. Q-10 has been reported as the predominant ubiquinone in *Brevundimonas* species (Segers *et al.*, 1994; Abraham *et al.*, 1999; Li *et al.*, 2004; Fritz *et al.*, 2005; Yoon *et al.*, 2006a, b). Strain DS-18^T had a cellular fatty acid profile that contained large amounts of unsaturated, straight-chain and 11-methyl fatty acids; the major fatty acids (>10% total fatty acids) were C_{18:1}ω7c and C_{16:0} (Table 2). This fatty acid profile was similar to those of *Brevundimonas* species (Table 2). The DNA G+C content of strain DS-18^T was 68.7 mol%.

Strain DS-18^T exhibited mean DNA–DNA relatedness levels of 15–32% to the type strains of *B. alba* (17%), *B. aurantiaca* (15%), *B. diminuta* (17%), *B. intermedia* (22%), *B. kwangchunensis* (32%), *B. subvibrioides* (30%), *B. terrae* (18%), *B. variabilis* (21%) and *M. bullata* (27%), all of which showed 16S rRNA gene sequence similarity values greater than 97% to strain DS-18^T. Strain DS-18^T was distinguishable from recognized *Brevundimonas* species by differences in several phenotypic characteristics

(Table 1 and Supplementary Table S1). The phylogenetic distinctiveness, together with genetic data and differential phenotypic properties, were sufficient to allocate strain DS-18^T to a species that is separate from recognized *Brevundimonas* species and *M. bullata* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-18^T should be placed in the genus *Brevundimonas* as a representative of a novel species, for which the name *Brevundimonas lenta* sp. nov. is proposed.

Description of *Brevundimonas lenta* sp. nov.

Brevundimonas lenta (len'ta. L. fem. adj. *lenta* sticky, referring to sticky colonies).

Cells are Gram-negative rods (0.3–0.5 × 0.6–3.5 μm). Motile by means of a single polar flagellum. Colonies on NA are circular, convex, glistening, sticky, greyish-yellow in colour and 0.8–1.0 mm in diameter after 7 days incubation

Table 2. Cellular fatty acid compositions (%) of strain DS-18^T and the type strains of *Brevundimonas* species

Strains: 1, strain DS-18^T (*B. lenta* sp. nov.); 2, *B. terrae* KSL-145^T; 3, *B. kwangchunensis* KSL-102^T; 4, *B. diminuta* LMG 2089^T; 5, *B. vesicularis* LMG 2350^T; 6, *B. subvibrioides* LMG 14903^T; 7, *B. bacterioides* LMG 15096^T; 8, *B. aurantiaca* LMG 18359^T; 9, *B. alba* LMG 18360^T; 10, *B. variabilis* LMG 18362^T (data in columns 2–10 from Yoon *et al.*, 2006b); 11, *B. nasdae* W1-2B^T; 12, *B. intermedia* DSM 4732^T (data in columns 11 and 12 from Li *et al.*, 2004); 13, *B. mediterranea* V4.BO.10^T (Fritz *et al.*, 2005). –, Not detected. Fatty acids that represented <0.5% in all strains were omitted.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
Straight-chain													
C _{14:0}	2.0	5.2	2.6	0.5	3.1	3.6	3.7	3.3	1.2	2.9	1.7	1.5	0.6
C _{15:0}	5.8	5.2	6.4	8.4	3.5	6.4	3.4	2.6	4.7	7.8	—	2.8	5.4
C _{16:0}	12.8	26.6	20.5	8.3	20.0	16.1	19.3	24.6	18.8	14.0	19.7	24.3	16.1
C _{17:0}	2.2	1.3	1.8	7.5	1.2	3.1	1.5	2.8	2.7	4.9	3.8	1.6	6.1
C _{18:0}	0.3	0.4	0.4	0.2	0.4	0.5	0.4	1.3	0.7	0.6	1.0	<1.0	0.9
Branched													
iso-C _{15:0}	—	0.1	—	—	—	—	—	0.1	—	—	—	—	—
Unsaturated													
C _{15:1} ω8c	0.2	0.5	0.3	2.6	—	0.2	0.1	—	0.4	0.6	—	—	—
C _{16:1} ω9c	—	—	—	—	—	—	0.4	—	1.9	—	—	—	8.4*
C _{17:1} ω6c	3.3	0.7	1.0	7.0	1.2	1.9	2.0	1.2	1.6	4.0	2.0	1.1	7.8
C _{17:1} ω8c	7.3	1.4	3.1	12.8	2.1	4.4	3.4	1.6	6.0	8.9	2.6	2.0	9.9
C _{18:1} ω7c	46.4	43.4	33.2	40.0	53.6	52.1	57.5	49.1	42.9	47.9	—	—	36.7*
C _{18:1} ω9c	—	—	—	—	—	—	0.2	—	1.3	—	—	—	—
Hydroxy													
C _{12:0} 3-OH	2.6	2.8	2.2	1.9	2.5	2.5	2.8	2.5	1.5	2.7	3.1	1.7	2.0
C _{16:0} 2-OH	—	—	0.3	—	—	0.9	—	0.4	0.3	0.3	—	—	—
Other													
11-Methyl C _{18:1} ω7c	8.9	7.2	22.1	—	4.9	1.2	0.4	7.9	6.3	—	—	—	4.2*
cyclo C _{19:0} ω8c	—	—	—	6.6	—	—	—	—	4.8	—	7.6	—	—
Summed features†													
1	0.6	0.2	0.3	1.2	0.2	0.5	0.2	0.2	0.8	0.9	—	—	—
3	6.3	4.0	5.3	1.4	7.2	4.1	3.8	2.2	3.5	3.1	2.5	7.5	—
7	—	—	—	—	—	—	—	—	—	—	51.6	49.4	—

*Referred to as C_{16:1}, C_{18:1} and 11-methyl C_{18:1}ω5t, respectively, by Fritz *et al.* (2005).

†Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 contains iso-C_{15:1} and/or C_{13:0} 3-OH. Summed feature 3 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 7 contains one or more of C_{18:1}ω7c, C_{18:1}ω9t and C_{18:1}ω12t.

at 25 °C. Optimal temperature for growth is 25 °C. Growth occurs at 4 and 34 °C, but not at 35 °C. Optimal pH for growth is between 6.5 and 7.0; growth occurs at pH 6.0 and 9.5, but not at pH 5.5 or 10.0. Growth occurs in the presence of 0–1% (w/v) NaCl; optimal growth occurs without NaCl. Anaerobic growth does not occur on NA or NA supplemented with nitrate. Tweens 20, 40 and 60 are hydrolysed, but casein, hypoxanthine, xanthine and tyrosine are not. H₂S and indole are not produced. Lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but lipase (C14), valine arylamidase, cystine arylamidase, α -chymotrypsin, α -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. Susceptible to polymyxin B, chloramphenicol, cephalothin, gentamicin, novobiocin, tetracycline, kanamycin and neomycin, but not to ampicillin, carbenicillin, lincomycin or oleandomycin. The predominant ubiquinone is Q-10. The major fatty acids (>10% of total fatty acids) are C_{18:1}ω7c and C_{16:0}. Other phenotypic characteristics are given in Table 1 and Supplementary Table S1.

The type strain is DS-18^T (=KCTC 12871^T =JCM 14602^T), isolated from soil from Dokdo, Korea. The DNA G+C content of the type strain is 68.7 mol% (determined by HPLC).

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