Brevundimonas terrae sp. nov., isolated from an alkaline soil in Korea

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A Gram-negative, rod-shaped, *Brevundimonas*-like bacterial strain, KSL-145^T, was isolated from an alkaline soil in Korea and subjected to a polyphasic taxonomic investigation. Strain KSL-145^T grew optimally at pH 7.5–8.0 and 30 °C without NaCl. It was characterized chemotaxonomically as containing Q-10 as the predominant ubiquinone and C_{18:1ω7c} and C_{16:0} as the major fatty acids. The DNA G + C content was 61.8 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain KSL-145^T fell within the radiation of the cluster comprising *Brevundimonas* species and *Mycoplana bullata*. The levels of 16S rRNA gene sequence similarity between strain KSL-145^T and the type strains of *Brevundimonas* species and *M. bullata* ranged from 95.3 to 98.7%. The mean DNA–DNA relatedness values between strain KSL-145^T and the type strains of *Brevundimonas diminuta* and *M. bullata*, the closest phylogenetic relatives, were 26 and 15%, respectively. Strain KSL-145^T could be differentiated from *Brevundimonas* species and *M. bullata* by differences in several phenotypic characteristics. On the basis of the phenotypic, phylogenetic and genetic data, strain KSL-145^T represents a novel species in the genus *Brevundimonas*, for which the name *Brevundimonas terrae* sp. nov. is proposed. The type strain is KSL-145^T (=KCTC 12481^T=JCM 13476^T).

The genus *Brevundimonas* was proposed through the reclassification of two *Pseudomonas* species as *Brevundimonas diminuta* and *Brevundimonas vesicularis* (Segers *et al.*, 1994). At the time of writing, the genus comprises 11 species with validly published names: *B. diminuta* and *B. vesicularis* (Segers *et al.*, 1994), *B. alba*, *B. aurantiaca*, *B. bacteroides*, *B. intermedia*, *B. subvibrioides* and *B. variabilis* (Abraham *et al.*, 1999), *B. nasdae* (Li *et al.*, 2004), *B. mediterranea* (Fritz *et al.*, 2005) and *B. kwangchunensis* (Yoon *et al.*, 2006). In this study, we report on the taxonomic characterization of a *Brevundimonas*-like bacterial strain, KSL-145^T, isolated from an alkaline soil (approximate pH 9.0–10.0) in Korea.

Strain KSL-145^T was isolated using the standard dilution plating technique at 30 °C on 10 × diluted nutrient agar (Difco) adjusted to pH 10.0. *B. diminuta* LMG 2089^T, which was used as a reference strain for DNA–DNA hybridization, was obtained from the Laboratorium voor Microbiologie, Universiteit Gent (Ghent, Belgium). *Mycoplana bullata* DSM 7126^T, which was used as a reference strain for DNA–DNA hybridization and phenotypic characterization, was obtained

from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The cell morphology was examined using light microscopy (E600; Nikon) and transmission electron microscopy. The presence of flagella was determined by transmission electron microscopy using cells from exponentially growing cultures. For the transmission electron microscopic observation, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined (after air-drying) with a Philips CM-20 transmission electron microscope. 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 trypticase soy agar (TSA; Difco). Growth in the absence of NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that no NaCl was used. Growth at various NaCl concentrations (0.5%, w/v, and 1.0–5.0%, w/v, using increments of 1.0%) was investigated in trypticase soy broth (Difco). The pH range for growth was determined in nutrient broth (Difco) adjusted to various pH values (initial pH 4.5–10.5, using increments 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 TSA and on TSA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and the hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine,

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Biolog assimilation data and cellular fatty acid compositions for strain KSL-145^T and related taxa are available as supplementary material in IJSEM Online.

urea and xanthine were determined as described by Cowan & Steel (1965). The hydrolysis of aesculin and the reduction of nitrate were studied as described previously (Lanyi, 1987). The assimilation of various substrates was determined by using the Biolog GN2 MicroPlate assay as recommended by the manufacturers. Sensitivity to antibiotics was tested on TSA plates, using antibiotic discs containing the following amounts (μg , unless otherwise indicated): polymyxin B, 100 U; streptomycin, 50; penicillin G, 20 U; chloramphenicol, 100; ampicillin, 10; cephalothin, 30; gentamicin, 30; novobiocin, 5; tetracycline, 30; kanamycin, 30; lincomycin, 15; oleandomycin, 15; neomycin, 30; and carbenicillin, 100. Enzyme activity was determined by using the API ZYM system (bioMérieux). Other physiological and biochemical tests were performed with the API 20E system (bioMérieux).

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in trypticase soy broth at 30 °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 performing a PCR with two universal primers as described previously (Yoon *et al.*, 1998). The sequencing of the amplified 16S rRNA gene and the 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 $\times$ 4.6 mm) column. For fatty acid analysis, cell mass of strain KSL-145^T was harvested from TSA plates after incubation for 3 days at 30 °C. The fatty acids were extracted and the fatty acid methyl esters prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by using the method of Tamaoka & Komagata (1984), with the 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. Five replicate hybridizations were performed for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values.

The morphological, cultural, physiological and biochemical characteristics of strain KSL-145^T are given in the species description (see below) or are shown in Table 1 or Supplementary Table S1 (available in IJSEM Online). The almost-complete 16S rRNA gene sequence of strain KSL-145^T determined in this study comprised 1418 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. The 16S rRNA gene sequence analyses showed that strain KSL-145^T is phylogenetically most closely related to *Brevundimonas* species and *M. bullata* (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain KSL-145^T joined the type strain of *B.*

diminuta at a bootstrap confidence value of 99.8 %, and this cluster joined the type strain of *M. bullata* at a bootstrap confidence value of 95.6 % (Fig. 1). The relationship between the cluster comprising strain KSL-145^T, *Brevundimonas* species and *M. bullata* and the cluster comprising *Caulobacter* species was supported by a relatively high bootstrap resampling value (79.0 %) (Fig. 1). Strain KSL-145^T exhibited 16S rRNA gene sequence similarity values of 98.7 and 98.0 % with respect to *B. diminuta* LMG 2089^T and *M. bullata* IAM 13153^T, respectively, and values of 95.3–96.9 % with respect to the other *Brevundimonas* species. The sequence similarities with respect to all other species included in the phylogenetic analysis were below 94.9 % (Fig. 1).

The results obtained from chemotaxonomic analyses were consistent with the results of 16S rRNA gene sequence analysis. The predominant isoprenoid quinone detected in strain KSL-145^T was Q-10, at a peak-area ratio of approximately 86 %; a minor amount of Q-8 (approx. 9 %) was also present. This predominant ubiquinone type was the same as those of *Brevundimonas* species (Segers *et al.*, 1994; Abraham *et al.*, 1999; Li *et al.*, 2004; Fritz *et al.*, 2005; Yoon *et al.*, 2006). The major fatty acids in strain KSL-145^T were C_{18:1 ω 7c} and C_{16:0}. This fatty acid profile was similar to those of *Brevundimonas* species (Abraham *et al.*, 1999; Li *et al.*, 2004; Fritz *et al.*, 2005) (see Supplementary Table S2 available in IJSEM Online). The DNA G+C content of strain KSL-145^T was 61.8 mol%, which is rather lower than those of recognized *Brevundimonas* species (Table 1).

Strain KSL-145^T exhibited mean DNA–DNA relatedness levels of 26 and 15 % with respect to *B. diminuta* LMG 2089^T and *M. bullata* DSM 7126^T, respectively, strains that showed 16S rRNA gene sequence similarity values of more than 97 % to strain KSL-145^T. This value indicates that strain KSL-145^T is a member of a genomic species that differs from *B. diminuta* and *M. bullata* (Wayne *et al.*, 1987). Strain KSL-145^T differed from the other *Brevundimonas* species, as well as from *B. diminuta* and *M. bullata*, in terms of several phenotypic characteristics (Table 1). The phylogenetic and genetic distinctiveness and differential phenotypic properties were sufficient to categorize strain KSL-145^T as a member of a species that is distinct from previously recognized *Brevundimonas* species and from *M. bullata* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). In the phylogenetic tree based on 16S rRNA gene sequences, *M. bullata* was more closely related to the genus *Brevundimonas* than to the clade comprising *Mycoplana dimorpha*, the type species of the genus *Mycoplana*. *M. bullata* may have to be reclassified as a member of another genus – perhaps the genus *Brevundimonas* – but such reclassification might not be appropriate at this time. Therefore, on the basis of the data presented, strain KSL-145^T should be classified within the genus *Brevundimonas* as a member of a novel species, for which the name *Brevundimonas terrae* sp. nov. is proposed.

Table 1. Differential phenotypic characteristics of strain KSL-145^T, *Brevundimonas* species and *M. bullata*

Taxa: 1, strain KSL-145^T; 2, *B. kwangchunensis* (data from Yoon *et al.*, 2006); 3, *B. diminuta* (Palleroni, 1984; Segers *et al.*, 1994; Li *et al.*, 2004); 4, *B. vesicularis* (Palleroni, 1984; Segers *et al.*, 1994; Li *et al.*, 2004); 5, *B. alba* (Poindexter, 1964); 6, *B. aurantiaca* (Poindexter, 1964; Li *et al.*, 2004); 7, *B. bacteroides* (Poindexter, 1964); 8, *B. intermedia* (Poindexter, 1964; Li *et al.*, 2004); 9, *B. subvibrioides* (Poindexter, 1964); 10, *B. variabilis* (Poindexter, 1964); 11, *B. nasdae* (Li *et al.*, 2004); 12, *B. mediterranea* (Fritz *et al.*, 2005); 13, *M. bullata* (unless indicated, data from Urakami *et al.*, 1990). Symbols: +, positive; –, negative; ND, no data available; W, weakly positive; V, variable; NG, no growth. Data in parentheses are for the type strain.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Oxidase	+	+	+*	+	ND	(+)	ND	(+)	ND	ND	+	ND	+ or W
Catalase	+	+	+	+	ND	(+)	ND	(+)	ND	ND	+	ND	W or –
Urease	–	–	–*	–	ND	(–)	ND	(–)	ND	ND	–	V (–)	NG
Arginine dihydrolase	–	–	–*	–	ND	(–)	ND	(–)	ND	ND	–	ND	ND
β -Galactosidase	–	–	–	–	ND	(–)	ND	(+)	ND	ND	+	–	ND
Nitrate reduction	–	–	–	–	–	–	V	–	–	–	–	–	W or –
Hydrolysis of:													
Aesculin	–	+	–	+	ND	(+)	ND	(+)	ND	ND	+	+	(–)†
Gelatin	–	V (–)	–*	–*	ND	(+)	ND	(–)	ND	ND	–	–	(–)†
Starch	–	–	–	–	–	+	+	+	+	V	–	ND	(–)†
Tween 80	+	+	–	–	ND	ND	ND	ND	ND	ND	–	ND	(–)†
Utilization of:‡													
Cellobiose	–	+	(–)	(+)	ND	(+)	(+)	(+)	(+)	ND	(+)	(+)	(+)
D-Fructose	–	V (+)	(–)	(–)	–	(–)	(–)	(–)	(–)	–	(–)	(–)	(+)
D-Galactose	–	–	(–)	(+)	+	(–)	(–)	(+)	(–)	+	(+)	(–)	(+)
D-Glucose	–	+	(–)	(+)	+	(+)	(+)	(+)	(+)	+	(+)	(+)	(+)
Lactose	–	–	(–)	(–)	–	(–)	(–)	(+)	(–)	–	(–)	(–)	(+)
Maltose	–	+	(–)	(+)	+	(+)	(+)	(+)	(–)	+	(+)	(+)	(+)
D-Mannose	–	+ or W	(–)	(–)	V	(–)	(–)	(+)	(–)	–	(–)	(–)	(+)
D-Trehalose	–	+	(–)	(–)	ND	(–)	(–)	(–)	(–)	ND	(–)	(–)	(+)
Turanose	–	+ or W	(–)	(–)	ND	(–)	(–)	(–)	(–)	ND	(–)	(–)	(+)
Tween 80	+	–	(–)	(+)	ND	(+)	(–)	(–)	(–)	ND	(+)	(+)	(+)
Acetic acid	–	–	(+)	(+)	+	(–)	(–)	(+)	(–)	+	(+)	(+)	(+)
α -Ketovaleic acid	–	–	(+)	(–)	ND	(–)	(–)	(–)	(–)	ND	(+)	(–)	(+)
Lactic acid	–	–	(–)	(+)	–	(–)	(–)	(–)	(–)	–	(+)	(–)	(+)
Propionic acid	+	–	(–)	(–)	ND	(–)	(–)	(–)	(–)	ND	(+)	(–)	(+)
Succinic acid	–	V (+)	(–)	(+)	+	(–)	(–)	(+)	(–)	+	(+)	(+)	(+)
L-Aspartic acid	+	V (+)	(+)	(–)	+	(–)	(–)	(–)	(–)	–	(+)	(–)	(+)
L-Glutamic acid	+	V (+)	(+)	(+)	+	(+)	(+)	(+)	(+)	+	(+)	(+)	(+)
L-Leucine	+	–	(–)	(–)	V	(–)	(–)	(–)	(–)	+	(+)	(–)	(+)
L-Proline	+	V (+)	(+)	(+)	V	(–)	(–)	(–)	(–)	+	(+)	(–)	(+)
L-Threonine	+	+	(–)	(+)	ND	(–)	(–)	(–)	(–)	ND	(+)	(+)	(+)
Glucose 1-phosphate	–	+	(–)	(+)	ND	(–)	(–)	(–)	(–)	ND	(–)	(–)	(+)
Glucose 6-phosphate	–	+	(–)	(–)	ND	(–)	(–)	(–)	(–)	ND	(–)	(–)	(+)
Susceptibility to:													
Streptomycin	+	+	ND	ND	+	+	+	+	+	+	ND	ND	(+)†
Penicillin G	–	+	ND	ND	+	+	+	+	+	+	ND	ND	(–)†
Occurrence of cyclo C _{19:0} ω8c	–	–	+	–	+	–	–	–	–	–	+	–	(–)
DNA G+C content (mol%)	61.8	68.4–68.7	66–68	65–66	ND	(65.6)	66§	(66.1)	67§	ND	66.5	67.1–67.5	66–68

*Result reported by Palleroni (1984) and Segers *et al.* (1994); different result for the type strain obtained by Li *et al.* (2004).

†Data from this study.

‡Data from Biolog GN2 MicroPlate assay taken from Yoon *et al.* (2006); data for *M. bullata* taken from this study for the type strain; data for *B. alba* and *B. variabilis* taken from Poindexter (1964).

§Determined for one isolate by Poindexter (1989).

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain KSL-145^T, *Brevundimonas* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50% are shown at branch points. *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

Description of *Brevundimonas terrae* sp. nov.

Brevundimonas terrae (ter'rae. L. gen. n. *terrae* of the soil).

Cells are Gram-negative, aerobic rods (0.4–0.6 × 1.0–3.0 µm). Motile by means of a single polar flagellum. Colonies on TSA are circular, smooth, glistening, slightly convex, greyish-yellow in colour and 2.0–3.0 mm in diameter after 3 days incubation at 30 °C. Optimal temperature for growth is 30 °C; growth occurs at 4 and 37 °C, but not at 38 °C. Optimal pH for growth is between 7.5 and 8.0; growth occurs at pH 6.5 and 10.0, but not at pH 6.0 or 10.5. Growth occurs in the presence of 0–2% (w/v) NaCl; optimal growth occurs without NaCl. Anaerobic growth does not occur on TSA or on TSA supplemented with nitrate. Casein and Tweens 20, 40 and 60 are hydrolysed, but 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, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but lipase (C14), valine arylamidase, cystine arylamidase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Susceptible to polymyxin B, chloramphenicol, gentamicin, novobiocin, tetracycline, kanamycin, oleandomycin and neomycin, but not to ampicillin, cephalothin, lincomycin or carbenicillin. The predominant ubiquinone is Q-10. The major fatty acids are C_{18:1ω7c} and C_{16:0}. The DNA G+C content is 61.8 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1 and Supplementary Table S1.

The type strain, KSL-145^T (= KCTC 12481^T = JCM 13476^T), was isolated from an alkaline soil in Kwangchun, Korea.

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