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

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Two Gram-negative, rod-shaped bacterial strains, KSL-102^T and KSL-110, were isolated from an alkaline soil in Korea, and their taxonomic positions were investigated by use of a polyphasic study. The two strains grew optimally at pH 7.0–8.0 and 30 °C without NaCl. They contained Q-10 as the predominant ubiquinone. The major fatty acids were C_{18:1ω7c} and C_{16:0} on trypticase soy agar, but 11-methyl C_{18:1ω7c} was also a major component when the two strains were cultivated on LMG medium no. 221. Their DNA G + C contents were 68.4–68.7 mol%. Strains KSL-102^T and KSL-110 exhibited three nucleotide differences in their 16S rRNA gene sequences and a mean DNA–DNA relatedness value of 85 %. Phylogenetic trees based on 16S rRNA gene sequences showed that the two strains fell within the evolutionary radiation encompassed by the genus *Brevundimonas*. Levels of 16S rRNA gene sequence similarity between the two strains and the type strains of recognized *Brevundimonas* species ranged from 96.3 to 98.4 %. DNA–DNA relatedness levels between the two strains and recognized *Brevundimonas* species were 8–21 %. On the basis of phenotypic, phylogenetic and genetic data, strains KSL-102^T and KSL-110 were classified in the genus *Brevundimonas* as members of a novel species, for which the name *Brevundimonas kwangchunensis* sp. nov. is proposed. The type strain is KSL-102^T (=KCTC 12380^T = DSM 17033^T).

The genus *Brevundimonas* was proposed based on the reclassification of two *Pseudomonas* species as *Brevundimonas diminuta* and *Brevundimonas vesicularis* (Segers *et al.*, 1994). Subsequently, several *Caulobacter* species or subspecies were transferred to the genus *Brevundimonas* as *Brevundimonas alba*, *B. aurantiaca*, *B. bacteroides*, *B. intermedia*, *B. subvibrioides* and *B. variabilis* (Abraham *et al.*, 1999). Recently, two further *Brevundimonas* species, *Brevundimonas nasdae* (Li *et al.*, 2004) and *Brevundimonas mediterranea* (Fritz *et al.*, 2005), have been described. At the time of writing, there are 10 *Brevundimonas* species with validly published names. The genus *Brevundimonas* is characterized chemotaxonomically by having Q-10 as the predominant ubiquinone, C_{18:1} and C_{16:0} as the major fatty acids and DNA G + C contents of 65–68 mol% (Segers *et al.*, 1994; Abraham *et al.*, 1999; Li *et al.*, 2004; Fritz *et al.*, 2005).

Phylogenetic analyses based on 16S rRNA gene sequences show that the genus falls within the *Alphaproteobacteria* (Abraham *et al.*, 1999; Anzai *et al.*, 2000; Li *et al.*, 2004; Fritz *et al.*, 2005). In this study, we report on the taxonomic characterization of two *Brevundimonas*-like bacterial strains, KSL-102^T and KSL-110, which were isolated from an alkaline soil (approximate pH 9.0–10.0) in Korea.

Strains KSL-102^T and KSL-110 were isolated by the standard dilution-plating technique at 30 °C on 10 × diluted nutrient agar (NA; Difco) with pH adjusted to 10.0. The type strains of 10 *Brevundimonas* species were used as reference strains. *B. diminuta* LMG 2089^T, *B. vesicularis* LMG 2350^T, *B. alba* LMG 18360^T, *B. aurantiaca* LMG 18359^T, *B. bacteroides* LMG 15096^T, *B. intermedia* LMG 18361^T, *B. subvibrioides* LMG 14903^T, *B. variabilis* LMG 18362^T and *B. mediterranea* LMG 21911^T were obtained from the Laboratorium voor Microbiologie Universiteit Gent (LMG), Gent, Belgium. *B. nasdae* DSM 14572^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. To investigate their morphological and physiological characteristics, strains KSL-102^T and KSL-110 were routinely cultivated at 30 °C on trypticase soy agar (TSA; Difco). Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. The presence of flagella was examined by

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Tables detailing Biolog assimilation data and the cellular fatty acid compositions of *Brevundimonas kwangchunensis* sp. nov. and other *Brevundimonas* species are available as supplementary material in IJSEM Online.

transmission electron microscopy using cells from exponentially growing cultures. 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 TSA. Growth at various NaCl concentrations was investigated in trypticase soy broth (TSB; Difco) and in TSB lacking NaCl. The pH range for growth was determined in nutrient broth (NB; Difco) that was adjusted to various pH values (initial pH 4.5–11.5 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization to various levels by the addition of HCl or Na₂CO₃ (below pH 10.5) and KOH (above pH 10.5). 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 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 by Lanyi (1987). Oxidation of various substrates was determined by using the Biolog GN2 MicroPlate assay as recommended by the manufacturer. Sensitivity to antibiotics was tested on TSA plates using discs containing the following antibiotics: 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) and oleandomycin (15 µg). Enzyme activity was determined by using the API ZYM system (bioMérieux). Other physiological and biochemical tests were performed with the API 20E system (bioMérieux). Morphological, cultural, physiological and biochemical characteristics of strains KSL-102^T and KSL-110 are given in the species description (see below) or are shown in Table 1 and Supplementary Table S1 in IJSEM Online.

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in TSB at 30 °C. Cell mass of the type strains of 10 *Brevundimonas* species was obtained by cultivation in recommended liquid media at 25 or 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 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). The 16S rRNA gene sequences of strains KSL-102^T and KSL-110 determined in this study comprised 1416 nt, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. The 16S rRNA gene sequences of the two strains were 99.8% similar (three nucleotide differences). Comparative 16S rRNA gene sequence analyses showed that strains KSL-102^T and KSL-110 are phylogenetically most closely affiliated to the genus *Brevundimonas* (Fig. 1). The relationship between the cluster comprising the two

strains and *Brevundimonas* species and the cluster comprising *Caulobacter* species was supported by a relatively high bootstrap resampling value (78.8%; Fig. 1). The 16S rRNA gene sequences of strains KSL-102^T and KSL-110 exhibited similarity values of 96.3–98.4% with respect to those of the type strains of recognized *Brevundimonas* species. Sequence similarities to all other species included in the phylogenetic analysis were less than 95.2% (Fig. 1).

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 methyl ester analysis, cell mass of strains KSL-102^T and KSL-110 (30 °C) and *B. diminuta* LMG 2089^T and *B. vesicularis* LMG 2350^T (25 °C) was harvested from agar plates after incubation for 3 days on TSA and for 7 days on LMG medium no. 221, and cell mass of *B. alba* LMG 18360^T, *B. aurantiaca* LMG 18359^T, *B. bacterioides* LMG 15096^T, *B. subvibrioides* LMG 14903^T and *B. variabilis* LMG 18362^T was harvested from agar plates after incubation for 7 days on LMG medium no. 221 at 30 °C. The fatty acid methyl esters were extracted and 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. Chemotaxonomic data support the result of monothetic phylogenetic classification of strains KSL-102^T and KSL-110 as members of the genus *Brevundimonas*. Strains KSL-102^T and KSL-110 contained ubiquinone with 10 isoprene units (Q-10) as the predominant respiratory lipoquinone. 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). The fatty acid profiles of strains KSL-102^T and KSL-110 were characterized by a common core of straight-chain, unsaturated, hydroxy fatty acids and 11-methyl C_{18:1}ω7c in similar amounts when the two strains were cultivated in identical medium of both TSA and LMG medium no. 221 (Supplementary Table S2 in IJSEM Online). The major fatty acids were C_{18:1}ω7c and C_{16:0} on TSA and C_{18:1}ω7c, 11-methyl C_{18:1}ω7c and C_{16:0} on LMG medium no. 221. The proportion of 11-methyl C_{18:1}ω7c increased and the proportion of C_{18:1}ω7c decreased when they were grown on LMG medium no. 221. The fatty acid profiles of the two strains were similar to those of *Brevundimonas* species analysed here (Supplementary Table S2 in IJSEM Online) and determined previously (Abraham *et al.*, 1999; Li *et al.*, 2004; Fritz *et al.*, 2005). Cyclo-C_{19:0}ω8c, which was detected in some *Brevundimonas* species, including *B. diminuta*, *B. nasdae* and *B. alba*, was not found in strains KSL-102^T and KSL-110. The DNA G + C contents of strains KSL-102^T and KSL-110 were 68.7 and 68.4 mol%, respectively, higher than those of recognized *Brevundimonas* species (Table 1).

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989) using

Table 1. Differential phenotypic characteristics of *Brevundimonas kwangchunensis* sp. nov. and other *Brevundimonas* species

Species: 1, *B. kwangchunensis*; 2, *B. diminuta* (data from Palleroni, 1984; Segers *et al.*, 1994; Li *et al.*, 2004); 3, *B. vesicularis* (Palleroni, 1984; Segers *et al.*, 1994; Li *et al.*, 2004); 4, *B. alba* (Poindexter, 1964); 5, *B. aurantiaca* (Poindexter, 1964; Li *et al.*, 2004); 6, *B. bacteroides* (Poindexter, 1964); 7, *B. intermedia* (Poindexter, 1964; Li *et al.*, 2004); 8, *B. subvibrioides* (Poindexter, 1964); 9, *B. variabilis* (Poindexter, 1964); 10, *B. nasdae* (Li *et al.*, 2004); 11, *B. mediterranea* (Fritz *et al.*, 2005). +, Positive; –, negative; ND, not determined; w, weakly positive; v, variable; data in parentheses are for the type strain. Cells of all species are Gram-negative and rod-shaped.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Oxidase	+	+	+	ND	(+)	ND	(+)	ND	ND	+	ND
Catalase	+	+	+	ND	(+)	ND	(+)	ND	ND	+	ND
Urease	–	–*	–	ND	(–)	ND	(–)	ND	ND	–	v (–)
Arginine dihydrolase	–	–*	–	ND	(–)	ND	(–)	ND	ND	–	ND
β-Galactosidase	–	–	–	ND	(–)	ND	(+)	ND	ND	+	–
Nitrate reduction	–	–	–	–	–	v	–	–	–	–	–
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	(–)	(–)
Acetic acid	–	(+)	(+)	+	(–)	(–)	(+)	(–)	+	(+)	(+)
Lactic acid	–	(–)	(+)	–	(–)	(–)	(–)	(–)	–	(+)	(–)
Succinic acid	v (+)	(–)	(+)	+	(–)	(–)	(+)	(–)	+	(+)	(+)
L-Aspartic acid	v (+)	(+)	(–)	+	(–)	(–)	(–)	(–)	–	(+)	(–)
L-Glutamic acid	v (+)	(+)	(+)	+	(+)	(+)	(+)	(+)	+	(+)	(+)
L-Leucine	–	(–)	(–)	v	(–)	(–)	(–)	(–)	+	(+)	(–)
L-Proline	v (+)	(+)	(+)	v	(–)	(–)	(–)	(–)	+	(+)	(–)
Glucose 1-phosphate	+	(–)	(+)	ND	(–)	(–)	(–)	(–)	ND	(–)	(–)
Glucose 6-phosphate	+	(–)	(–)	ND	(–)	(–)	(–)	(–)	ND	(–)	(–)
Sensitivity to:											
Streptomycin	+	ND	ND	+	+	+	+	+	+	ND	ND
Penicillin G	+	ND	ND	+	+	+	+	+	+	ND	ND
Occurrence of cyclo C _{19:0ω8c}	–	+	–	+	–	–	–	–	–	+	–
DNA G+C content (mol%)	68.4–68.7	66–68	65–66	ND	(65.6)	66‡	(66.1)	67‡	ND	66.5	67.1–67.5

*Data taken from Palleroni (1984) and Segers *et al.* (1994); different results for the type strains were reported by Li *et al.* (2004).

†Data from Biolog GN2 MicroPlate assay in this study; data for *B. alba* and *B. variabilis* were taken from Poindexter (1964).

‡Reported for one isolate by Poindexter (1989).

photobiotin-labelled DNA probes and microdilution wells at 60 °C. Hybridization was performed with five replications 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. Mean DNA–DNA relatedness between strains KSL-102^T and KSL-110 was 85 % when their DNAs were used individually as labelled DNA probes for cross-hybridization. This

value indicates that the two strains are members of the same genomic species (Wayne *et al.*, 1987). Strains KSL-102^T and KSL-110 were similar to each other with regard to most phenotypic properties. However, the two strains differed in the oxidation of some substrates, indicating that they may represent ecotypes of the same species, as suggested by Konstantinidis & Tiedje (2005). The two strains exhibited levels of DNA–DNA relatedness of 8–21 %

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Brevundimonas kwangchunensis* strains KSL-102^T and KSL-110, *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 out-group (not shown). Bar, 0.01 substitutions per nucleotide position.

to the type strains of the 10 recognized *Brevundimonas* species.

The phylogenetic distinctiveness and DNA–DNA relatedness data were sufficient to categorize strains KSL-102^T and KSL-110 as members of a species that is distinct from all recognized *Brevundimonas* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). There were some differences between the two strains and *Brevundimonas* species in their phenotypic characteristics (Table 1). Therefore, on the basis of the data presented, strains KSL-102^T and KSL-110 should be classified in the genus *Brevundimonas* as members of a novel species, for which the name *Brevundimonas kwangchunensis* sp. nov. is proposed.

Description of *Brevundimonas kwangchunensis* sp. nov.

Brevundimonas kwangchunensis (kwang.chun.en'sis. N.L. fem. adj. *kwangchunensis* of Kwangchun, from where the first strains were isolated).

Cells are Gram-negative, aerobic rods (0.4–0.6 µm × 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 1.5–2.0 mm in diameter after 3 days incubation at 30 °C. Colonies on LMG medium no. 221 are circular, smooth, glistening, slightly convex, yellowish white in colour and 1.0–2.0 mm in diameter after 3 days incubation at 30 °C. Optimal temperature for growth is 30 °C. Growth occurs at 10 and 36 °C, but not at 4 or 37 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 6.0 and 9.5, but not at pH 5.5 or 10.0. Growth occurs in the presence of 0–2% (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, 60 and 80 are hydrolysed. Hypoxanthine, xanthine and tyrosine are not hydrolysed. 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, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Sensitive to chloramphenicol, cephalothin, novobiocin, tetracycline and kanamycin and weakly sensitive to polymyxin B, but resistant to ampicillin, gentamicin, lincomycin and oleandomycin. The predominant ubiquinone is Q-10. The major fatty acids are C_{18:1ω7c} and C_{16:0} on TSA; 11-methyl C_{18:1ω7c} is a major component when cultivated on LMG medium no. 221. The DNA G+C content is 68.4–68.7 mol% (68.7 mol% for type strain) (determined by HPLC). Other phenotypic characteristics are given in Table 1 and Supplementary Tables S1 and S2 in IJSEM Online.

The type strain, KSL-102^T (=KCTC 12380^T=DSM 17033^T), was isolated from an alkaline soil in Kwangchun, Korea. Strain KSL-110 is a reference strain.

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