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New Taxa in *Alphaproteobacteria*: *Brevundimonas olei* sp. nov., an Esterase-Producing Bacterium

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A polyphasic taxonomic approach was used to characterize a Gram-negative, non-motile bacterium, designated MJ15^T, that was isolated from soil of a GS-Caltex Oil reservoir in Korea. As shown by comparative 16S rRNA gene sequence analysis, strain MJ15^T belongs to genus *Brevundimonas*. The 16S rRNA gene sequence similarities ranged from 95.6-99.2% between strain MJ15^T and validated representatives of the genus *Brevundimonas*. With respect to *Brevundimonas* species, strain MJ15^T exhibited DNA-DNA relatedness values below 40.7%. The G+C content of the genomic DNA was 61.7 mol%. Strain MJ15^T contained ubiquinone Q-10. The major fatty acids were $C_{16:0}$ (27.7%), $C_{19:0}$ cyclo *ω8c* (23.2%), summed feature 8 (containing $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*) (28.5%), and major hydroxyl fatty acid was $C_{12:0}$ 3OH (3.7%). Based upon its phenotypic and genotypic properties, as well as its phylogenetic distinctiveness, strain MJ15^T (KCTC 22461^T; JCM 16237^T) should be classified in the genus *Brevundimonas* as the type strain of a novel species. The name *Brevundimonas olei* sp. nov. is proposed for this new species.

***Keywords*:** taxonomy, 16S rRNA gene, *Alphaproteobacteria*, *Brevundimonas olei*

The When two *Pseudomonas* species, *Brevundimonas diminuta*, and *Brevundimonas vesicularis*, were reclassified, the genus *Brevundimonas* was created (Segers *et al*., 1994). At this time, the genus is comprised of 21 species with validly published names: *Brevundimonas diminuta* (Segers *et al*., 1994), *B. vesicularis* (Segers *et al*., 1994), *B. alba* (Abraham *et al*., 1999), *B. aurantiaca* (Abraham *et al*., 1999), *B. bacteroides* (Abraham *et al*., 1999), *B. intermedia* (Abraham *et al*., 1999), *B. subvibrioides* (Abraham *et al*., 1999), *B. variabilis* (Abraham *et al*., 1999), *B. nasdae* (Li *et al*., 2004), *B. mediterranea* (Fritz *et al*., 2005), *B. kwangchunensis* (Yoon *et al*., 2006a), *B. terrae* (Yoon *et al*., 2006b), *B. aveniformis* (Ryu *et al*., 2007), *B. lenta* (Yoon *et al*., 2007), *B. basaltis* (Choi *et al*., 2009), *B. halotolerans*, *B. poindexterae*, *B. staleyi* (Abraham *et al*., 2009), *B. naejangsanensis*, *B. bullata* (Kang *et al*., 2009), and *B. vancanneytii* (Estrela and Abraham, 2009).

A Gram-negative and non-motile strain MJ15^T was isolated in the course of screening microorganisms from an oil reservoir. Based upon analysis of the 16S rRNA gene sequence, strain MJ15^T was considered to be a *Brevundimonas*-like strain. After strain MJ15^T was subjected to a polyphasic taxonomic investigation, the results indicated that strain MJ15^T should be placed in a novel species in the genus *Brevundimonas*.

Materials and Methods

Isolation of bacterial strains and culture conditions

Strain MJ15^T was isolated from the surface soil of an oil reservoir. To incubate the strain, 1 g of the soil was immersed in 50 ml saline (0.85% NaCl) solution, vortexed, serially diluted and 100 μl of the aliquot was inoculated on ten times diluted R2A agar (Difco, USA). Purification of single colonies on the plates was completed by transferring them onto new plates and incubating them once again under the conditions previously described. Routine cultures on LB agar at 30°C were conducted on the isolates and then maintained as a glycerol suspension (20%, w/v) at -70°C. The type strains of *Brevundimonas* species were obtained from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea) and Belgian Coordinated Collections of Microorganisms (LMG, Belgium) and used as reference strains for DNA-DNA hybridization and other experiments.

Phenotypic and biochemical characteristics

As described by Buck (1982), the Gram reaction was performed by using the non-staining method. Light microscopy (Nikon E600) and transmission electron microscopy (TEM) were used to examine cell morphology. The following procedure was used to conduct TEM. Bacteria were grown on LB agar at 30°C for 18 h. Cells were gently resuspended in 1 drop of deionized H_2O, and samples were placed on carbon- and Formvar-coated nickel grids for 30 sec. Grids were floated on 1 drop of 0.1% (w/v) aqueous uranyl acetate, blotted dry, and then viewed with a Carl Zeiss LEO912AB electron microscope at 100 kV under standard operating conditions. Catalase and oxidase tests were performed (Cappuccino and Sherman, 2002). According to the instructions of the manufacturer (bioMérieux, France), physiological characteristics were determined with API 20E, API 20NE, API ID 32 GN, and API Zym galleries. Assessment of growth at different temperatures (4, 10, 15, 25, 30, 37, and 42°C) and various pH values (pH 5.0-10.0 at intervals of 0.5 pH units) was determined on Luria-Bertani agar (LB, Difco) after 5 days incubation. Evaluation of growth on nutrient agar (NA, Difco), trypticase soy agar (TSA, Difco)

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and Luria-Bertani agar (LB, Difco) was completed at 30°C.

Isoprenoid quinones and cellular fatty acids

Chloroform/methanol was used to extract isoprenoid quinones (2:1, v/v). The isoprenoid quinines were then purified by TLC (thin-layer chromatography) and subsequently analyzed by HPLC (Collins and Jones, 1981; Shin *et al*., 1996). Cells were allowed to grow on TSA for 48 h at 30°C, and two loops of the well-grown cells were harvested in order to perform fatty acid methyl ester analysis. Preparation, separation, and identification of fatty acid methyl esters were done with the Sherlock Microbial Identification System (MIS), produced by MIDI, Inc., Newark, DE., USA (Sasser, 1990).

PCR amplification, 16S rRNA sequencing and phylogenetic analysis

Genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent, Korea) for the phylogenetic analysis of strain MJ15^T. PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). SeqMan software (DNASTAR Inc.) was used to compile the nearly complete sequence of the 16S rRNA gene. The 16S rRNA gene sequences of the related taxa were obtained from GenBank and edited using the BioEdit program (Hall, 1999). Multiple alignments were performed with the CLUSTAL X program (Thompson *et al*., 1997). The Kimura two-parameter model (Kimura, 1983) was used to calculate evolutionary distances. The phylogenic

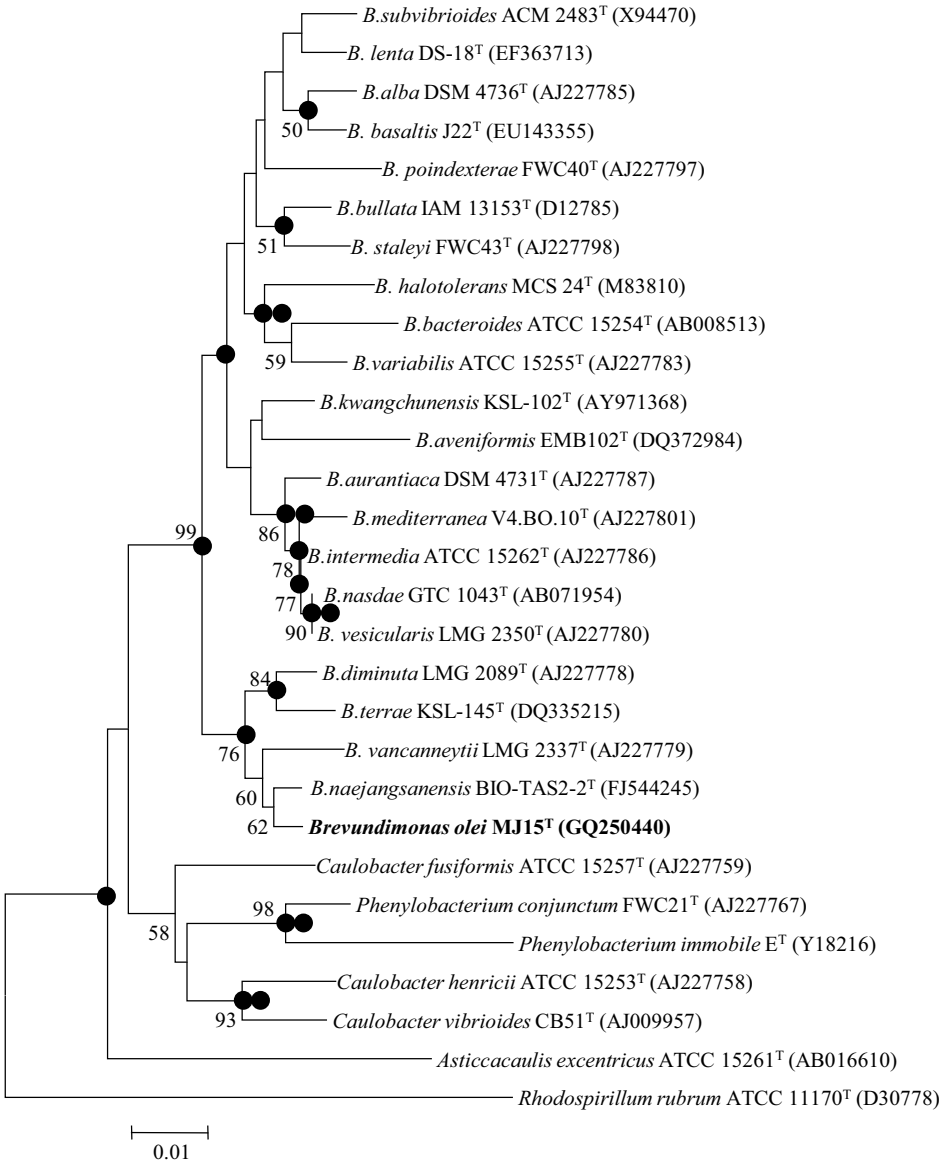

Fig. 1. Phylogenetic tree based upon 16S rRNA gene sequences (1,401 nt) showing the phylogenetic relationships between strain MJ15^T and closely related *Brevundimonas* type strains. Bootstrap values of >50% (percentages of 1,000 replications) are shown at branch points. A filled circle indicates the common nodes recovered from either the maximum-parsimony algorithm or the maximum-likelihood tree. Sequences used for comparative study are included in parentheses. Filled double-circles indicate that corresponding nodes were recovered in both the maximum-parsimony tree and maximum-likelihood tree. Neighbor-joining method was used. The bar represents 0.01 substitutions per nucleotide position.

tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) and maximum-parsimony (Fitch, 1971) in MEGA3 Program (Kumar *et al.*, 2004). Furthermore, to obtain confidence levels for the branches, we conducted bootstrap analyses with 1,000 replicates (Felsenstein, 1985) and constructed a maximum-likelihood tree using the PHYLIP Program (Choi *et al*., 2000; Brinkman *et al*., 2001).

Determination of DNA G+C content

Genomic DNA was extracted and purified with the QIAGEN Genomic-tip system 100/G (QIAGEN) and was then enzymatically degraded into nucleosides for the determination of the DNA G+C content. The nucleosides were analyzed using HPLC as described previously (Tamaoka and Komagata, 1984; Mesbah *et al*., 1989).

DNA-DNA hybridization

The DNA-DNA hybridization was performed fluorometrically using photobiotin-labelled DNA probes and micro-dilution wells according to the method developed by Ezaki *et al*. (1989). The hybridization was replicated five times for each sample. The highest and lowest values obtained for each sample were excluded. The remaining three values were utilized in the calculation of hybridization values. The DNA relatedness values quoted are expressed as means of these three values.

The NCBI GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MJ15^T is GQ250440.

Result and Discussion

Morphological and phenotypic characteristics

Strain MJ15^T was Gram-negative and cell morphology showed rods of 0.4-0.6 μm wide and 1.9-2.2 μm long. Table 1 lists the phenotypic and chemotaxonomic characteristics that differentiate strain MJ15^T from other *Brevundimonas* species.

Cellular fatty acids and isoprenoid quinones

Strain MJ15^T contained a higher content of $C_{16:0}$ (27.7%), $C_{19:0}$ cyclo *ω8c* (23.2%), summed feature 8 (containing $C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6c*) (28.5%), and moderate amounts of $C_{17:0}$ (4.0%), $C_{12:0}$ 3OH (3.7%), $C_{17:1}$ *ω8c* (3.4%), and summed feature 3 (containing $C_{16:1}$ *ω6c*/ $C_{16:1}$ *ω7c*) (3.1%). Although there were differences in the proportions of fatty acids between species were listed in Table 2, these fatty acid profiles were fit into the genus description, and similar to those of other Brevundimonas species. Strain MJ15^T contained ubiquinone Q-10 as the major respiratory quinone commonly found in *Alphaproteobacteria.*

Phylogenetic analysis

A nearly complete 16S rRNA gene sequence of strain MJ15^T (1,401 nt) was obtained. The closest relatives of strain MJ15^T were *B. naejangsanensis* BIO-TAS2-2^T (99.3%), *B. vancanneytii* LMG 2337^T (98.3%), *B. diminuta* LMG 2089^T (98.3%), B. *intermedia* ATCC 15262^T (98.2%), *B. vesicularis* LMG 2350^T (98.2%), *B. nasdae* GTC 1043^T (98.0%), *B. aurantiaca* DSM 4731^T (97.9%), *B. terrae* KSL-145^T (97.7%), *B. mediterranea* V4.BO.10^T (97.6%), *B. bullata* IAM 13153^T (97.1%), and *B. halotolerans* MCS24^T (97.0%) based upon 16S rRNA gene sequence similarity.

DNA G+C content

The DNA G+C content of strain MJ15^T is 61.8 mol%.

DNA-DNA hybridization

DNA-DNA hybridizations were performed to differentiate strain MJ15^T from closely related *Brevundimonas* species. Indicating that it is not related to them at the species level, Strain MJ15^T exhibited relatively low levels of DNA-DNA relatedness with respect to *B. diminuta* KCTC 12488^T (40.7%), *B. vesicularis* KCTC 12421^T (36.4%), *B. mediterranea* KCTC12441^T (36.3%), *B. intermedia* KCTC 12440^T (30.4%), *B. vancanneytii* LMG 2337^T (28.5%), *B. naejangsanensis* KCTC 22631^T (20.0%), *B. bullata* KACC 13177^T (17.0%), *B. halotolerrans* LMG 25346^T (9.1%), *B. kwangchunensis* KCTC 12380^T (6.5%), *B. poindexterae* LMG 25261^T (6.2%), *B. aurantiaca* KACC 12011^T (5.8%), *B. nasdae* KCTC 12493^T (5.6%), *B. terrae* KCTC 12481^T (1.2%) (Wayne *et al*., 1987).

Taxonomic conclusion

The results of the polyphasic analyses (i.e. phylogenetic and genetic distinctiveness) combined with the differential phenotypic properties suggest that strain MJ15^T represents a new species within the genus *Brevundimonas.* The name *Brevundimonas olei* sp. nov. is proposed for this new species.

Description of *Brevundimonas olei* sp. nov.

Brevundimonas olei (o'le. L. gen. neut. n. *olei* of/from oil, as the organism was isolated from oil-contaminated soil).

Cell is Gram-negative and non-motile. It is 0.4-0.6 μm in width and 1.9.-2.2 μm in length. After 3 days on R2A, colonies were 1-2 mm in diameter, smooth, pale-yellow colored, circular, and convex. It is oxidase and catalase positive. It grows between 25 and 37°C, but not at 4°C. The optimum growth temperature is 30°C on LB broth. The pH growth range is between pH 6.0 and 7.0, with an optimum pH of 7.0 in LB broth. It does not reduce nitrate to nitrogen gas. Growth occurs on R2A agar, nutrient agar, TSA, MacConkey agar, and LB agar.

Activities were exhibited by the cells for acid phosphatase, alkaline phosphatase, α-chymotrypsin esterase (C4), esterase (C8), *β*-galactosidase (PNPG), leucine arylamidase, naphtol-AS-BI-phosphohydrolase, protease (gelatin hydrolysis), trypsin, and valine arylamidase. No activities were shown by the cells for *N*-acetyl-*β*-glucosaminidase, arginine dihydrolase, cystine arylamidase, *α*-fucosidase, *α*-galactosidase, *β*-galactosidase (ONPG), *α*-glucosidase (starch hydrolysis), *β*-glucosidase (esculin hydrolysis), *β*-glucosidase, *β*-glucuronidase, lipase (C14), *α*-mannosidase, and urease.

Production of acid occurred from D-fructose, D-fucose, gentiobiose, D-lactose, maltose, D-melibiose, L-rhamnose, L-sorbose, N-acetylglucosamine, salicin, and starch. However, production of acid did not occur from 2-ketogluconate, 5-ketogluconate, gluconate, D-arabinose, L-arabinose, D-cellobiose, D-fucose, D-galactose, D-xylose, D-mannose, melezitose, D-raffinose, ribose, D-sucrose, D-tagatose, D-trehalose, turanose, D-xylose, L-xylose, *α*-methyl-D-glucoside, *α*-methyl-D-mannoside, *β*-methyl-D-xyloside, D-adonitol (Ribitol), D-arabitol, L-arabitol, dulcitol (galactitol), erythritol, glycerol, inositol, mannitol, sorbitol, xylitol, amygdalin, arbutin, esculin, inulin, or glycogen.

Table 1. Differential phenotypic characteristics of type strain MJ15^T. and closely related type strains of genus *Brevundimonas*.
Strains: 1, *B. olei* MJ15^T; 2, *B. aurantiaca* KACC 12011^T; 3, *B. bullata* KACC 13177^T; 4, *B. diminuta* KCTC 12488^T; 5, *B. halotolerrans* LMG 25346^T; 6, *B. intermedia* KCTC 12440^T; 7, *B. kwangchunensis* KCTC 12380^T; 8, *B. mediterranea* KCTC12441^T; 9, *B. naejangsanensis* KCTC 22631^T; 10, *B. nasdae* KCTC 12493^T; 11, *B. poindexterae* LMG 25261^T; 12, *B. terrae* KCTC 12481^T; 13, *B. vancanneytii* LMG 2337^T; and 14, *B. vesicularis* KCTC 12421^T.
Data were obtained in this study under identical growth conditions. Symbols: +, positive; -, negative; w, weakly positive.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Motility	-	+	+	+		+	+	+	+	+		+	+	+
Morphology	rod	rod	rod	short rod	rod	rod	rod	rod	rod	short rod	rod	rod	short rods	short rod
Colony color (Yellow, White)	pale Yellow	Yellow	yellow	yellow	white	Cream	yellow	cream White	grayish yellow	slightly Yellow	tan	greyish yellow	whitish yellow	Orange
Oxidase	-	+	+	+	-	+	+	+	+	+	-	+	+	+
Catalase	+	+	-	+	+	+	+	-	+	+	-	+	-	+
Nitrate reduction	+	-	-	-	-	-	-	+	-	-	-	-	-	-
Enzyme activity														
N–Acetyl-*β*-glucosaminidase	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Acid phosphatase	+	+	+	+	-	+	+	+	+	+	+	+	+	w
Arginine dihydrolase	-	-	w	-	-	-	-	-	-	-	-	-	-	-
α-Chymotrypsin	+	w	-	+	-	w	w	w	+	-	-	w	w	+
Esterase (C4)	+	w	w	w	-	w	w	+	-	w	w	w	-	+
Esterase (C8)	+	+	-	w	w	+	+	+	w	w	w	+	w	w
β-Galactosidase (ONPG)	-	-	-	-	-	+	-	-	-	-	-	-	-	+
β-Galactosidase (PNPG)	+	w	-	-	-	w	-	w	+	+	+	-	w	+
α-Glucosidase (starch hydrolysis)	-	w	+	-	+	-	w	w	-	-	-	-	w	-
β-Glucosidase (Esculin hydrolysis)	-	-	-	-	-	+	w	w	w	+	+	+	-	w
β-Glucosidase	-	w	-	-	-	+	-	+	-	-	-	-	-	w
Leucine arylamidase	+	+	-	w	+	+	+	+	+	-	+	+	+	w
Naphtol-AS-BI-phosphohydrolase	+	+	w	+	-	+	+	+	+	-	+	+	+	w
Protease (gelatin hydrolysis)	+	-	-	-	w	-	-	-	-	-	-	-	-	+
Trypsin	+	+	-	+	-	w	+	+	+	-	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Valine arylamidase	+	+	-	-	w	-	w	-	-	-	w	-	+	-
Assimilation														
3-Hydroxybenzoate	+	-	-	-	-	-	-	-	+	-	-	-	+	-
4-Hydroxybenzoate	+	w	+	w	-	-	-	-	-	-	+	-	+	-
D,L-3-Hydroxybutyrate	+	-	+	+	+	-	+	w	+	-	w	+	w	+

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2-Ketogluconate (α)	+	-	+	-	w	+	-	+	-	-	w	-	+	-
Acetate	+	-	-	w	-	-	+	w	-	+	-	-	-	-
Adipate	+	w	w	+	+	-	-	-	-	+	-	-	w	-
Caprate	+	-	+	-	-	-	+	-	-	-	-	+	-	-
Citrate	+	+	+	-	-	+	-	-	-	-	w	+	+	-
Gluconate	+	+	w	w	w	+	+	+	+	-	-	-	w	-
Itaconate	+	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Malate	+	w		-	w	-	-	+	+	+	-	-	w	w
Malonate	+	-	+	+	-	+	+	+	+	-	+	+	-	-
Phenyl acetate	+	+	-	-	w	-	-	-	-	-	-	-	+	-
Propionate	+	w	-	-	-	+	-	-	-	-	-	-	+	-
n-Valerate	+	w	-	-	-	+	+	-	-	-	-	-	-	-
L-Arabinose	+	w	-	-	-	+	-	-	-	-	+	+	+	-
L-Fucose	-	+	-	-	-	+	-	+	-	-	-	-	-	-
D-Glucose	+	+	w	-	-	w	+	+	-	-	-	-	-	-
D-Maltose	+	w	w	w	-	-	+	-	w	w	-	w	-	w
D-Mannose	+	+	-	+	w	+	-	-	-	-	-	-	-	-
D-Melibiose	+	+	w	-	+	-	-	-	-	-	w	-	-	-
L-Rhamnose	+	+	+	+	w	+	-	-	-	-	-	-	w	-
D-Ribose	-	+	w	+	w	w	-	-	-	-	-	-	w	w
D-Sucrose	+	+	+	+	-	-	+	+	-	+	-	-	-	+
myo-Inositol	-	+	+	+	-	-	+	w	-	+	-	-	-	w
D-Mannitol	+	+	+	+	+	+	+	-	+	+	+	-	w	+
D-Sorbitol	+	+	-	-	-	-	-	-	-	-	+	-	+	-
L-Alanine	+	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Histidine	+	+	+	+	w	+	-	-	+	-	+	-	w	-
L-Proline	+	-	-	+	-	-	w	-	+	-	-	+	-	+
L-Serine	+	+	+	-	-	+	+	+	+	-	-	-	+	-
N-Acetyl-D-glucosamine	+	+	+	+	w	w	-	-	-	-	+	-	w	-
Salicin	+	+	w	-	-	+	+	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-	-	-	-	-	-	+	-
G+C content	61.7	65.6	66.7	67.0	64.6	66.1	68.4-68.7	67.3	67.0	66.5	67.0	61.8	66.3	65-66

Table 2. Cellular fatty acid profiles of strain MJ15^{T} ^{T} and closely related species of genus *Brevundimonas*.
Strains: 1, *Brevundimonas olei* MJ15^{T}; 2, *B. aurantiaca* KACC 12011^{T}; 3, *B. bullata* KACC 13177^{T}; 4, *B. diminuta* KCTC 12488^{T}; 5, *B. halotolerrans* LMG 25346^{T}; 6, *B. intermedia* KCTC 12440^{T}; 7, *B. kwangchunensis* KCTC 12380^{T}; 8, *B. mediterranea* KCTC12441^{T}; 9, *B. naejangsanensis* KCTC 22631^{T}; 10, *B. nasdae* KCTC 12493^{T}; 11, *B. poindexterae* LMG 25261^{T}; 12, *B. terrae* KCTC 12481^{T}; 13, *B. vancanneytii* LMG 2337^{T}; and 14, *B. vesicularis* KCTC 12421^{T}.
All data of validated type strains were grown on TSA at 30°C for 2 days for fatty acid analysis and all data were obtained in this study. Fatty acids less than 1.0% of the total fatty acids are noted as trace (Tr). Therefore, percentages do not add up to 100%. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. The *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. †Summed feature contained fatty acids, which could not separated by GLC with the Microbial Identification System (MIDI). ND, Not determined.

Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Saturated														
11:0 3OH	ND	ND	Tr	Tr	Tr	Tr	Tr	Tr	ND	Tr	ND	Tr	ND	Tr
12:0	Tr	ND	Tr	Tr	Tr	Tr	Tr	ND	Tr	Tr	0.2	Tr	Tr	Tr
12:0 3OH	3.7	1.8	2.8	3.1	Tr	2.2	1.5	1.3	4.0	2.7	2.9	3.1	2.8	2.5
13:0	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	ND	Tr	0.8	Tr	Tr	Tr
14:0	1.4	2.9	4.8	1.3	1.2	1.5	1.9	1.2	1.3	2.3	3.0	5.2	1.7	3.1
15:0 iso	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.2	Tr	Tr	Tr
16:0	27.7	17.4	18.7	24.3	14.4	20.9	20.0	17.8	29.8	22.9	24.2	31.9	32.4	19.7
16:0 2OH	ND	ND	Tr	Tr	ND	Tr	Tr	ND	Tr	Tr	ND	ND	Tr	Tr
17:0	4.0	5.2	Tr	5.7	4.3	5.2	6.8	3.9	Tr	1.6	1.9	ND	3.0	5.1
18:0	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.5	Tr	Tr	Tr
19:0 cyclo ω8*c*	23.2	ND	9.7	10.4	ND	ND	ND	2.3	27.0	4.3	ND	ND	19.6	ND
20:2 ω6,9*c*	Tr	ND	Tr	Tr	ND	ND	ND	ND	Tr	1.1	1.1	ND	Tr	ND
Unsaturated														
12:1 3OH	Tr	Tr	1.6	Tr	1.2	Tr	1.5	Tr	Tr	1.3	ND	Tr	Tr	Tr
15:1 ω8*c*	Tr	Tr	Tr	Tr	2.3	Tr	Tr	Tr	ND	Tr	0.7	1.2	Tr	Tr
17:1 ω6*c*	1.2	3.7	1.9	2.1	3.6	4.0	2.2	3.4	ND	2.1	1.2	1.3	Tr	3.6
17:1 ω8*c*	3.4	4.6	6.4	3.8	9.8	5.5	7.6	8.2	Tr	Tr	3.0	2.6	1.7	5.0
18:1 ω5*c*	1.9	Tr	Tr	1.7	Tr	1.2	Tr	Tr	1.7	2.3	1.0	1.1	1.3	1.3
18:1 ω7*c*, 11-methyl	Tr	7.8	3.8	ND	3.4	9.0	14.7	5.3	Tr	Tr	14.1	6.4	2.3	3.4
Summed Feature 1 (15:1 iso H / 13:0 3OH)	Tr	Tr	Tr	0.4	Tr	Tr	Tr	Tr	Tr	Tr	0.4	Tr	Tr	Tr
Summed Feature 3 (16:1 ω6*c* / 16:1 ω7*c*)	3.1	3.3	10.6	1.4	4.7	6.9	4.0	13.1	3.7	5.2	1.7	3.7	2.2	5.3
Summed Feature 7 (19:1 ω6*c* / unk .846/19cy)	ND	ND	ND	ND	ND	Tr	Tr	ND	ND	Tr	ND	Tr	ND	ND
Summed Feature 8 (18:1 ω7*c* /18:1 ω6*c*)	28.5	51.1	34.7	43.2	52.3	39.9	37.1	40.4	29.1	50.4	42.3	42.3	30.5	48.6

Cells used the following substrates for growth: 3-hydroxybenzoate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, 2-ketogluconate(α), acetate, adipate, caprate, citrate, gluconate, itaconate, D,L-lactate, L-malate, malonate, phenyl acetate, propionate, propionate, suberate, n-valerate, L-arabinose, D-glucose, D-maltose, D-mannose, D-melibiose, L-rhamnose, D-sucrose, D-mannitol, D-sorbitol, L-alanine, L-histidine, L-proline, L-serine, *N*-acetyl D-glucosamine, and salicin. Cells did not use the following substrates for growth: 5-ketogluconate, D-ribose, D-trehalose, D-turanose, myo-inositol, and glycogen.

The major fatty acids were $C_{16:0}$ (27.7%), $C_{19:0}$ cyclo ω8*c* (23.2%), summed feature 8 (containing $C_{18:1}$ ω7*c*/$C_{18:1}$ ω6*c*) (28.5%) and major hydroxyl fatty acid was $C_{12:0}$ 3OH (3.7%). DNA G+C content is 61.7 mol%. Isolation of the type strain, MJ15^{T} (=KCTC 22461^{T} =JCM 16237^{T}), was made from soil of a GS-Caltex, Daejeon Oil reservoir, Daejeon City, Korea.

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References

Abraham, W.R., A.B. Estrela, D.I. Nikitin, J. Smit, and M. Vancanneyt. 2009. Proposal of *Brevundimonas halotolerans* sp. nov., *Brevundimonas poindexterae* sp. nov. and *Brevundimonas staleyi* sp. nov., prosthecate bacteria from aquatic habitats. *Int. J. Syst. Evol. Microbiol*. DOI 10.1099/ijs.0.016832-0.

Abraham, W.R., C. Strömpl, H. Meyer, S. Lindholst, E.R.B. Moore, R. Christ, M. Vancanneyt, B.J. Tindali, A. Bennasar, J. Smit, and M. Tesar. 1999. Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. *Int. J.*

Syst. Bacteriol. 49, 1053-1073.

Brinkman, F.S., I. Wan, R.E. Hancock, A.M. Rose, and S.J. Jones. 2001. PhyloBLAST: facilitating phylogenetic analysis of BLAST results. *Bioinformatics* 17, 385-387.

Buck, J.D. 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* 44, 992-993.

Cappuccino, J.G. and N. Sherman. 2002. Microbiology: a Laboratory Manual, 6th edn. Benjamin Cummings, San Francisco, USA.

Choi, J.H., H.Y. Jung, H.S. Kim, and H.G. Cho. 2000. PhyloDraw: a phylogenetic tree drawing system. *Bioinformatics* 16, 1056-1058.

Choi, J. H., M.S. Kim, S.W. Roh, and J.W. Bae. 2010. *Brevundimonas basaltis* sp. nov., isolated from black sand. *Int. J. Syst. Evol. Microbiol*. 60, 1488-1492.

Collins, M.D. and D. Jones. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol. Rev.* 45, 316-354.

Estrela, A.B. and W.R. Abraham. 2009. Proposal of *Brevundimonas vancanneytii* sp. nov. isolated from blood of a patient with endocarditis. *Int. J. Syst. Evol. Microbiol*. DOI 10.1099/ijs.0.015651-0.

Ezaki, T., Y. Hashimoto, and E. Yabuuchi. 1989. Fluorometric DNA-DNA hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Evol. Microbiol.* 39, 224-229.

Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* 39, 783-791.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20, 406-416.

Fritz, I., C. Strömpl, D.I. Nikitin, A. Lysenko, and W.R. Abraham. 2005. *Brevundimonas mediterranea* sp. nov., a non-stalked species from the Mediterranean Sea. *Int. J. Syst. Evol. Microbiol.* 55, 479-486.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acid. Symp. Ser.* 41, 95-98.

Kang, S.J., N.S. Choi, J.H. Choi, J.S. Lee, J.H. Yoon, and J.J. Song. 2009. *Brevundimonas naejangsanensis* sp. nov., a proteolytic bacterium isolated from soil, and reclassification of *Mycoplana bullata* into the genus *Brevundimonas* as *Brevundimonas bullata* comb. nov. *Int. J. Syst. Evol. Microbiol*. DOI 10.1099/ijs.0.011700-0.

Kim, M.K., W.T. Im, H. Ohta, M. Lee, and S.T. Lee. 2005. *Sphingopyxis granuli* sp. nov., a β-glucosidase producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J. Microbiol.* 43, 152-157.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Brief. Bioinform.* 5, 150-163.

Li, Y., Y. Kawamura, N. Fujiwara, T. Naka, H. Liu, X. Huang, K. Kobayashi, and T. Ezaki. 2004. *Sphingomonas yabuuchiae* sp. nov. and *Brevundimonas nasdae* sp. nov., isolated from the Russian space laboratory Mir. *Int. J. Syst. Evol. Microbiol.* 54, 819-825.

Mesbah, M., U. Premachandran, and W.B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* 39, 159-167.

Ryu, S.H., M. Park, J.R. Lee, P.Y. Yun, and C.O. Jeon. 2007. *Brevundimonas aveniformis* sp. nov., a stalked species isolated from activated sludge. *Int. J. Syst. Evol. Microbiol.* 57, 1561-15 65.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406-425.

Sasser, M. 1990. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. MIDI Technical Note 101. MIDI Inc., Newark, DE, USA.

Segers, P., M. Vancanneyt, B. Pot, U. Torck, B. Hoste, D. Dewettinck, E. Falsen, K. Kersters, and P. Devos. 1994. Classification of *Pseudomonas diminuta* Leifson and Hugh 1954 and *Pseudomonas vesicularis* Busing, Doll, and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov., respectively. *Int. J. Syst. Bacteriol.* 44, 499-510.

Shin, Y.K., J.S. Lee, C.O. Chun, H.J. Kim, and Y.H. Park. 1996. Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^T. *J. Microbiol. Biotechnol.* 6, 68-69.

Tamaoka, J. and K. Komagata. 1984. Determination of DNA base composition by reversed phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* 25, 125-128.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876-4882.

Wayne, L.G., D.J. Brenner, R.R. Colwell, P.A.D. Grimont, O. Kandler, M.I. Krichevsky, L.H. Moore, and *et al*. 1987. International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37, 463-464.

Yoon, J.H., S.J. Kang, J.S. Lee, and T.K. Oh. 2006b. *Brevundimonas terrae* sp. nov., isolated from an alkaline soil in Korea. *Int. J. Syst. Evol. Microbiol.* 56, 2915-2919.

Yoon, J.H., S.J. Kang, J.S. Lee, H.W. Oh, and T.K. Oh. 2007. *Brevundimonas lenta* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.* 57, 2236-2240.

Yoon, J.H., S.J. Kang, H.W. Oh, J.S. Lee, and T.K. Oh. 2006a. *Brevundimonas kwangchunensis* sp. nov., isolated from an alkaline soil in Korea. *Int. J. Syst. Evol. Microbiol.* 56, 613-617.