Brevundimonas basaltis sp. nov., isolated from black sand

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A Gram-negative, aerobic, rod-shaped, motile *Brevundimonas*-like bacterial strain, J22^T, was isolated from black sand collected from Soesoggak, Jeju Island, Korea. Growth of strain J22^T was observed in R2A medium at temperatures between 10 and 42 °C (optimum 30 °C), between pH 6.5 and 10.5 (optimum pH 7.5) and at a NaCl concentration between 0 and 4 % (w/v) (optimum 0–1 %). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain J22^T belonged to the genus *Brevundimonas*, with high sequence similarities of >97 % to the sequence of the type strains *Brevundimonas alba* CB88^T, *Brevundimonas lenta* DS-18^T, *Brevundimonas variabilis* CB17^T, *Mycoplana bullata* TK0051^T, *Brevundimonas kwangchunensis* KSL-102^T, *Brevundimonas intermedia* CB63^T, *Brevundimonas subvibrioides* CB81^T and *Brevundimonas bacteroides* CB7^T. Strain J22^T exhibited DNA–DNA relatedness values of less than 22.2 % with the phylogenetically related species of the genus *Brevundimonas*. The DNA G+C content of strain J22^T was 66.3 mol%. The predominant cellular fatty acids were $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega9c$; $C_{12:0}$ 3-OH was present, which chemotaxonomically characterizes the members of the genus *Brevundimonas*. Phylogenetic, genomic and biochemical characteristics served to differentiate this isolate from recognized members of the genus *Brevundimonas*. Strain J22^T (=KCTC 22177^T=JCM 15911^T) should be classified as a novel species in the genus *Brevundimonas*, for which the name *Brevundimonas basaltis* sp. nov. is proposed.

The genus *Brevundimonas* was proposed by reclassification of two *Pseudomonas* species as *Brevundimonas diminuta* and *Brevundimonas vesicularis* by Segers *et al.* (1994). Several species, including *Brevundimonas alba*, *Brevundimonas aurantiaca*, *Brevundimonas bacteroides*, *Brevundimonas intermedia*, *Brevundimonas subvibrioides*, *Brevundimonas terrae* and *Brevundimonas variabilis*, were transferred from the genus *Caulobacter* to the genus *Brevundimonas* (Abraham *et al.*, 1999). At present the genus consists of 16 identified species. Members of the genus *Brevundimonas* are Gram-negative, have a 61.8–68.7 mol% G+C content, and are characterized chemotaxonomically by the presence of $C_{12:0}$ 3-OH fatty acids and the presence of ubiquinone Q-10 (Yoon *et al.*, 2006). In this paper, we describe the morphological, biochemical and phylogenetic characteristics of *Brevundimonas*-like strain J22^T.

Strain J22^T was isolated from black sand from Soesoggak, a coastline surrounded on three sides by basalt, located in the south of Jeju island (33° 15′ 16″ N, 126° 37′ 52″ E), Korea. It was isolated by the standard dilution-plating method on R2A agar at 30 °C for 3 days. Cell morphology of strain J22^T was observed by light microscopy (ECLIPSE 80*i*; Nikon). The presence of flagella was also observed using the flagella staining method (Heimbrook *et al.*, 1989). Motility was examined using motility test medium (BBL) and observed as cell growth spreading out from the line of inoculation in the tube. Growth at different temperatures (4, 10, 15, 20, 25, 30, 37 and 42 °C), NaCl concentration (0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 %, w/v) and pH (5.0–10.0 at intervals of 0.5 pH units) was monitored on R2A agar at 30 °C for up to 5 days. Growth on tryptic soy agar (TSA), marine agar (MA), nutrient agar (NA), Luria broth (LB) agar (all purchased from BBL) and *Caulobacter* medium agar (DSMZ Medium 595) was also evaluated (at 30 °C). Hydrolysis of DNA and casein was tested using DNase test agar (BBL) and skim milk (BBL), respectively, as described by Atlas (1993). Hydrolysis of cellulose was tested using the method of Gerhardt *et al.* (1994). Catalase and oxidase activities of the strain were determined in 3 % (v/v) hydrogen peroxide solution and in 1 % (w/v) *p*-tetramethyl phenylenediamine (bioMérieux), respectively. Other enzyme activities and utilization of different carbon sources were assessed using commercial API 20NE and API ZYM kits (bioMérieux) and the Biolog GN2 MicroPlate assay, according to the manufacturers' protocols.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain J22^T is EU143355.

The 16S rRNA gene sequence of strain J22^T was amplified by the colony PCR method with two universal primers for bacteria (Baker *et al.*, 2003). 16S rRNA gene sequence analysis was performed using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) and an automated DNA analyser system (PRISM 3730XL DNA analyser; Applied Biosystems) according to the manufacturer's instructions. The 16S rRNA gene sequence of strain J22^T was compared with known 16S rRNA sequences of other strains of the genus *Brevundimonas* in the GenBank database (NCBI). The 16S rRNA gene sequence of strain J22^T was aligned with that of closely related strains of the genus *Brevundimonas* by the multiple sequence alignment program CLUSTAL_X v.1.83 (Thompson *et al.*, 1997). The phylogenetic relationship between strain J22^T and the representative species of the genus *Brevundimonas* was defined using MEGA 4 (Tamura *et al.*, 2007). The phylogenetic consensus tree was constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Felsenstein, 1981) and maximum-likelihood (Kluge & Farris, 1969) methods. Genomic DNA was extracted from strain J22^T and eight reference strains, in addition to *Escherichia coli* K-12 as a calibration reference, according to Sambrook *et al.* (1989). In order to evaluate the genomic relationships, DNA–DNA hybridizations were performed fluorimetrically using the method of Ezaki *et al.* (1989) and the improved method proposed by Hirayama *et al.* (1996) with dry-adsorption immobilization. The G+C contents were determined using the fluorimetric method proposed by Gonzalez & Saiz-Jimenez (2002) using SYBR Green I and a real-time PCR thermocycler. For fatty acid analysis, cell biomass of strain J22^T and related species was collected from R2A agar plates after incubation for 2 days at 30 °C. Cellular fatty acids were extracted according to the protocol of Sasser (1990). Cellular fatty acid composition was determined by gas chromatography (Hewlett Packard 6890) and the Microbial Identification System.

The colonies of strain J22^T were creamy, convex and circular (0.5–2.0 mm diameter) with an entire edge and a smooth surface after 3 days on R2A agar plates at 30 °C. Cells were rod-shaped, Gram-negative, motile and flagellated. Strain J22^T grew at temperatures in the range of 10–42 °C, with 0–4 % NaCl and at pH 5.5–10.0. Optimum conditions for growth of strain J22^T were at 30 °C, 0 % NaCl and pH 7.5. Strain J22^T grew on R2A, *Caulobacter* medium and LB, but not on MA, TSA or NA. Strain J22^T was catalase-positive and oxidase-negative. DNA was hydrolysed, but casein and cellulose were not. Physiological and biochemical characteristics of strain J22^T and related species of *Brevundimonas* are shown in Table 1. Additional characteristics are given in the species description below.

As a result of the phylogenetic analysis, strain J22^T was classified within the genus *Brevundimonas* and, in particular, as highly related to *B. alba* CB88^T (Fig. 1). The 16S rRNA gene sequence of J22^T had 98.95–95.96 % similarity to those of other members of the genus *Brevundimonas* and showed highest similarity to those of the type strain *B. alba* CB88^T (98.95 %), *B. lenta* DS-18^T (98.58 %), *B. variabilis* CB17^T (98.06 %), *Mycoplana bullata* TK0051^T (97.75 %), *B. kwangchunensis* KSL-102^T (97.61 %), *B. intermedia* CB63^T (97.16 %), *B. subvibrioides* CB81^T (97.05 %) and *B. bacteroides* CB7^T (97.04 %). However, DNA–DNA hybridization values between strain J22^T and other *Brevundimonas* strains were less than 22.2 %: 22.2 % with *B. lenta* DS-18^T, 21.3 % with *B. kwangchunensis* KSL-102^T, 18.6 % with *B. alba* CB88^T, 17.9 % with *Mycoplana bullata* TK0051^T, 13.8 % with *B. variabilis* CB17^T, 13.6 % with *B. subvibrioides* CB81^T, 11.1 % with *B. bacteroides* CB7^T and 8.6 % with *B. intermedia* CB63^T. The G+C content of the genomic DNA of strain J22^T was 66.3 mol%, consistent with G+C content of other *Brevundimonas* species, which

Table 1. Physiological and biochemical characteristics that differentiate strain J22^T from related species of the genus *Brevundimonas*

Strains: 1, strain J22^T; 2, *B. alba* CB88^T; 3, *B. lenta* DS-18^T; 4, *B. variabilis* CB17^T; 5, *B. kwangchunensis* KSL-102^T; 6, *B. intermedia* CB63^T; 7, *B. subvibrioides* CB81^T; 8, *B. bacteroides* CB7^T; 9, *Mycoplana bullata* TK0051^T. Data are from this study. Characteristics that were all negative: reduction of nitrates to nitrites, indole production, glucose fermentation, hydrolysis of urea, utilization of arabinose, mannitol, *N*-acetylglucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid, arginine hydrolase, β-glucuronidase, α-mannosidase and α-fucosidase. All strains were positive for esterase lipase (C8). Symbols: –, negative; +, positive; w, weak reaction.

Characteristic	1	2	3	4	5	6	7	8	9
Hydrolysis of:									
Aesculin	+	+	+	+	+	+	+	+	–
Gelatin	–	+	+	+	–	–	+	+	–
Utilization of:									
Glucose	–	–	–	–	+	+	–	–	–
Mannose	–	–	–	–	–	+	–	–	–
Maltose	–	–	–	–	–	+	–	–	–
Malic acid	–	–	–	–	+	–	–	–	–
Galactosidase	+	+	–	+	–	–	–	–	–
Alkaline phosphatase	w	–	+	+	+	+	+	+	+
Esterase (C4)	w	+	+	+	+	+	+	–	+
Lipase (C14)	+	–	–	–	–	–	–	–	w
Leucine arylamidase	+	+	+	+	+	+	+	–	+
Valine arylamidase	+	+	–	–	+	+	+	–	+
Cysteine arylamidase	+	–	–	–	–	–	–	–	w
Trypsin	+	–	+	–	+	+	+	+	+
α-Chymotrypsin	+	–	–	–	w	+	w	–	+
Acid phosphatase	–	–	+	–	+	+	–	–	+
Naphthol-AS-BI-phosphohydrolase	w	–	+	–	+	+	w	w	+
α-Galactosidase	+	–	–	–	–	–	–	–	–
β-Galactosidase	–	–	–	–	–	+	–	–	–
α-Glucosidase	w	+	+	–	+	+	+	+	+
β-Glucosidase	–	–	–	–	+	+	–	w	–
N-Acetyl-β-glucosaminidase	–	–	–	–	–	+	–	–	–

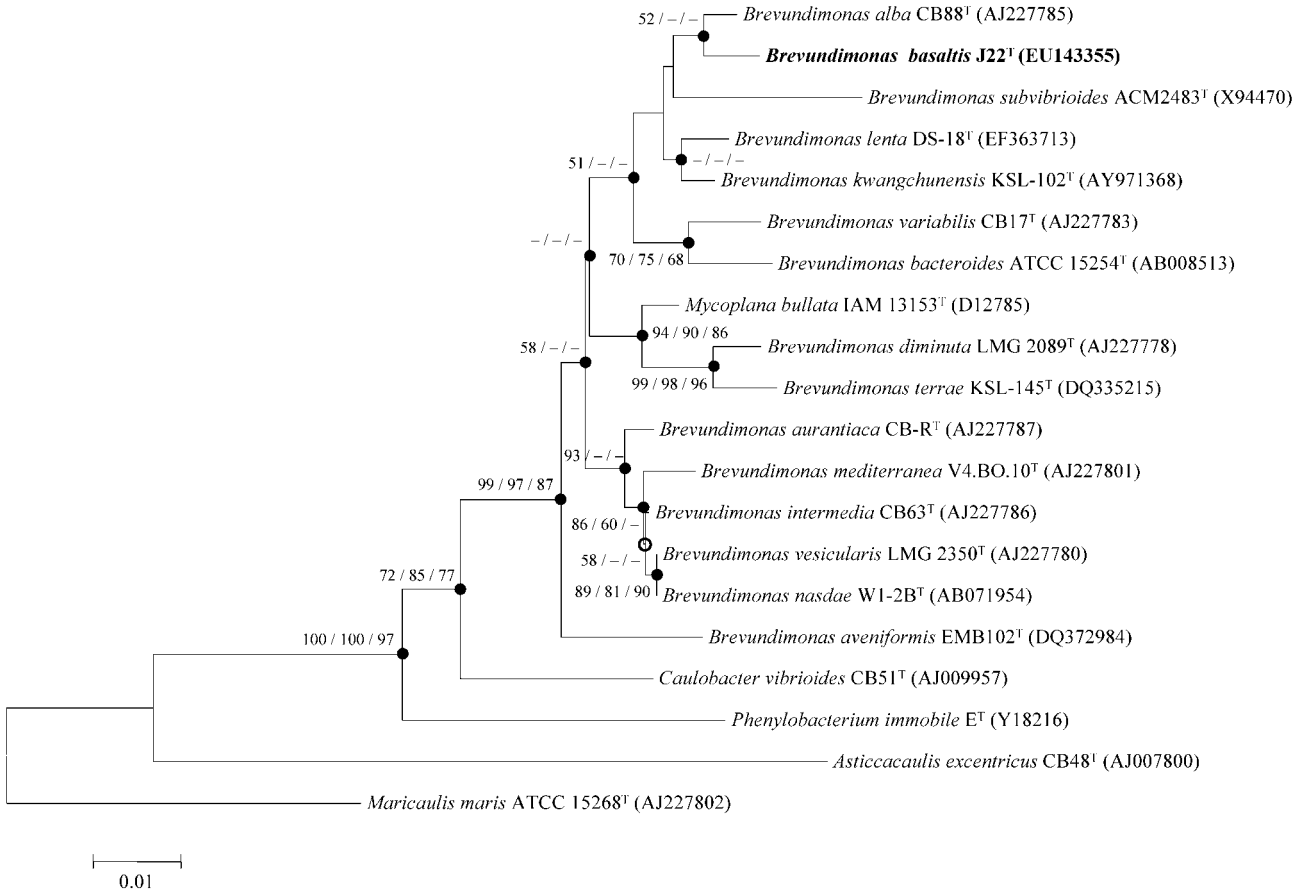

Fig. 1. Phylogenetic consensus tree of strain J22^T and type strains of related taxa based on 16S rRNA gene sequence. The tree was constructed using the neighbour-joining, maximum-parsimony and maximum-likelihood methods and numbers at nodes represent bootstrap values (based on 1000, 1000 and 300 resamplings). Filled circles and the empty circle indicate generic branches that were present among neighbour-joining, maximum-parsimony and maximum-likelihood algorithms, and both neighbour-joining and maximum-parsimony algorithms, respectively. The GenBank accession number of each species is enclosed in parentheses. *Maricaulis maris* ATCC 15268^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

ranges from 61.8 to 68.7 mol%. The major fatty acids detected in strain J22^T were the unsaturated fatty acid $C_{18:1}\omega7c$ (46.4 %) and the saturated fatty acid $C_{16:0}$ (19.3 %); the hydroxyl-fatty acid $C_{12:0}$ 3-OH (2.7 %) was present, which chemotaxonomically characterizes the members of the genus *Brevundimonas*. The fatty acid profiles of strain J22^T and the eight reference strains were very similar. The detailed fatty acid composition is shown in Table 2.

Strain J22^T can be distinguished from related species by lipase (C14) and cysteine arylamidase activity and α-galactosidase utilization and differs from *B. alba* CB88^T in its non-hydrolysis of casein and cellulose. Strain J22^T was classified within the genus *Brevundimonas* on the basis of 16S rRNA gene sequence and, in particular, as highly related to *B. alba* CB88^T (98.95 %). However, DNA–DNA hybridization values between strain J22^T and other *Brevundimonas* strains were less than 22 %; the value was 19 % with *B. alba* CB88^T. This showed that its closest phylogenetic relative, *B. alba*, could not be assigned to the same species (<20 %) (Stackebrandt & Goebel, 1994). On the basis of the phenotypic, genetic and chemotaxonomic analyses, strain J22^T represents a separate novel species of the genus *Brevundimonas*, for which the name *Brevundimonas basaltis* sp. nov. is proposed.

Description of *Brevundimonas basaltis* sp. nov.

Brevundimonas basaltis (ba.sal′tis. L. masc. gen. n. *basaltis* of basalt, pertaining to the source of isolation).

Cells are Gram-negative rods and motile via flagella. Colonies are circular, convex, entire, creamy in colour, and 0.5–2.0 mm in diameter after cultivation for 3 days at 30 °C. Grows at 10–42 °C (optimum 30 °C), with 0–4 % (w/v) NaCl (optimum 0 %) and at pH 5.5–10.0 (optimum pH 7.5). Growth occurs on R2A, *Caulobacter* medium and LB. Catalase-positive. DNA and aesculin are hydrolysed,

Table 2. Fatty acid composition of strain J22^T and related reference strains

Strains: 1, strain J22^T; 2, *B. alba* CB88^T; 3, *B. lenta* DS-18^T; 4, *B. variabilis* CB17^T; 5, *B. kwangchunensis* KSL-102^T; 6, *B. intermedia* CB63^T; 7, *B. subvibrioides* CB81^T; 8, *B. bacteroides* CB7^T; 9, *Mycoplana bullata* TK0051^T. Data are from this study. The values are shown as percentages of the total fatty acids. ND, Not detected; tr, trace (less than 1.0 %). All entries of fatty acids found in amounts <1.0 % are omitted.

Fatty acids	1	2	3	4	5	6	7	8	9
Saturated									
$C_{14:0}$	tr	1.6	3.4	3.1	2.6	2.0	5.5	3.7	1.0
$C_{15:0}$	3.1	2.4	5.3	2.7	5.1	4.1	4.2	2.5	3.9
$C_{16:0}$	19.3	22.5	22.5	19.2	21.2	21.6	18.6	13.6	20.4
$C_{17:0}$	1.8	1.3	1.7	2.5	1.5	3.7	1.4	1.1	1.6
Hydroxy									
$C_{12:0}$ 3-OH	2.7	1.9	2.4	2.8	2.1	3.1	2.8	2.7	1.8
Unsaturated									
$C_{15:1}\omega8c$	1.5	ND	ND	tr	tr	tr	ND	tr	tr
$C_{16:1}\omega9c$	8.1	2.4	ND	ND	ND	ND	ND	tr	ND
$C_{17:1}\omega8c$	4.1	3.1	3.5	3.5	3.0	2.7	2.9	3.4	3.1
$C_{17:1}\omega6c$	tr	ND	1.2	1.4	1.0	1.8	1.1	2.2	1.5
$C_{18:1}\omega9c$	1.3	1.7	ND	ND	ND	ND	ND	ND	ND
$C_{18:1}\omega7c$	46.4	37.5	39.0	57.3	36.2	39.6	54.9	60.5	50.7
$C_{18:1}\omega5c$	1.8	1.1	tr	tr	1.1	tr	tr	1.2	1.4
11-Methyl $C_{18:1}\omega7c$	3.3	8.5	14.0	ND	19.3	12.1	tr	1.6	2.7
Cyclo $C_{19:0}\omega8c$	ND	12.4	ND	ND	ND	ND	ND	ND	4.6
Summed feature*									
3	2.8	3.7	5.8	4.9	5.3	5.4	5.1	4.5	4.6

*Summed feature 3 contained $C_{16:1}\omega7c$, $C_{15:0}$ 2-OH iso.

but casein, cellulose, urea and gelatin are not. Indole is not produced. Positive for activity of esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin and α-galactosidase. Negative for activity of acid phosphatase, β-galactosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, β-glucuronidase, α-mannosidase and α-fucosidase, and negative reactions are obtained for utilization of glucose, mannose, maltose, malic acid, arabinose, mannitol, *N*-acetylglucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid (API 20NE and API ZYM). Utilizes dextrin, β-hydroxybutyric acid, α-ketoglutaric acid, quinic acid, L-alaninamide and L-aspartic acid (Biolog GN2). Other organic substrates are not utilized. Major fatty acids are $C_{16:0}$ (19.3 %) and $C_{18:1}\omega7c$ (46.4 %). The DNA G+C content is 66.3 mol%.

The type strain, J22^T (=KCTC 22177^T=JCM 15911^T), was isolated from black sand taken from Soesoggak Beach on Jeju, Republic of Korea.

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