Burkholderia sediminicola sp. nov., isolated from freshwater sediment

Ju Hyoung Lim, Sang-Hoon Baek and Sung-Taik Lee

Correspondence
Sung-Taik Lee
e_stlee@kaist.ac.kr

Environmental and Molecular Microbiology Lab., Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 335 Gwahangno (373-1 Guseong-dong), Yuseong-gu, Daejeon 305-701, Republic of Korea

A Gram-negative, motile and rod-shaped bacterium, designated HU2-65W^T, was isolated from freshwater sediment. The strain possessed ubiquinone 8 as the predominant isoprenoid quinone and contained major amounts of ω7-*cis*-octadecenoic acid and hexadecanoic acid in its cell envelope, which are properties shared by members of the genus *Burkholderia*. On the basis of 16S rRNA gene sequence similarity, strain HU2-65W^T was most closely related to the type strain of *Burkholderia xenovorans* (98.4 %). The DNA G+C content of strain HU2-65W^T was 61.2 mol%, and DNA–DNA relatedness values to type strains of closely related species were found to be much lower than 70 %, indicating that the strain represents a separate genomic species within the genus *Burkholderia*. Strain HU2-65W^T was also differentiated from other species of the genus by physiological and biochemical characteristics. Consequently, strain HU2-65W^T is considered to represent a single, novel species of the genus *Burkholderia*, for which the name *Burkholderia sediminicola* sp. nov. is proposed, with the type strain HU2-65W^T (=KCTC 22086^T =LMG 24238^T).

The genus *Burkholderia* has been recognized as a group with great diversity and at present contains more than 40 species with validly published names, which have been isolated from a wide array of ecological niches, ranging from soil to animal clinical samples (Coenye & Vandamme, 2003). During attempts to investigate the culturability and diversity of bacteria in freshwater sediment, an aerobic bacterium, designated HU2-65W^T, was isolated from an extinction-culture plate and was then subjected to taxonomic studies. The genotypic, phenotypic and chemotaxonomic traits provided evidence that strain HU2-65W^T belongs to a novel species in the genus *Burkholderia*.

Sediment samples were collected from Lake Hakha (36° 16′ 60″ N 127° 18′ 32″ E), a typical freshwater lake in South Korea. The sediment was homogenized by using a Nissei homogenizer and the suspension was serially diluted with freshwater medium, which was collected at the same sampling site the day prior to sampling and was then filter-sterilized. Dilution-to-extinction arrays (Button *et al.*, 1993) were made in 96-well plates to reach a cell concentration of 1.1 cells per volume according to the total count in the sediment sample. The plates were incubated in a GasPak EZ anaerobic container (BD) for 3 months at ambient temperature in a cycle consisting of 12 h light and 12 h darkness. In order to detect non-turbidity growth in extinction wells (Martens-Habbena & Sass, 2006), portions of cell suspensions were stained with 4′,6′-diamidino-2-phenylindole (DAPI) and fluorescence was measured by a microplate reader Flx 800 (BioTek). One single colony was isolated on a 1/10 R2A agar plate to which bacterial suspension was transferred from a well showing bacterial growth. The isolate was then routinely propagated aerobically on R2A agar at 28 °C for 2 days.

Colonies were directly applied to 16S rRNA gene PCR and amplified fragments were then sequenced using the primers 9F (Stackebrandt & Liesack, 1993), 341F (Muyzer *et al.*, 1993), 536R and 907F (Lane *et al.*, 1985), 1100R (Lane, 1991) and 1512R (Weisburg *et al.*, 1991). The 16S rRNA gene sequence of strain HU2-65W^T was compared with all 16S rRNA gene sequences available in GenBank, and alignment of sequences was carried out with the CLUSTAL_X program (Thompson *et al.*, 1997). Gaps at the 5′ and 3′ ends and ambiguous bases were removed from the alignment using the BioEdit software (Hall, 1999). DNA for determination of base composition and hybridization analysis was extracted and purified using the Qiagen Genomic-tip system 100/G. Enzymic degradation of DNA into nucleosides was carried out as described previously (Mesbah *et al.*, 1989). DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) in black MaxiSorp FluoroNunc module plates (Nunc). Before hybridization, biotinylated probe DNAs were sheared by ultrasonication using a Bioruptor

Abbreviation: PHA, polyhydroxyalkanoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Burkholderia sediminicola* HU2-65W^T is EU035613.

(Cosmo). Hybridization was performed at 49.4 °C in the presence of 50 % (v/v) formamide with the ionic strength of 0.3 M NaCl and carried out reciprocally with five replications per sample.

Cell morphology and motility were examined by phase-contrast microscopy. Catalase and oxidase activities were determined by using standard methods (Cowan & Steel, 1965). The Gram reaction was performed according to Buck (1982). Substrate assimilation and some biochemical characters were determined using API 32GN, API 20NE and API ZYM galleries (bioMérieux) according to the manufacturer's protocol. Polyhydroxyalkanoate (PHA) accumulation was tested by viable-colony staining (Spiekermann *et al.*, 1999) under nitrogen-depletion conditions supplemented with 2 % (w/v) glucose as an excess carbon source (Wieczorek *et al.*, 1996). Presence of the PHA synthase gene was investigated by amplifying an internal region of *phaC* with the primer set I-179L/I-179R (Solaiman *et al.*, 2000). Amylase and DNase activities were detected on starch agar and DNase test agar (Difco), respectively. Carbohydrate oxidation/fermentation (O/F) test was performed according to Hugh & Leifson (1953). Antibiotic susceptibility was evaluated by MIC determination (Andrews, 2001), whereby 12 successive dilutions of antibiotics in R2A broth were used. Isoprenoid quinones were determined as described previously (Komagata & Suzuki, 1987) using Sep-Pak Vac silica cartridges (Waters). For quantitative analysis of cellular fatty acid compositions, cells were harvested after 24 h incubation at 28 °C on TSA (Difco), which was based on the culture conditions used for closely related *Burkholderia* strains (Coenye *et al.*, 2001; Goris *et al.*, 2002, 2004), and cellular fatty acid methyl esters were prepared and identified following the protocol of the Sherlock Microbial Identification System (MIDI). All phenotypic tests described above were performed at least twice with 2 days incubation, unless otherwise specified.

The 16S rRNA gene sequence of strain HU2-65W^T (1473 bp) showed the highest similarity to members of the genus *Burkholderia*; the type strains of *Burkholderia xenovorans*, *Burkholderia bryophila* (Vandamme *et al.*, 2007), *Burkholderia fungorum* and *Burkholderia terricola* showed 98.4, 98.3, 98.2 and 98.1 % similarity, respectively, to strain HU2-65W^T. In the phylogenetic tree constructed by the neighbour-joining algorithm (Saitou & Nei, 1987), strain HU2-65W^T formed a coherent cluster within the *Burkholderia graminis* group (Goris *et al.*, 2004) (Fig. 1). The DNA G+C content of strain HU2-65W^T was 61.2 mol%, which is very similar to values for closely related species. DNA–DNA relatedness values in reciprocal hybridizations were much lower than 70 % between strain HU2-65W^T and the following closely related members of the genus *Burkholderia*: *B. xenovorans* KACC 12026^T (43 and 47 %, reciprocally), *B. bryophila* LMG 23644^T (33 and 34 %), *B. fungorum* KACC 12023^T (49 and 23 %), *B. terricola* KACC 12025^T (41 %) and *Burkholderia ginsengisoli* KCTC 12389^T (47 and 56 %), indicating that strain HU2-65W^T represents a separate genomic species belonging to the genus *Burkholderia*.

Morphological, physiological and biochemical characteristics of strain HU2-65W^T were consistent with its classification within the genus *Burkholderia* (Table 1). Cells were Gram-negative, motile, slightly curved rods and the strain grew optimally at 25–30 °C and pH 6. The strain was able to accumulate PHA on a nitrogen-depletion medium supplemented with 2 % (w/v) glucose, which was confirmed by the presence of the *phaC* gene, of which a 540 bp internal region (Solaiman *et al.*, 2000) was amplified and sequenced (GenBank accession no. EU035614). Resistance up to 100 μg ampicillin ml^{-1} was observed, but the strain did not grow with kanamycin, streptomycin or tetracycline, even at concentrations of 1 μg ml^{-1}. Strain HU2-65W^T was unable to assimilate L-arabinose, as found previously in *B. xenovorans* strains (Goris *et al.*, 2004). The strain possessed ubiquinone Q-8 as the predominant isoprenoid quinone, which is typical of members of the genus *Burkholderia*. The high levels of ω7-*cis*-octadecenoic acid ($C_{18:1}\omega7c$) and hexadecanoic acid ($C_{16:0}$) were in good agreement with cellular fatty acid

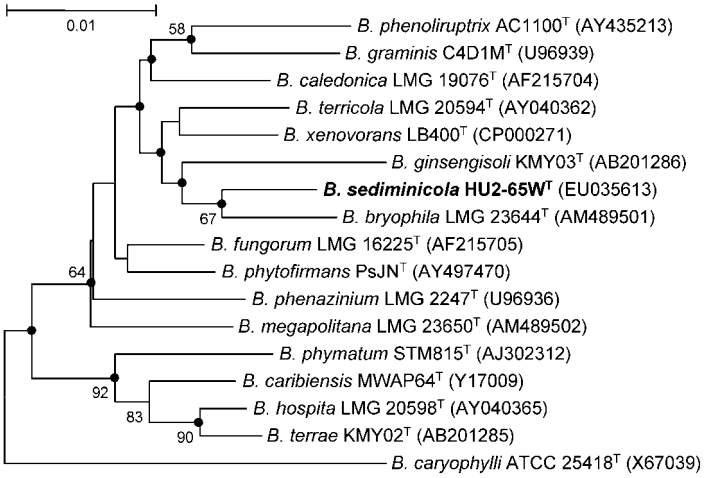

Fig. 1. Neighbour-joining tree showing relationships between strain HU2-65W^T and closely related taxa belonging to the genus *Burkholderia*. Bootstrap values of 50 % or more (using 1000 replications) are indicated at branch points. Filled circles indicate that the corresponding nodes were also recovered in maximum-parsimony trees. Accession numbers are shown in parentheses. Bar, 0.01 expected substitutions per nucleotide position.

Table 1. Phenotypic characteristics that distinguish strain HU2-65W^T from closely related *Burkholderia* species

Taxa: 1, strain HU2-65W^T; 2, *B. xenovorans* (3 strains included); 3, *B. bryophila* (14); 4, *B. fungorum* (9); 5, *B. terricola* (17); 6, *B. ginsengisoli* (1). Data for reference species were taken from Coenye *et al.* (2001, 2004), Fain & Haddock (2001), Goris *et al.* (2002, 2004), Lim *et al.* (2003), Kim *et al.* (2006) and Vandamme *et al.* (2007). The following features are present in all strains: growth at 30 °C, activity of acid and alkaline phosphatase and leucine arylamidase and assimilation of *N*-acetyl-D-glucosamine, D-glucose, DL-lactate and mannitol. The following features are absent in all strains: growth at NaCl concentrations above 3 % (w/v) and activity of chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, trypsin and tryptophanase. +, All strains positive; −, all strains negative; V (+), strain dependent, positive for type strain; V (–), strain dependent, negative for type strain; ND, no data available.

Characteristic	1	2	3	4	5	6
Nitrate reduction	−	V (–)	−	+	+	−
Growth at 37 °C	−	V (–)	−	+	V (+)	+
Acid production in O/F medium with D-glucose	−	−	−	+	V (+)	−
Activity of C_{14}-lipase	+	−	−	V (–)	−	−
Assimilation of:						
L-Arabinose	−	−	+	V (+)	+	ND
D-Gluconate	−	+	+	+	+	+
D-Mannose	−	+	+	+	+	+
Major fatty acid composition (%)*						
$C_{16:0}$	20.8	18.2	ND	14.7±0.9	14.3±1.3	16.1
$C_{17:0}$ cyclo	7.0	5.1	ND	5.1±1.6	14.0±5.2	24.9
$C_{19:0}$ cyclo $\omega 8c$	2.9	3.6	ND	2.5±0.7	5.5±3.0	15.1
$C_{18:1}\omega 7c$	31.3†	27.3	ND	35.6±2.1	27.8±8.8	4.2
Summed feature 3‡	8.0	8.5	ND	8.1±1.1	8.2±2.8	11.7
Summed feature 4§	17.0	19.1	ND	13.6±1.9	9.1±4.9	2.3

*Some values are represented as means±SD.
†Composed of $C_{18:1}\omega 7c/\omega 9t/\omega 12t$ and/or $C_{18:1}\omega 7c/\omega 9c/\omega 12t$.
‡Composed of $C_{12:0}$ ALDE with ECL 10.928/iso-$C_{16:1}$ I/$C_{14:0}$ 3-OH.
§Composed of iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega 7c$.

profiles of previously described *Burkholderia* species. The presence of 3-hydroxyhexadecanoic acid ($C_{16:0}$ 3-OH) was also consistent with a unique characteristic shared by members of the genus *Burkholderia* (Gillis *et al.*, 1995). Phenotypic characteristics of strain HU2-65W^T and those of the most closely related species are summarized in Table 1.

The genotypic and phenotypic data given above clearly demonstrate that strain HU2-65W^T represents a novel *Burkholderia* species, for which we propose the name *Burkholderia sediminicola* sp. nov.

Description of *Burkholderia sediminicola* sp. nov.

Burkholderia sediminicola (se.di.mi.ni.co′la. L. n. *sedimen*, *-inis* sediment; L. suff. *-cola* inhabitant, dweller; N.L. n. *sediminicola* sediment-dweller, referring to the source of the type strain).

Rod-like cells occur as single units or in pairs with rounded ends and are 0.6–1.4 μm long by 0.3–0.5 μm wide. Colonies grown on R2A for 36 h are circular and convex with an entire margin, 0.6–1 mm in diameter, translucent and creamy white in colour. No slime formation is observed. Temperature range for growth is 15–30 °C; no growth occurs at 42 °C. Cells grow at pH 6.0–8.0 and are unable to denitrify. Best growth is obtained without NaCl, but growth is possible in the presence of 1.5 % (w/v) NaCl. None of D-fructose, D-glucose, maltose or D-xylose is oxidized or fermented in O/F medium. Tests for catalase, C_4-esterase, C_8-ester lipase, naphthol-AS-BI-phosphohydrolase and oxidase activities are positive. Tests for amylase, arginine dihydrolase, cystine arylamidase, DNase, gelatinase, *N*-acetyl-β-glucosaminidase, β-glucosidase, β-glucuronidase, urease and valine arylamidase activities are negative. β-Galactosidase activity is substrate dependent, i.e. 4-nitrophenyl β-D-galactopyranoside is hydrolysed but 2-naphthyl β-D-galactopyranoside is not. Carbenicillin, cephalothin, lincomycin, penicillin G and rifampicin do not inhibit growth at concentrations of up to 100 μg ml^{-1}. Resistance is shown to up to 50 μg novobiocin, 20 μg hygromycin B, 15 μg oleandomycin or 5 μg chloramphenicol ml^{-1}, but growth is inhibited by erythromycin, gentamicin or neomycin even at concentrations of 1 μg ml^{-1}. L-Alanine, citrate, L-fucose, histidine, 4-hydroxybenzoate, 3-hydroxybutyrate, 2-ketogluconate, malate, phenylacetate, L-proline, D-ribose and D-sorbitol are utilized as sole carbon sources; acetate, adipate, caprate,

glycogen, 3-hydroxybenzoate, inositol, itaconate, 5-keto-gluconate, malonate, maltose, melibiose, propionate, rhamnose, salicin, L-serine, suberate, sucrose and valerate are not. The following fatty acids are present in relatively small amounts: $C_{16:0}$ 3-OH (6.2 %), $C_{16:0}$ 2-OH (2.2 %), $C_{14:0}$ (1.4 %), $C_{16:1}$ 2-OH (1.3 %), $C_{18:1}$ 2-OH (0.8 %), $C_{18:0}$ (0.7 %) and $C_{18:1}\omega5c$ (0.5 %). Additional characteristics are listed in Table 1 and described elsewhere in the text.

The type strain is HU2-65W^T (=KCTC 22086^T =LMG 24238^T), which was isolated from freshwater sediment.

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