The Journal of Microbiology (2012) Vol. 50, No. 5, pp. 855–859
DOI 10.1007/s12275-012-1554-2

NOTE

Burkholderia denitrificans sp. nov., Isolated from the Soil of Dokdo Island, Korea§

Chang-Muk Lee[1], Hang-Yeon Weon[2], Sang-Hong Yoon[1], Soo-Jin Kim[2], Bon-Sung Koo[1], and Soon-Wo Kwon[2]*

[1]Functional Biomaterial Division, [2]Korean Agricultural Culture Collection (KACC), Agricultural Microbiology Team, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea

(Received November 4, 2011 / Accepted May 18, 2012)

A novel, Gram-negative, bacterial strain KIS30-44^T was identified from wet forest soil collected on the Korean island of Dokdo. Growth of the strain was observed at 15–30°C, pH 5–9, 0–3% NaCl, and 950 mM KNO_3. KIS30-44^T reduced nitrate to nitrogen gas. Analysis of the 16S rRNA gene sequence showed that KIS30-44^T was phylogenetically related to *Burkholderia sacchari*, *Burkholderia mimosarum*, and *Burkholderia oxyphila* (98.1%, 98.0%, and 98.0% sequence similarity, respectively). The genomic G+C content was 63.5 mol%. KIS30-44^T exhibited less than 52% DNA-DNA relatedness with the type strains of 9 closely related *Burkholderia* species. The major isoprenoid quinone was Q-8. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and two unknown aminolipids. The major fatty acids in KIS30-44^T were $C_{16:0}$, $C_{18:1}$ *ω7c* and summed feature 3 (iso-$C_{15:0}$ 2-OH and $C_{16:1}$ *ω7c*), and the strain contained half the amount of $C_{17:0}$ cyclo found in the 9 closely related *Burkholderia* species. The results of these phenotypic, 16S rRNA gene sequence, DNA-DNA hybridization, and chemotaxonomic data indicate that KIS30-44^T represents a novel species within the genus *Burkholderia*, for which the name *Burkholderia denitrificans* (Type strain KIS30-44^T =KACC 12733^T =DSM 24336^T) is proposed.

Keywords: *Burkholderia denitrificans*, 16S rRNA gene sequence, taxonomy

The genus *Burkholderia* (previously part of *Pseudomonas*) includes physiologically invasive, resistant to toxic inorganic compounds, and resilient bacteria that have been identified from widely diverse environmental and ecological niches, such as contaminated soils and human organs (Ulrich *et al.*, 2004; Rosen *et al.*, 2011). Since the genus was first reported (Yabuuchi *et al.*, 1992), 55 *Burkholderia* type strains have been deposited into international culture collections (RDP release 10, Aug. 9, 2011). Most members of the genus *Burkholderia* are characterized as Gram-negative, motile, and obligatorily aerobic (or facultatively anaerobic) rod-shaped bacteria, including some environmentally important species. *Burkholderia xenovorans* is particularly noteworthy, in that it can degrade polychlorinated biphenyls and chlorinated pesticides using aromatic compounds as sole sources of carbon and energy (Daugulis and Rehmann, 2008; Sylvestre *et al.*, 2011). Moreover, the genus *Burkholderia*, along with *Pseudomonas*, *Xanthomonas*, *Bacillus*, and *Streptomyces*, is known to play an important role in recycling nitrogen in the environment, reducing nitrogen burdens in various types of contaminated soils (Cheneby *et al.*, 2000). Here, we describe a taxonomic study of a new strain of *Burkholderia* sp. KIS30-44^T, which was isolated from soil collected on Dokdo Island, Korea.

The wet forest soil collected from Dokdo Island was homogenized, serially diluted with NaCl solution (0.85%, w/v), and spread on R2A agar plates (BBL) containing 0.02% cycloheximide (Sigma, USA). The plates were incubated at 28°C for 5 days, and the strain KIS30-44^T was recovered. Single colonies were maintained as glycerol suspensions (20%, w/v) at -70°C. We routinely cultivated the KIS30-44^T strain on R2A agar at 30°C.

Cell morphology was examined by using phase-contrast (AXIO; Zeiss, Germany) and transmission electron (LEO model 912AB) microscopy with cells grown for 2 days on R2A agar at 30°C. Growth at various temperatures (5–42°C) was measured on R2A agar plates. The optimum pH for growth was examined in R2A broth adjusted to various pH values (pH 4–10 at intervals of 1.0 pH unit). Anaerobic growth was investigated using incubation in the GasPak Unaerobic System (BBL, USA) at 28°C for 15 days. Abiotic salt tolerance was tested at different NaCl concentrations (0, 1, 2, 3, and 5%, w/v) in R2A broth. For nitrate and nitrite reduction tests, each isolate was inoculated into 3 culture tubes (25 ml) containing 13 ml of R2A medium supplemented with either $NaNO_2$ (10–50 mM) or KNO_3 (10–1,000 mM), and the media were overlaid with sterile paraffin oil (approximately 1 ml) in order to exclude atmospheric oxygen. The reduction of nitrate and nitrite was monitored by an ion

*For correspondence. E-mail: swkwon1203@korea.kr; Tel.: +82-31-299-1860; Fax: +82-31-299-1869
§Supplemental material for this article may be found at http://www.springer.com/content/120956.

chromatograph (Model DX-320, Personal IC; Dionex, USA) equipped with a conductivity detector and anion exchange column (IonPac AS14; Dionex) as described (Gjerde and Fritz, 1987). N_2 gas generated by KNO_3 reduction was collected from cell cultures using Durham tubes at 30°C for 4 days. The physiological and biochemical properties of KIS30-44^T were determined using API ZYM, API 20NE, and API ID 32GN test kits (bioMérieux, France) following the manufacturer's instructions. API ZYM test strips were assayed after a 4 h incubation at 28°C, whereas other API test strips were examined after 5 days at 28°C.

The G+C content of the total DNA was analyzed by reverse-phase HPLC (Supelcosil LC-18; Supelco), as described by Mesbah *et al.* (1989) and Ezaki *et al.* (1989). Isoprenoid quinones were analyzed by HPLC as described by Groth *et al.* (1996). The polar lipid profile was determined according to the method of Minnikin *et al.* (1984). Cellular fatty acids were determined from a culture grown on R2A at 30°C for 48 h. Whole-cell fatty acids were extracted, methylated, and analyzed following previously described procedures (Sasser, 1990). The fatty acid methyl esters were identified and quantified by using the TSBA database (ver. 6.0) of the Sherlock Microbial Identification System (MIDI). Genomic DNA was extracted using a commercial kit (Promega, USA).

PCR-mediated amplification of the 16S rRNA gene and direct sequencing of the purified product were carried out as described previously (Lee *et al.*, 2008). The resulting 16S rRNA sequence (1,467 bp) was aligned using the ARB software package (Ludwig *et al.*, 2004) and was added to the alignment of the SILVA SSURef 106 database (release April 2011) (Pruesse *et al.*, 2007). The aligned nucleotide positions, with 30% and 50% conservation and without filters, were used for phylogenetic analyses using Mega version 4.0 software (Tamura *et al.*, 2007). Phylogenetic trees were inferred using neighbor-joining with Kimura's two-parameter model and maximum parsimony. We used the EzTaxon server to compare sequence similarities among strains (http://www.eztaxon.org/; Chun *et al.*, 2007).

To determine genomic relatedness, the filter hybridization method was performed according to Seldin and Dubnau (1985). Probe labeling was conducted using a nonradioactive DIG high-prime system (Roche, Switzerland); hybridized DNA was visualized using a DIG luminescent detection kit (Roche). DNA-DNA relatedness was quantified using Bio-1D image analysis software (Vilber Lourmat).

KIS30-44^T cells were Gram-negative, motile, facultatively anaerobic, short rods (Supplementary data Fig. S1). Strain KIS30-44^T grew on R2A, nutrient agar (Difco, USA), and trypticase soy agar (Difco), but not on MacConkey agar. In nitrate reduction tests, strain KIS30-44^T reduced nitrate to nitrite and nitrite to nitrogen gas. The maximum abiotic stress tolerance of strain KIS30-44^T was 500 mM NaCl, 950 mM KNO_3, and 50 mM $NaNO_2$.

16S rRNA gene sequence analysis of strain KIS30-44^T revealed that this novel strain had the highest nucleotide similarity with members of the *Burkholderiaceae* family of β-proteobacteria, including *B. sacchari* IPT101^T (98.1%), *B. mimosarum* PAS44^T (98.0%), *B. oxyphila* OX-01^T (98.0%), *B. ferrariae* FeGl01^T (97.8%), *B. unamae* MTl-641^T (97.7%), *B. heleia* SA42^T (97.7%), *B. nodosa* R-25485^T (97.5%), *B. kururiensis* JCM 10599^T (97.1%), *B. silvatlantica* SRMrh-20^T (97.0%), *B. bannensis* E25^T (97.0%), and *B. tropica* Ppe8^T (97.0%). The relationships between strain KIS30-44^T and other members of the genus *Burkholderia* were evident in the phylogenetic tress constructed using the neighbor-joining algorithm (Fig. 1). Strain KIS30-44^T formed a cluster with *B. ferrariae*, *B. heleia*, *B. mimosarum*, *B. nodosa*, *B. oxyphila*, *B. sacchari*, *B. silvatlantica*, *B. tropica*, *B. bannensis*,

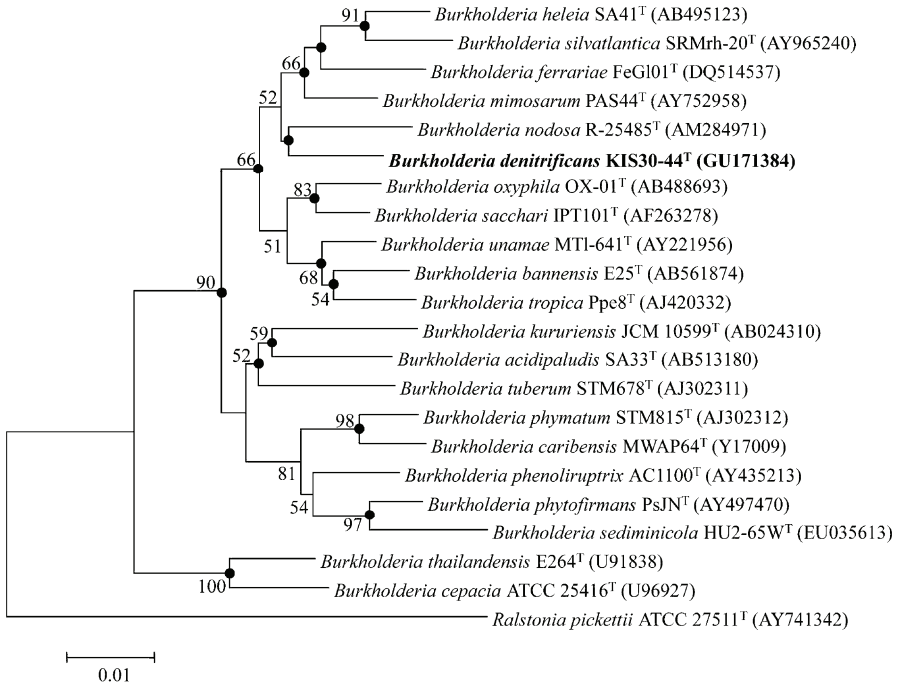

Fig. 1. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain KIS30-44^T. The nucleotide positions were aligned without filtering. Numbers at branch nodes indicate the percentage of 1,000 bootstrap resamplings. Bootstrap values greater than 50% are shown at branch points. The dots on the nodes indicate that the corresponding branches were also recovered in trees generated with the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics of KIS30-44^T and type strains of closely related *Burkholderia* species

Strains: 1, *Burkholderia* sp. KIS30-44^T; 2, *Burkholderia ferrariae* DSM 18251^T; 3, *Burkholderia heleia* KACC 16324^T; 4, *Burkholderia kururiensis* DSM 13646^T; 5, *Burkholderia mimosarum* LMG 23256^T; 6, *Burkholderia nodosa* LMG 23741^T; 7, *Burkholderia oxyphila* KACC 16325^T; 8, *Burkholderia sacchari* DSM 17165^T; 9, *Burkholderia silvatlantica* LMG 23149^T; 10, *Burkholderia unamae* DSM 17197^T. All data were obtained in this study unless otherwise indicated. All strains are positive for acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and urease. All strains assimilated *N*-acetylglucosamine, adipic acid, L-alanine, L-arabinose, L-fucose, D-glucose, L-histidine, 4-hydroxybenzoic acid, 3-hydroxybutyric acid, inositol, lactic acid, D-mannitol, D-mannose, phenylacetic acid, potassium gluconate, potassium 2-ketogluconate, propionic acid, D-ribose, L-serine, sodium acetate, D-sorbitol and valeric acid. All strains are negative for *N*-acetyl-β-glucosaminidase, aesculin hydrolysis, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, gelatin hydrolysis, α-glucosidase, β-glucosidase, β-glucuronidase, indole production, α-mannosidase and trypsin. No strains assimilate D-maltose, potassium 5-ketogluconate, and sodium malonate. +, positive; (+), weakly positive; -, negative; ND, not determined.

Characteristics	1	2	3	4	5	6	7	8	9	10
Catalase	-/+	+/+[a]	+/+[b]	+/+[c]	+/+[d]	+/+[e]	+/-[f]	+/+[g]	+/+[h]	+/+[i]
Motility	+	ND	-[b]	-[c]	ND	ND	-[f]	+[g]	ND	+[i]
Nitrate reduction	+	+	+	+	+	+	-	+	+	+
Glucose fermentation	-	-	+	-	-	-	-	-	-	-
Assimilation of:										
Capric acid	-	-	+	-	-	-	+	+	+	+
Glycogen	-	-	+	-	+	-	-	-	-	-
3-Hydroxybenzoic acid	-	+	-	-	+	-	+	-	-	+
Itaconic acid	-	-	-	-	-	-	+	+	+	+
Malic acid	-	+	+	-	-	+	+	+	+	-
D-Melibiose	-	-	-	-	-	-	-	-	-	+
L-Proline	-	+	+	+	+	+	+	+	+	+
L-Rhamnose	+	+	+	+	-	+	-	-	+	+
D-Saccharose	+	-	-	-	+	-	-	+	+	-
Salicin	-	-	-	-	-	+	-	-	-	+
Suberic acid	-	-	+	-	-	-	+	+	-	+
Trisodium citrate	-	+	+	-	-	+	+	+	+	-
Enzymatic activities of:										
Alkaline phosphatase	+	+	+	+	-	+	+	+	-	+
Arginine dihydrolase	-	+	+	+	-	+	+	+	+	+
β-Galactosidase	-	+	+	+	+	+	-	-	-	+
Lipase (C14)	-	-	-	-	-	-	-	-	-	(+)
Valine arylamidase	+	-	(+)	(+)	(+)	+	(+)	+	(+)	+
DNA G+C content (mol%)	63.5	62.7[a]	64.0[b]	64.8[c]	64.8[d]	62.8[e]	64.0[f]	ND	ND	ND

Data from [a] Valverde *et al.* (2006), [b] Aizawa *et al.* (2010), [c] Zhang *et al.* (2000), [d] Chen *et al.* (2006), [e] Chen *et al.* (2007), [f] Otsuka *et al.* (2011), [g] Brämer *et al.* (2001), [h] Perin *et al.* (2006), [i] Caballero-Mellado *et al.* (2004).

and *B. unamae*. This cluster was also supported by other neighbor-joining trees based on maximum-parsimony algorithms (Fig. 1).

DNA-DNA relatedness experiments, conducted in triplicate to compare strain KIS30-44^T with 9 type strains whose 16S rRNA sequence similarities to KIS30-44^T were higher than 97%, showed a maximum of 52% DNA-DNA relatedness to *B. sacchari* DSM 17165^T (reciprocal 54%), whereas 36–51% DNA relatedness values were observed for the other 8 *Burkholderia* type stains (*B. mimosarum* LMG 23256^T, *B. oxyphila* KACC 16325^T, *B. ferrariae* DSM 18251^T, *B. unamae* DSM 17197^T, *B. heleia* KACC 16324^T, *B. nodosa* LMG 23741^T, *B. kururiensis* DSM 13646^T, and *B. silvatlantica* LMG 23149^T).

Phenotypic comparisons of selective characteristics of the tested strains are shown in Table 1. Compared to its closest phylogenetic relatives, *B. sacchari* and *B. mimosarum*, strain KIS30-44^T was unique in its enzymatic assimilation of L-rhamnose. Moreover, the phenotypic characteristics of strain KIS30-44^T were different from known *Burkholderia* in the expression of arginine dihydrolase and β-galactosidase, and KIS30-44^T was unable to assimilate many substrates.

The DNA G+C content of strain KIS30-44^T was 63.5 mol%. The major isoprenoid quinone was Q-8. The polar lipid profile consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and two unknown aminolipids (ALs) (Supplementary data Fig. S2). The whole-cell fatty acids consisted mainly of $C_{16:0}$ (35.8%), summed feature 3 (iso-$C_{15:0}$ 2-OH and $C_{16:1}$ *ω7c*) (17.5%), and $C_{18:1}$ *ω7c* (10.6%). In comparison with other *Burkholderia* species, strain KIS30-44^T contained smaller amounts of $C_{17:0}$ cyclo and $C_{19:0}$ cyclo *ω8c* in its fatty acid composition (Table 2).

On the basis of morphological and 16S rRNA gene sequence analyses, strain KIS30-44^T can be considered a member of the genus *Burkholderia*. However, our data demonstrating the DNA-DNA relatedness, physiological characteristics, and fatty acid profile of strain KIS30-44^T support the novelty of this isolate. Strain KIS30-44^T can be clearly differentiated from 3 other closely related *Burkholderia* type strains by its assimilation activities toward L-proline and L-rhamnose and its variations in fatty acid composition (for $C_{17:0}$ cyclo and summed feature 3). Therefore, we conclude that strain KIS30-44^T represents a novel species of the genus *Burkholderia*, for which the name *Burkholderia denitrificans* sp. nov. is

Table 2. Fatty acid compositions of KIS30-44^T and closely related *Burkholderia* species
Strains: 1, *Burkholderia* sp. KIS30-44^T; 2, *Burkholderia ferrariae* DSM 18251^T; 3, *Burkholderia heleia* KACC 16324^T; 4, *Burkholderia kururiensis* DSM 13646^T; 5, *Burkholderia mimosarum* LMG 23256^T; 6, *Burkholderia nodosa* LMG 23741^T; 7, *Burkholderia oxyphila* KACC 16325^T; 8, *Burkholderia sacchari* DSM 17165^T; 9, *Burkholderia silvatlantica* LMG 23149^T; 10, *Burkholderia unamae* DSM 17197^T. All the data were obtained from this study. Fatty acids representing less than 1.0 % are omitted.

Fatty acids	1	2	3	4	5	6	7	8	9	10
$C_{13:1}$ at 12-13	1.7	2.7	1.4	3.3	3.3	1.6	1.2	1.6	1.7	1.7
$C_{14:0}$	5.6	5.1	4.6	4.9	4.9	3.9	4.2	2.5	5.1	5.0
$C_{16:0}$	35.8	21.2	35.9	21.6	34.0	22.7	25.4	25.6	28.8	20.5
$C_{16:0}$ 2-OH	-	3.6	-	2.2	-	1.3	2.2	1.2	1.8	2.1
$C_{16:0}$ 3-OH	3.3	5.0	3.2	2.1	2.2	3.1	3.3	2.4	3.0	3.1
$C_{16:1}$ 2-OH	-	1.9	-	1.0	-	-	-	-	-	1.6
$C_{17:0}$ cyclo	8.7	19.3	32.3	13.5	28.2	21.2	24.1	25.4	28.8	19.2
$C_{18:0}$	-	-	-	1.0	-	-	-	-	-	1.0
$C_{18:1}$ *ω7c*	10.6	8.1	-	16.7	5.7	20.0	-	18.1	2.6	17.0
$C_{18:1}$ *ω9c*	-	-	-	-	-	-	-	-	-	-
$C_{19:0}$ cyclo *ω8c*	9.4	17.7	10.9	22.0	8.6	13.5	21.1	10.7	19.4	17.9
Summed feature 2[a]	4.3	10.1	5.5	5.4	7.3	5.7	5.3	5.7	5.8	5.5
Summed feature 3[a]	17.5	3.5	1.8	3.6	4.4	3.8	-	4.6	-	2.8

[a] Summed feature 2 comprises $C_{12:0}$ aldehyde, an unknown fatty acid of equivalent chain length 10.928, $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I. Summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ *ω7c*.

proposed.

Description of *Burkholderia denitrificans* sp. nov

Burkholderia denitrificans (de.ni.tri'fi.cans, N.L. v. *denitrifico* to denitrify, N.L. part. Adj. *denitrificans* denitrifying).

Cells are Gram-negative, facultative anaerobic, slightly curved, short rods, motile, and measuring 1.6–2.3 μm long by 0.6–0.8 μm wide. Colonies grown on R2A are circular, convex, and cream-colored with a clear margin. The temperature range for growth is 15–30°C; no growth occurs at 35°C. Growth occurs in the absence of NaCl and in the presence of 1.5% (w/v) NaCl, but not at NaCl concentrations greater than 3.0% (w/v). KIS30-44^T reduces nitrate under anaerobic conditions and is negative for *N*-acetyl-β-glucosaminidase, aesculin hydrolysis, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, gelatin hydrolysis, glucose fermentation, α-glucosidase, β-glucosidase, β-glucuronidase, indole production, α-mannosidase, and trypsin. According to the API 20NE and API ID 32GN test strips, KIS30-44^T assimilates *N*-acetylglucosamine, adipic acid, L-alanine, L-arabinose, L-fucose, D-glucose, L-histidine, 4-hydroxybenzoic acid, 3-hydroxybutyric acid, inositol, lactic acid, D-mannitol, D-mannose, phenylacetic acid, potassium gluconate, potassium 2-ketogluconate, propionic acid, L-rhamnose, D-ribose, D-saccharose, L-serine, sodium acetate, D-sorbitol, and valeric acid, but does not assimilate D-maltose, potassium 5-ketogluconate, and sodium malonate. The major isoprenoid quinone is Q-8. The polar lipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and two unknown aminolipids. The major fatty acids(>10% of the total fatty acids) are $C_{16:0}$, summed feature 3 (iso-$C_{15:0}$ 2-OH and $C_{16:1}$ *ω7c*), and $C_{18:1}$ *ω7c*.

The type strain KIS30-44^T (=KACC 12733^T =DSM 24336^T) was isolated from forest soil collected on Dokdo Island, Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KIS30-44^T is GU171384.

Authors thank Dr. Jin-Ho Kim and Dr. Chul-Man Choi for ion chromatography analysis. This work is supported by a grant from the National Academy of Agricultural Science, Rural Development Administration (Project No. PJ00864902) and the Korea Institute of Planning and Evaluation for Technology (IPET) project 110037-03-1-HD110.

References

Aizawa, T., Ve, N.B., Nakajima, M., and Sunairi, M. 2010. *Burkholderia heleia* sp. nov., a nitrogen-fixing bacterium isolated from an aquatic plant, *Eleocharis dulcis*, that grows in highly acidic swamps in actual acid sulfate soil areas of Vietnam. *Int. J. Syst. Evol. Microbiol.* **60**, 1152–1157.

Brämer, C.O., Vandamme, P., da Silva, L.F., Gomez, J.G., and Steinbuchel, A. 2001. Polyhydroxyalkanoate-accumulating bacterium isolated from soil of a sugar-cane plantation in Brazil. *Int. J. Syst. Evol. Microbiol.* **51**, 1709–1713.

Caballero-Mellado, J., Martinez-Aguilar, L., Paredes-Valdez, G., and Santos, P.E. 2004. *Burkholderia unamae* sp. nov., an N2-fixing rhizospheric and endophytic species. *Int. J. Syst. Evol. Microbiol.* **54**, 1165–1172.

Chen, W.M., de Faria, S.M., James, E.K., Elliott, G.N., Lin, K.Y., Chou, J.H., Sheu, S.Y., Cnockaert, M., Sprent, J.I., and Vandamme, P. 2007. *Burkholderia nodosa* sp. nov., isolated from root nodules of the woody Brazilian legumes *Mimosa bimucronata* and *Mimosa scabrella*. *Int. J. Syst. Evol. Microbiol.* **57**, 1055–1059.

Chen, W.M., James, E.K., Coenye, T., Chou, J.H., Barrios, E., de Faria, S.M., Elliott, G.N., Sheu, S.Y., Sprent, J.I., and Vandamme, P. 2006. *Burkholderia mimosarum* sp. nov., isolated from root nodules of *Mimosa* spp. from Taiwan and South America. *Int. J. Syst. Evol. Microbiol.* **56**, 1847–1851.

Cheneby, D., Philippot, L., Hartmann, A., Henault, C., and Germon, J. 2000. 16S rDNA analysis for characterization of denitrifying bacteria isolated from three agricultural soils. *FEMS Microbiol. Ecol.* **34**, 121–128.

Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., and Lim, Y.W. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int.*

J. Syst. Evol. Microbiol. **57**, 2259–2261.

Daugulis, A.J. and Rehmann, L. 2008. Enhancement of PCB degradation by *Burkholderia xenovorans* LB400 in biphasic systems by manipulating culture conditions. *Biotechnol. Bioeng.* **99**, 521–528.

Ezaki, T., Hashimoto, Y., and Yabuuchi, E. 1989. Fluorometric deoxyribonucleic acid deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane-filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* **39**, 224–229.

Gjerde, D.T. and Fritz, J.S. 1987. Ion chromatography. A. Hèuthig Verlag, Heidelberg; New York, N.Y., USA.

Groth, I., Schumann, P., Weiss, N., Martin, K., and Rainey, F.A. 1996. *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int. J. Syst. Bacteriol.* **46**, 234–239.

Lee, C.M., Weon, H.Y., Hong, S.B., Jeon, Y.A., Schumann, P., Kroppenstedt, R.M., Kwon, S.W., and Stackebrandt, E. 2008. *Cellulomonas aerilata* sp. nov., isolated from an air sample. *Int. J. Syst. Evol. Microbiol.* **58**, 2925–2929.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., and *et al.* 2004. ARB: a software environment for sequence data. *Nucleic Acids Res.* **32**, 1363–1371.

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

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.

Otsuka, Y., Muramatsu, Y., Nakagawa, Y., Matsuda, M., Nakamura, M., and Murata, H. 2011. *Burkholderia oxyphila* sp. nov., a bacterium isolated from acidic forest soil that catabolizes (+)-catechin and its putative aromatic derivatives. *Int. J. Syst. Evol. Microbiol.* **61**, 249–254.

Perin, L., Martinez-Aguilar, L., Paredes-Valdez, G., Baldani, J.I., Estrada-de Los Santos, P., Reis, V.M., and Caballero-Mellado, J. 2006. *Burkholderia silvatlantica* sp. nov., a diazotrophic bacterium associated with sugar cane and maize. *Int. J. Syst. Evol. Microbiol.* **56**, 1931–1937.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glockner, F.O. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**, 7188–7196.

Rosen, B.P., Yoshinaga, M., and Cai, Y. 2011. Demethylation of methylarsonic acid by a microbial community. *Environ. Microbiol.* **13**, 1205–1215.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. *In* Inc., M. (ed.), MIDI Technical Note 101, Newark, DE, USA.

Seldin, L. and Dubnau, D. 1985. Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus marcerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int. J. Syst. Bacteriol.* **35**, 151–154.

Sylvestre, M., Kumar, P., Mohammadi, M., Viger, J.F., Barriault, D., Gomez-Gil, L., Eltis, L.D., and Bolin, J.T. 2011. Structural insight into the expanded PCB-degrading abilities of a biphenyl dioxygenase obtained by directed evolution. *J. Mol. Biol.* **405**, 531–547.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596–1599.

Ulrich, R.L., DeShazer, D., Brueggemann, E.E., Hines, H.B., Oyston, P.C., and Jeddeloh, J.A. 2004. Role of quorum sensing in the pathogenicity of *Burkholderia pseudomallei*. *J. Med. Microbiol.* **53**, 1053–1064.

Valverde, A., Delvasto, P., Peix, A., Velazquez, E., Santa-Regina, I., Ballester, A., Rodriguez-Barrueco, C., Garcia-Balboa, C., and Igual, J.M. 2006. *Burkholderia ferrariae* sp. nov., isolated from an iron ore in Brazil. *Int. J. Syst. Evol. Microbiol.* **56**, 2421–2425.

Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T., and Arakawa, M. 1992. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiol. Immunol.* **36**, 1251–1275.

Zhang, H., Hanada, S., Shigematsu, T., Shibuya, K., Kamagata, Y., Kanagawa, T., and Kurane, R. 2000. *Burkholderia kururiensis* sp. nov., a trichloroethylene (TCE)-degrading bacterium isolated from an aquifer polluted with TCE. *Int. J. Syst. Evol. Microbiol.* **50**, 743–749.