Burkholderia diazotrophica sp. nov., isolated from root nodules of Mimosa spp.

Shih-Yi Sheu,[1] Jui-Hsing Chou,[2] Cyril Bontemps,[3] Geoffrey N. Elliott,[4] Eduardo Gross,[5] Fabio Bueno dos Reis Junior,[6] Rémy Melkonian,[7] Lionel Moulin,[7] Euan K. James,[8] Janet I. Sprent,[9] J. Peter W. Young[10] and Wen-Ming Chen[2]

Correspondence
Wen-Ming Chen
p62365@ms28.hinet.net

[1]Department of Marine Biotechnology, National Kaohsiung Marine University, 142 Hai-Chuan Road, Nan-Tzu, Kaohsiung City 811, Taiwan, ROC

[2]Laboratory of Microbiology, Department of Seafood Science, National Kaohsiung Marine University, 142 Hai-Chuan Road, Nan-Tzu, Kaohsiung City 811, Taiwan, ROC

[3]Génétique et Microbiologie, UMR UHP-INRA 1128, IFR 110 EFABA, Université de Lorraine, Faculté des Sciences et Technologies, BP 239, 54506 Vandœuvre-lès-Nancy, France

[4]The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, UK

[5]Depto. de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, km 16, Ilhéus, 45662-900 BA, Brazil

[6]Embrapa Cerrados, Planaltina, 73301-970 DF, Brazil

[7]IRD, UMR LSTM, 34060 Montpellier, France

[8]The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

[9]Division of Plant Sciences, University of Dundee at JHI, Invergowrie, Dundee DD2 5DA, UK

[10]Department of Biology, University of York, York YO10 5DD, UK

Five strains, JPY461[T], JPY359, JPY389, DPU-3 and STM4206 were isolated from nitrogen-fixing nodules on the roots of Mimosa spp. and their taxonomic positions were investigated using a polyphasic approach. All five strains grew at 15–40 °C (optimum, 30–37 °C), at pH 4.0–8.0 (optimum, pH 6.0–7.0) and with 0–1 % (w/v) NaCl [optimum, 0 % (w/v)]. On the basis of 16S rRNA gene sequence analysis, a representative strain (JPY461[T]) showed 97.2 % sequence similarity to the closest related species Burkholderia acidipaludis SA33[T], a similarity of 97.2 % to Burkholderia terrae KMY02[T], 97.1 % to Burkholderia phymatum STM815[T] and 97.1 % to Burkholderia hospita LMG 20598[T]. The predominant fatty acids of the five novel strains were summed feature 2 (comprising $C_{16:1}$ iso I and/or $C_{14:0}$ 3-OH), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$, $C_{16:0}$ 3-OH, $C_{17:0}$ cyclo, $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$. The major isoprenoid quinone was Q-8 and the DNA G+C content of the strains was 63.0–65.0 mol%. The polar lipid profile consisted of a mixture of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminophospholipid, an unidentified aminolipid and several unidentified phospholipids. The DNA–DNA relatedness of the novel strain with respect to recognized species of the genus Burkholderia was less than 54 %. On the basis of 16S rRNA and recA gene sequence similarities, chemotaxonomic and phenotypic data, the five strains represent a novel species in the genus Burkholderia, for which the name Burkholderia diazotrophica sp. nov. is proposed with the type strain, JPY461[T] (=LMG 26031[T]=BCRC 80259[T]=KCTC 23308[T]).

The genus of Burkholderia, belonging to the family Burkholderiaceae of the Betaproteobacteria was proposed by Yabuuchi et al. (1992), and at the time of writing included more than 50 recognized species (Suárez-Moreno et al., 2012). Members of the genus Burkholderia are characterized

as Gram-negative, aerobic, non-spore-forming, non-fermentative, straight rod-shaped, and catalase-positive bacteria, and most species are motile by using a single polar flagellum or a tuft of polar flagella. They have a high metabolic versatility, having $C_{16:0}$ 3-OH as the cellular hydroxyl fatty acid, and have a DNA G+C content of 59.–69.5 mol% (Gillis *et al.*, 1995). Species of the genus *Burkholderia* have been isolated from humans (cystic fibrosis), rhizosphere soil, root nodules, animals, plants, water and hospital equipment (Vandamme *et al.*, 2007; Suárez-Moreno *et al.*, 2012). Although the genus *Burkholderia* is largely known through studies on its various pathogenic representatives, it is also widely reported that species of the genus *Burkholderia* have been isolated from root nodules, and that they can form N_2-fixing symbioses with some legumes, particularly *Mimosa* spp. (Chen *et al.*, 2001, 2003a, b, 2005a, b, 2006, 2007, 2008; Barrett & Parker, 2005, 2006; Bontemps *et al.*, 2010; Mishra *et al.*, 2012).

Recently, 143 nodule symbionts from 49 native species of *Mimosa* in Brazil were sampled and surveyed for their symbiotic diversity (Bontemps *et al.*, 2010). Sequences of the16S rRNA and *recA* genes of these isolates showed that 141 isolates were members of the genus *Burkholderia*. Three members of one novel group from the study of Bontemps *et al.* (2010), strains JPY461[T], JPY359 and JPY389, as well as strain DPU-3 isolated in Taiwan and strain STM4206 isolated in French Guiana, were subjected to a taxonomic study using a polyphasic approach. Amongst the Brazilian strains, strain JPY461[T] was isolated from root nodules on *Mimosa candollei* (syn. *M. quadrivalvis* var. *leptocarpa*) which were collected in the Chapada dos Veiadeiros in Goias, Central Brazil; strain JPY359 was isolated from nodules on *Mimosa tenuiflora* collected from trees growing along a roadside in Bahia, NE Brazil; and strain JPY389 was isolated from nodules on *Mimosa pudica* growing by a roadside in the Chapada Diamantina, Bahia, NE Brazil. The non-Brazilian strains, DPU-3 and STM4206, were isolated from nodules of *M. pudica* plants that were used in 'trapping' experiments with *M. pudica* rhizosphere soil sampled from Taitung riverside in Taiwan and from a coastal garden in East Cayenne, French Guiana (Mishra *et al.*, 2012), respectively. The three Brazilian strains and strain STM4206 have all been shown to nodulate and fix nitrogen in association with *M. pudica* (Bontemps *et al.*, 2010; Mishra *et al.*, 2012).

The five strains were grown on yeast extract-mannitol (YEM) agar plates (Vincent, 1970) and incubated at 25 °C, unless otherwise indicated. Subculturing was performed on YEM agar at 25 °C for 2 days. Strains were stored at −80 °C in YEM broth with 20 % (v/v) glycerol or by lyophilization. *Burkholderia hospita* LMG 20598[T], *Burkholderia kururiensis* KP23[T], *Burkholderia caribensis* CCUG 42847[T] and *Burkholderia terrae* KMY02[T] were obtained from the Belgian Coordinated Collections of Microorganisms (BCCM), and *Burkholderia acidipaludis* SA33[T] was obtained from the NITE Biological Research Center (NBRC). The other reference strains, *Burkholderia mimosarum* PAS44[T], *Burkholderia phymatum* STM815[T], *Burkholderia sabiae* Br3407[T], *Burkholderia*

tuberum STM678[T] and *Burkholderia nodosa* Br3437[T] have been described previously by our group (Vandamme *et al.*, 2002; Chen *et al.*, 2006, 2007, 2008). All type strains were used as reference strains for phenotypic and genotypic tests.

Bacterial cells were observed by phase-contrast microscopy (DM2000; Leica) using cells grown on YEM agar at 25 °C for 2 days. Motility was tested by the hanging drop method (Murray *et al.*, 1994). The Gram Stain Set S kit (BD Difco) and the Ryu non-staining KOH method (Powers, 1995) were used for testing the Gram reaction. Poly-β-hydroxybutyrate granule accumulation was observed under light microscopy after staining of the cells with Sudan black. Colony morphology was observed on YEM agar using a stereoscopic microscope (SMZ 800; Nikon).

The pH range for growth was determined by measuring the optical densities (wavelength 600 nm) of nutrient broth (NB; BD Difco) cultures. The pH was adjusted prior to sterilization to pH 4–10 (at intervals of 1.0 pH unit) using appropriate biological buffers (Breznak & Costilow, 1994): citrate/Na_2HPO_4 buffer for pH range 4.0–5.0; phosphate buffer for pH range 6.0–7.0; Tris buffer for pH range 8.0–9.0; no buffer for pH 10.0,. Post-sterilization controls revealed only minor changes in pH of the buffers. The NaCl requirement was determined using NB containing 0, 0.5 and 1.0–10.0 % (w/v) NaCl (at 1.0 % intervals). Growth at various temperatures (4–50 °C) was examined in YEM broth. Cellular growth was determined by measuring the turbidity (OD_{600}) of cultures grown at various pH values, NaCl concentrations and temperatures. Anaerobic growth was determined after incubating strains on YEM agar in the Oxoid AnaeroGen system (Miller *et al.*, 1995).

Strains were examined for a broad range of phenotypic properties. Activities of catalase, oxidase, DNase, urease and lipase (corn oil), and hydrolysis of starch, casein and Tweens 20, 40, 60 and 80 were determined using standard methods (Smibert & Krieg, 1994). Additional biochemical tests were performed using the API 20NE and API ZYM kits (bioMérieux) and carbon source utilization was evaluated using the GN2 MicroPlate (Biolog). All commercial phenotypic tests were performed according to the manufacturer's recommendations. Although four of the strains have previously been shown to form N-fixing nodules on *M. pudica* (Bontemps *et al.*, 2010; Mishra *et al.*, 2012), nodulation tests on this host were repeated with all five strains under sterile conditions using the tube method of Gibson (1963). Nitrogen fixation assays (acetylene reduction assays), were carried out on plants 28 days after inoculation according to the method of James & Crawford (1998).

Antibiotic sensitivity of strains JPY359, JPY389 JPY461[T], DPU-3, STM4206 and of the reference strains were analysed by the disc diffusion method after spreading cell suspensions (0.5 McFarland) on NB agar. The following antibiotic discs (Oxoid) were used: ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), novobiocin (30 μg), rifampicin (5 μg), penicillin G (10 μg), streptomycin (10

μg), sulfamethoxazole (23.75 μg) plus trimethoprim (1.25 μg), and tetracycline (30 μg). The effect of antibiotics on cell growth was assessed after 2 days incubation at 25 °C and susceptibility was scored based on the distance from the edge of the clear zone to the disc.

The 16S rRNA and *recA* gene sequences of strains JPY359, JPY389 and JPY461^T have been reported by Bontemps *et al.* (2010) and those of strain STM4206 by Mishra *et al.* (2012). Genomic DNA of strain DPU-3 was isolated by a bacterial genomic kit (DP02-150; GeneMark Technology), and the 16S rRNA and *recA* gene sequences were obtained and analysed as described previously by Bontemps *et al.* (2010). The 16S rRNA gene sequences were compared against 16S rRNA gene sequences available from the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012), the Ribosomal Database Project (Maidak *et al.*, 2001) and the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi.). Analyses of the sequence data were performed by using the software package BioEdit (Hall, 1999) and MEGA software, version 5 (Tamura *et al.*, 2011), after multiple alignments of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (corrected according to Kimura's two-parameter model; Kimura, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). The maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were generated by using the treeing algorithms contained in the PHYLIP software package (Felsenstein, 1993). In each case bootstrap values were calculated based on 1000 replications.

The phylogenetic tree based on 16S rRNA gene sequence comparisons (Fig. 1 and Fig. S1, available in IJSEM Online) showed that strains JPY359, JPY389, JPY461^T, DPU-3 and STM4206 formed an independent phylogenetic line within the genus *Burkholderia* in the family *Burkholderiaceae* of the class *Betaproteobacteria*. The overall topologies of the phylogenetic trees obtained with the maximum-likelihood

and maximum-parsimony methods were similar (data not shown). The 16S rRNA gene sequences of the five strains showed high similarity (more than 98.8 %) to each other. Sequence similarity calculations (over 1400 bp) indicated that strain JPY461^T was closely related to *B. acidipaludis* SA33^T (97.2 % 16S rRNA gene sequence similarity), *B. terrae* KMY02^T (97.2 %), *B. phymatum* STM815^T (97.1 %) and *B. hospita* LMG 20598^T (97.1 %). Lower sequence similarities (<97 %) were found with the representative members of all other species of the genus *Burkholderia* listed in Fig. 1.

According to the pairwise *recA* gene sequence comparisons, the similarity of strain JPY461^T with the other four strains, JPY359, JPY389, DPU-3 and STM4206, ranged from 99.8 to 100 %. The highest similarity values of the representative strain JPY461^T (94.5 %) was to *B. phymatum* STM815^T, and the levels of the *recA* gene sequence similarity between strain JPY461^T and other species with validly published names within the *Betaproteobacteria* were below 94.5 %. A phylogenetic tree based on *recA* gene sequences was constructed as described above and this also showed that the five strains formed a deep monophyletic cluster within the genus *Burkholderia* (Fig. S2).

Whole genome DNA–DNA hybridization experiments were performed at 55 °C with photobiotin-labelled probes as described by Ezaki *et al.* (1989). DNA–DNA hybridization experiments were performed between strain JPY461^T and strains JPY359, JPY389, DPU-3 and STM4206, and with one of the four closest relatives, *B. acidipaludis* SA33^T, *B. terrae* KMY02^T, *B. phymatum* STM815^T, and *B. hospita* LMG 20598^T, respectively. The degree of DNA–DNA relatedness was calculated from triplicate experiments. The DNA–DNA relatedness values between strains JPY461^T, JPY359, JPY389, DPU-3 and STM4206 were 77–100 %, indicating that the five strains are members of the same genomic species (Wayne *et al.*, 1987). In addition, strain JPY461^T showed
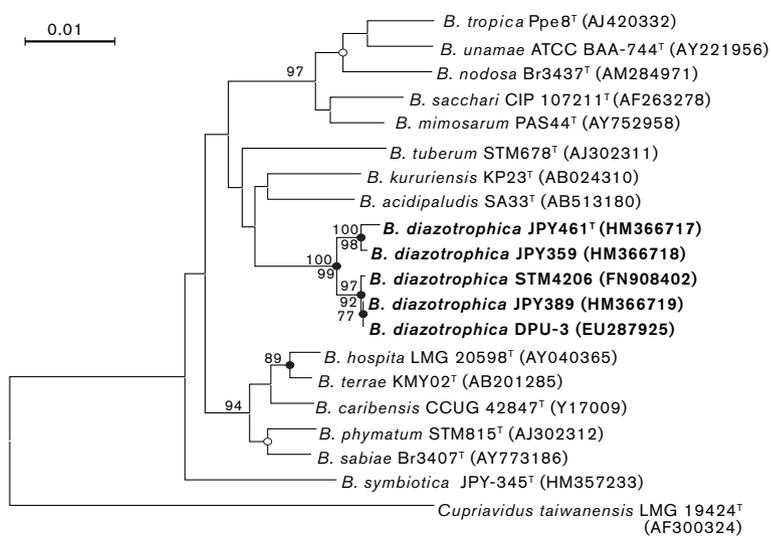

Fig. 1. Neighbour-joining phylogenetic tree of the novel strains (*Burkholderia diazotrophica* sp. nov.) and related bacteria, based on 16S rRNA gene sequence comparisons. Numbers at nodes are bootstrap values >70 % based on the neighbour-joining (above nodes) and maximum-parsimony (below nodes) tree-making algorithms. Filled circles indicate branches of the tree that were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. *Cupriavidus taiwanensis* LMG 19424^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position. The full tree from which Fig. 1 was taken is available as Fig. S1.

DNA–DNA relatedness values of $54\pm5\%$, $47\pm3\%$, $38\pm1\%$, and $40\pm1\%$ with *B. acidipaludis* SA33[T], *B. terrae* KMY02[T], *B. phymatum* STM815[T] and *B. hospita* LMG 20598[T], respectively. Since the recommended DNA–DNA relatedness threshold value for the definition of a species is 70 % (Wayne *et al.*, 1987), these results indicated that strain JPY461[T] did not belong to any known species of the genus *Burkholderia*.

The fatty acid profiles of strains JPY359, JPY389, JPY461[T], DPU-3, STM4206, *B. mimosarum* PAS44[T], *B. phymatum* STM815[T], *B. sabiae* Br3407[T], *B. tuberum* STM678[T], *B. nodosa* Br3437[T], *B. terrae* KMY02[T], *B. acidipaludis* SA33[T], *B. hospita* LMG 20598[T], *B. caribensis* CCUG 42847[T] and *B. kururiensis* KP23[T] were determined using cells grown on YEM agar at 25 °C for 3 days. The physiological age of the different bacterial cultures at the time of harvest was standardized by selecting a sector from a quadrant streak on YEM agar plates according to the MIDI protocol (http://www.microbialid.com/PDF/TechNote_101.pdf). In this study, the different species of the genus *Burkholderia* exhibited very similar growth rates on YEM agar. Fatty acid methyl esters were prepared and separated according to the standard protocol of the Sherlock Microbial Identification System, version 6.0 (MIDI), analysed by GC (5890 Series II; Hewlett Packard) and identified by using the RTSBA6.00 database of the Microbial Identification System (Sasser, 1990). The fatty acid profiles of strains JPY359, JPY389, JPY461[T], DPU-3 and STM4206 were similar to those of the other species of the genus *Burkholderia*, although there were differences in the proportions of some components (Table 1). The major fatty acids ($>5\%$) of strains JPY359, JPY389, JPY461[T], DPU-3 and STM4206 were summed feature 2 (comprising $C_{16:1}$ iso I and/or $C_{14:0}$ 3-OH; $5.2\pm0.3\%$), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; $6.2\pm0.8\%$), $C_{16:0}$ ($18.2\pm0.9\%$), $C_{16:0}$ 3-OH ($5.2\pm0.3\%$), $C_{17:0}$ cyclo ($8.4\pm0.6\%$), $C_{18:1}\omega7c$ ($32.2\pm1.8\%$) and $C_{19:0}$ cyclo $\omega8c$ ($8.4\pm0.7\%$).

Isoprenoid quinones were extracted and purified according to the method of Collins (1985) and were analysed by HPLC. Strains JPY359, JPY389, JPY461[T], DPU-3 and STM4206 had Q-8 as their main respiratory quinone. The DNA G+C content of strains JPY359, JPY389, JPY461[T], DPU-3 and STM4206, determined by HPLC according to Mesbah *et al.* (1989), was 63.0–65.0 mol%.

Polar lipids were extracted and analysed by two-dimensional TLC according to Embley & Wait (1994). Molybdophosphoric acid was used for detection of all lipids, ninhydrin reagent for lipids containing free amino groups, Zinzadze reagent for phosphorus-containing lipids and α-naphthol reagent for glycolipids. Strains JPY359, JPY389, JPY461[T], DPU-3 and STM4206 exhibited a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), an unidentified aminophospholipid (APL), an unidentified aminolipid (AL), and several unidentified phospholipids (PL) (see Fig. S3 for profiles of JPY461[T] and the reference strains; data of JPY359, JPY389, DPU-3 and STM4206 are not shown). Strain JPY461[T] exhibited a very similar polar lipid profile to its closest relatives, *B. mimosarum* PAS44[T], *B. phymatum* STM815[T], *B. sabiae* Br3407[T], *B. tuberum* STM678[T], *B. nodosa* Br3437[T], *B. terrae* KMY02[T], *B. acidipaludis* SA33[T], *B. hospita* LMG 20598[T], *B. caribensis* CCUG 42847[T] and *B. kururiensis* KP23[T], and the major polar lipids for all were PE, PG, DPG and APL1 (Fig. S3).

Table 1. Cellular fatty acid contents of the novel strains and related species of the genus *Burkholderia*

Taxa: 1, *B. diazotrophica* sp. nov. ($n=5$); 2, *B. mimosarum* PAS44[T]; 3, *B. phymatum* STM815[T]; 4, *B. sabiae* Br3407[T]; 5, *B. tuberum* STM678[T]; 6, *B. nodosa* Br3437[T]; 7, *B. terrae* KMY02[T]; 8, *B. acidipaludis* SA33[T]; 9, *B. hospita* LMG 20598[T]; 10, *B. caribensis* CCUG 42847[T]; 11, *B. kururiensis* KP23[T]. Strains were grown on YEM agar at 25 °C for 3 days. Values are mean percentages ($\pm$ SD where appropriate) of total fatty acids. The fatty acids for which the mean amount for all taxa was <1 % are not given. −, Not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
$C_{14:0}$	4.5 ± 0.3	4.3	4.3	5.1	4.1	3.9	4.3	4.6	3.3	4.1	5.0
$C_{16:0}$	18.2 ± 0.9	22.5	20.8	20.8	24.7	20.0	18.0	27.8	22.9	19.4	17.0
$C_{16:0}$ 2-OH	3.4 ± 0.3	2.7	3.2	2.0	2.5	2.7	2.9	3.0	2.1	2.8	3.0
$C_{16:0}$ 3-OH	5.2 ± 0.3	5.8	5.2	4.8	5.4	5.2	5.5	5.6	4.7	5.4	5.6
$C_{16:1}$ 2-OH	1.9 ± 0.2	1.6	2.2	1.3	1.2	1.8	2.9	1.9	3.4	1.9	2.3
$C_{17:0}$ cyclo	8.4 ± 0.6	13.3	16.6	11.2	19.7	14.9	11.0	16.4	11.7	11.7	7.0
$C_{18:0}$	2.4 ± 0.1	1.0	1.5	3.9	1.1	−	1.5	3.3	1.2	1.5	1.4
$C_{18:1}\omega7c$	32.2 ± 1.8	21.7	11.2	18.2	10.9	20.0	24.6	15.6	28.7	16.0	28.6
$C_{18:1}$ 2-OH	1.2 ± 0.1	1.7	2.0	1.2	−	1.5	1.5	1.7	1.2	1.5	1.6
$C_{19:0}$ cyclo $\omega8c$	8.4 ± 0.7	9.5	14.8	11.7	17.5	11.2	10.7	11.3	5.4	11.6	11.5
Summed Feature 2*	5.2 ± 0.3	6.0	5.3	5.3	6.1	5.8	5.5	6.0	5.0	4.6	5.6
Summed Feature 3*	6.2 ± 0.8	3.5	2.7	6.6	1.7	3.7	5.9	2.8	10.7	3.5	7.3

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 2 comprises $C_{14:0}$ 3-OH, $C_{16:1}$ iso I. Summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$.

However, these species of the genus *Burkholderia* had differences in some minor components, such as several unidentified aminolipids and phospholipids. The results suggested that there are some differences in the polar lipid profiles among these 11 species, although they belong to the same genus and have very similar polar lipid profiles overall.

Detailed results of the biochemical characterization and antibiotic sensitivity tests are given in the species description and in Table 2. The novel species can be distinguished from representatives of its close phylogenetic relatives by using a combination of phenotypic attributes, especially nitrate reduction, activities of urease and *β*-galactosidase, oxidation of various substrates and susceptibility to some antibiotics.

On the basis of the data obtained from 16S rRNA and *recA* gene sequence comparisons, the novel species occupies a distinct position within the genus *Burkholderia*. The phylogenetic insight is supported by the unique combination of chemotaxonomic and biochemical characteristics of the novel strains. It is clear from the phylogenetic and phenotypic data that the five strains constitute a novel species of the genus *Burkholderia*. The name *Burkholderia diazotrophica* sp. nov., is proposed for this taxon.

Description of *Burkholderia diazotrophica* sp. nov.

Burkholderia diazotrophica (di.a.zo.tro′phi.ca. Gr. prefix *di* two, double; N.L. n. *azotum* nitrogen; Gr. adj. *trophikos* nursing, tending or feeding; M.L. fem. adj. *diazotrophica* one that feeds on dinitrogen).

Cells are Gram-stain-negative, motile, aerobic, non-spore-forming rods. Poly-*β*-hydroxybutyrate accumulation is observed. After 24 h growth on YEM agar at 25 °C, the mean cell size is approximately 0.8–1.0 μm in diameter and 1.0–2.0 μm in length. Colonies on YEM agar are yellow-pigmented, circular, smooth and convex with entire edges. The colony size is approximately 1.0–1.3 mm in diameter on YEM agar after 48 h incubation at 25 °C. Growth

Table 2. Comparison of phenotypic characteristics of strain JPY461[T] with related species of the genus *Burkholderia*

Strains:1, *B. diazotrophica* (*n*=5); 2, *B. mimosarum* PAS44[T]; 3, *B. phymatum* STM815[T]; 4, *B. sabiae* Br3407[T]; 5, *B. tuberum* STM678[T]; 6, *B. nodosa* Br3437[T]; 7, *B. terrae* KMY02[T]; 8, *B. acidipaludis* SA33[T]; 9, *B. hospita* LMG 20598[T]; 10, *B. caribensis* CCUG 42847[T]; 11, *B. kururiensis* KP23[T]. Data for reference strains were obtained in this study with the exception of sources of isolation and DNA G+C contents, which were taken from Chen *et al.* (2006), Vandamme *et al.* (2002), Chen *et al.* (2008), Vandamme *et al.* (2002), Chen *et al.* (2007), Yang *et al.* (2006), Aizawa *et al.* (2010), Goris *et al.* (2002), Achouak *et al.* (1999) and Zhang *et al.* (2000) for strains 2–11, respectively. All strains are Gram-negative, non-spore-forming, rod-shaped and positive for oxidase and catalase activities. All strains were sensitive to chloramphenicol, kanamycin, streptomycin, nalidixic acid, tetracycline, sulfamethoxazole plus trimethoprim and gentamicin. +, Positive; −, negative; S, sensitive; R, resistant; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Isolation source	Nodules	Nodules	Nodules	Nodules	Nodules	Nodules	Soil	Plant	Soil	Bulk vertisol	TCE-polluted water
Nitrate reduction	+	+	+	+	−	+	−	+	+	−	−
Urease	+	+	−	+	−	+	+	+	+	+	+
β-Galactosidase	+	−	+	+	+	+	+	−	+	+	+
Oxidation of (GN2 MicroPlate):											
Dextrin	+	+	+	+	−	−	−	+	+	+	−
N-Acetyl-D-galactosamine	−	−	+	−	−	+	+	−	−	−	+
α-Lactose	−	−	−	+	−	−	+	−	+	+	−
Lactulose	−	−	−	+	+	−	+	−	+	+	+
D-Psicose	+	+	+	−	+	+	+	+	+	+	+
Sucrose	+	−	+	−	−	−	−	−	−	−	−
Trehalose	+	−	−	+	−	+	+	+	−	+	−
Succinamic acid	+	−	+	+	+	+	+	+	+	+	+
Inosine	+	−	+	−	−	−	+	−	+	+	+
Susceptibility to:											
Penicillin G	S	S	S	S	S	S	S	S	R	S	S
Novobiocin	S	S	S	S	S	S	S	S	R	S	S
Ampicillin	S	S	S	S	S	S	S	S	R	S	S
Rifampicin	R	R	R	S	R	R	R	R	R	R	R
Nodulation and nitrogen fixation on *Mimosa pudica*	+	+	+	+	+	+	−	ND	−	−	−
DNA G+C content (mol%)	63.0–65.0	64.8	62.1	64.5	62.8	62.8	62.0	64.0	62.0	63.1	64.8

occurs at 15–40 °C (optimum, 30–37 °C), at pH 4.0–8.0 (optimum, pH 6.0–7.0) and with 0–1% (w/v) NaCl (optimum, 0 %). Nodulation of *M. pudica* is present. Nitrogen fixation is positive. Catalase- and oxidase-positive. Positive result in tests for hydrolysis of Tweens 20, 40, 60 and 80, but negative result in tests for hydrolysis of DNA, starch, chitin, casein, gelatin, aesculin, corn oil and alginate. In API 20NE tests, positive reactions for nitrate reduction, urease and β-galactosidase activities, and assimilation of glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, gluconate, malate and citrate, and negative reactions for indole production, glucose fermentation, arginine dihydrolase activity, aesculin and gelatin hydrolysis and assimilation of maltose, caprate, adipate and phenyl-acetate. In the API ZYM kit, alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present and C14 lipase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucouronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. In all strains, the following compounds are utilized as sole carbon sources in the GN2 MicroPlate: dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, α-D-glucose, D-mannitol, D-mannose, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, pyruvic acid methyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, β-hydroxybutyric acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, quinic acid, bromosuccinic acid, succinamic acid, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, DL-carnitine, inosine, thymidine, 2-aminoethanol, glycerol and DL-α-glycerol phosphate. None of the strains oxidized α-cyclodextrin, *N*-acetyl-D-galactosamine, gentiobiose, α-lactose, lactulose, melibiose, methyl β-D-glucoside, α-hydroxybutyric acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, phenyethylamine or putrescine. Resistant to rifampicin and sensitive to chloramphenicol, gentamicin, kanamycin, penicillin G, ampicillin, novobiocin, tetracycline, streptomycin, sulfamethoxazole plus trimethoprim and nalidixic acid. The major fatty acids (>5 %) are summed feature 2 (comprising $C_{16:1}$ iso I and/or $C_{14:0}$ 3-OH), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}$ $\omega6c$), $C_{16:0}$, $C_{16:0}$ 3-OH, $C_{17:0}$ cyclo $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$. The DNA G+C content is 63.0–65.0 mol%. The major respiratory quinone is Q-8. The polar lipid profile consists of a mixture of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminophospholipid, an unidentified aminolipid and several unidentified phospholipids.

The type strain is strain JPY461[T] (=LMG 26031[T]=BCRC 80259[T]=KCTC 23308[T]), which was isolated from root nodules on *Mimosa candollei* (syn. *M. quadrivalvis* var. *leptocarpa*) in the state of Goias, Brazil.

Acknowledgements

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