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

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Five strains, JPY461T, 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 (JPY461T) showed 97.2 % sequence similarity to the closest related species *Burkholderia acidipaludis* SA33T, a similarity of 97.2 % to *Burkholderia terrae* KMY02T, 97.1 % to *Burkholderia phymatum* STM815T and 97.1 % to *Burkholderia hospita* LMG 20598T. The predominant fatty acids of the five novel strains were summed feature 2 (comprising C16:1iso I and/or C14:0 3-OH), summed feature 3 (comprising C16:1ω7c and/or C16:1ω6c), C16:0, C16:0 3-OH, C17:0 cyclo, C18:1ω7c and C19:0 cyclo ω8c. 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, diphasatidylglycerol, 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, JPY461T (=LMG 26031T=BCRC 80259T=KCTC 23308T).

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 C16:0 3-OH as the cellular hydroxyl fatty acid, and have a DNA G+C content of 59.3–69.5 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 N₂-fixing symbioses with some legumes, particularly *Mimosa* spp. (Chen et al., 2001, 2003a, b, 2005a, 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 the 16S 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 JPY461T, 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 JPY461T was isolated from root nodules on *Mimosa candollei* (syn. *M. quadrivalvis* var. *leptocarpa*) which were collected in the Chapada dos Veadeiros in Goiás, 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 hospital* LMG 20598T, *Burkholderia kururienisi* KP23T, *Burkholderia caribensis* CCGC 42847T and *Burkholderia terrae* KMY02T were obtained from the Belgian Coordinated Collections of Microorganisms (BCCM), and *Burkholderia acidipaludis* SA33T was obtained from the NITE Biological Research Center (NBRC). The other reference strains, *Burkholderia mimosarum* PAS44T, *Burkholderia phymatum* STM815T, *Burkholderia sabiae* Br3407T, *Burkholderia tuberum* STM678T and *Burkholderia nodosa* Br3437T 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 5 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/Na₂HPO₄ 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 (OD₆00) 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-fixer 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 JPY461T, 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), cefotaxime (30 μg), and oxytetracycline (30 μg). The results are consistent with the phenotypic characteristics of the isolates.
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 JPY461T 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 calculated from triplicate experiments. The DNA–DNA relatedness values were below 90 %. The highest similarity values of the representative strain JPY461T (95.5 %) was to B. phymatum STM815T, and the levels of the recA gene sequence similarity between strain JPY461T 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 JPY461T and strains JPY359, JPY389, DPU-3 and STM4206, and with one of the four closest relatives, B. acidipaludis SA33T, B. terrae KMY02T, B. phymatum STM815T, and B. hospita LMG 20598T, respectively. The degree of DNA–DNA relatedness was calculated from triplicate experiments. The DNA–DNA relatedness values between strains JPY461T, 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 JPY461T 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 19424T 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±5 %, 47±3 %, 38±1 %, and 40±1 % 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\(_{14:1}\) 3-OH; 5.2±0.3 %), summed feature 3 (comprising C\(_{16:1}\) iso 7c and/or C\(_{16:1}\) 10c; 6.2±0.8 %), C\(_{16:0}\) (18.2±0.9 %), C\(_{16:0}\) 3-OH (5.2±0.3 %), C\(_{17:0}\) cyclo (8.4±0.6 %) and C\(_{19:0}\) cyclo o8c (8.4±0.7 %).

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 amionolipid (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 content 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 (±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\(_{16:1}\) 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\(_{16:1}\) 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\(_{18:1}\) iso 7c | 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 o8c | 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 |

*aSummed 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\(_{16:1}\) iso 7c and/or C\(_{16:1}\) 10c. *
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 JPY461T with related species of the genus *Burkholderia*

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<td>Penicillin G</td>
<td>S</td>
<td>S</td>
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<td>S</td>
<td>S</td>
<td>R</td>
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<td>Novobiocin</td>
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<td>Ampicillin</td>
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<td>Rifampicin</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
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<tr>
<td>Nodulation and nitrogen fixation on <em>Mimosa pudica</em></td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>-</td>
<td>ND</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>63.0–65.0</td>
<td>64.8</td>
<td>62.1</td>
<td>64.5</td>
<td>62.8</td>
<td>62.8</td>
<td>62.0</td>
<td>64.0</td>
<td>62.0</td>
<td>63.1</td>
<td>64.8</td>
</tr>
</tbody>
</table>
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. padica* 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-acetylgalosaminic acid, galactose, malate and citrate, and negative reactions for indole production, glucose fermentation, arginine dihydrolase activity, aesculin and gelatin hydrolysis and assimilation of malose, 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-Bl-phosphohydrolase activities are present and C14 lipase, cystine arylamidase, trypsin, α-chymotrypsin, z-galactosidase, β-galactosidase, β-glucuronidase, z-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, z-mannosidase and z-fucosidase activities are absent. In all strains, the following compounds are utilized as sole carbon sources in the GN2 MicroPlate: dextrin, glycolon, Tween 40, Tween 80, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellulbiose, D-fructose, D-galactose, z-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, z-ketogluutaric acid, DL-lactic acid, malonic acid, quinic acid, bromosuccinic acid, succinamic acid, D-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycylic L-aspartic acid, glycylic L-glutamic acid, L-histidine, hydroxy-L-proline, L-phenylalanine, L-proline, L-prolylglutamic 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, L-lactose, lactulose, melibiose, methyl β-D-glucoside, z-hydroxybutyric acid, itaconic acid, z-ketobutyric acid, z-ketovaleric acid, phenethylamine 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 C16:1iso and/or C14:1v3-OH), summered feature 3 (comprising C16:1ω7c and/or C16:1ω6c), C16:0ω8c C16:0 3-OH, C17:0 cyclo C18:1ω7c and C19:0 cyclo ω8c. 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, dihydroxyglycerol, unidentified aminophospholipid, an unidentified aminolipid and several unidentified phospholipids.

The type strain is strain JPY461T (=LMG 26031T =BCRC 80259T =KCTC 23308T), 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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**References**


