**Burkholderia monticola** sp. nov., isolated from mountain soil

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An ivory/yellow, Gram-stain-negative, short-rod-shaped, aerobic bacterial strain, designated JC2948T, was isolated from a soil sample taken from Gwanak Mountain, Republic of Korea. 16S rRNA gene sequence analysis indicated that strain JC2948T belongs to the genus *Burkholderia*. The test strain showed highest sequence similarities to *Burkholderia tropica* LMG 22274T (97.6 %), *Burkholderia acidiphilus* NBRC 101816T (97.5 %), *Burkholderia tuberum* LMG 21444T (97.5 %), *Burkholderia sprentiae* LMG 27175T (97.4 %), *Burkholderia terricola* LMG 20594T (97.3 %) and *Burkholderia diazotrophica* LMG 26031T (97.1 %). Based on average nucleotide identity (ANI) values, the new isolate represents a novel genomic species as it shows less than 90 % ANI values with other closely related species. Also, other phylosiological and biochemical comparisons allowed the phenotypic differentiation of strain JC2948T from other members of the genus *Burkholderia*. Therefore, we suggest that this strain should be classified as the type strain of a novel species of the genus *Burkholderia*. The name *Burkholderia monticola* sp. nov. (type strain, JC2948T = JCM 19904T = KACC 17924T) is proposed.

The nearly full sequence (1463 bp) of the 16S rRNA gene was amplified and sequenced as described previously (Chun & Goodfellow, 1995). The sequencing results underwent preliminary analysis against the EzTaxon database (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012) to determine any closely related strains with validly published names. The 16S rRNA gene sequence was aligned using the EzEditor program (Jeon et al., 2014) and the results were used to reconstruct phylogenetic trees. The neighbour-joining (NJ; Saitou & Nei, 1987) and maximum-likelihood (ML; Felsenstein, 1981) methods were carried out using MEGA.

Soil samples were collected from Mount Gwanak in Seoul, Republic of Korea, in 2012, and a number of bacteria were isolated using a standard dilution plating method on Nutrient agar (NA; Difco). Single colonies were randomly selected based on their morphology and cultured on new NA plates at 30 °C for 1 day under aerobic conditions. The isolate, designated JC2948T, was routinely subcultured under the same conditions and was stored at −80 °C in Nutrient broth (NB; Difco) supplemented with 40 % (v/v) glycerol for preservation. *Burkholderia tropica* LMG 22274T, *Burkholderia sprentiae* LMG 27175T and *Burkholderia tuberum* LMG 21444T were selected as reference type strains for polyphasic analysis (Vandamme et al., 2002; Reis et al., 2004; De Meyer et al., 2013). All strains were cultivated under the same conditions.

**Abbreviations:** ANI, average nucleotide identity; DPG, diphosphatidylglycerol; GTR, general time-reversible; NJ, neighbour-joining; ML, maximum-likelihood; PE, phosphatidylethanolamine.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain JC2948T is KF155692. The GenBank Bioproject ID and Gold Project ID for the draft genome sequence of strain JC2948T are PRJNA240348 and Gp0090081, respectively.

Two supplementary figures and one supplementary table are available with the online Supplementary Material.

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version 6.06 (Sohpal et al., 2010). The NJ tree was constructed according to the Juke & Cantor one-parameter model (Jukes & Cantor, 1969) and an ML tree was computed by the general time-reversible (GTR) model of nucleotide substitution with four discrete gamma rate categories (Lanave et al., 1984). Bootstrap analysis with 1000 replicates was performed for the reliability of tree topologies. Bayesian inference was performed with MrBayes 3.2.2 (Ronquist & Huelsenbeck, 2003) by running four Markov chains for 5,000,000 generations with a sampling frequency of 100 and burning an initial 1,250,000 generations under the GTR model with a gamma-distributed rate variation. On the basis of 16S rRNA gene sequence analyses, strain JC2948T showed highest similarity to B. tropica LMG 22274T (97.58%; Reis et al., 2004), followed by B. acidipaludis NBRC 101816T (97.47%; Aizawa et al., 2010), B. tuberum LMG 21444T (97.46%; Vandamme et al., 2002), B. sprentiae LMG 27175T (97.37%; De Meyer et al., 2013), B. terricola LMG 20594T (97.25%; Goris et al., 2002) and B. diazotrophica LMG 26031T (97.07%; Sheu et al., 2013). The phylogenetic tree indicated that strain JC2948T formed a distinctive phyletic branch within the radiation of the genus Burkholderia (Fig. 1).

Based on the newly proposed cut-off of 16S rRNA gene sequence similarity for species delineation (98.65%, Chun & Rainey, 2014), strain JC2948T was clearly distinguished from other species of the genus Burkholderia. However, the 16S rRNA gene sequence similarities between strain JC2948T and most related type strains were higher than 97%, the conventional threshold for species delineation. Thus, levels of DNA–DNA relatedness were calculated using average nucleotide identity (ANI) values. The genome sequence of strain JC2948T was determined by using Illumina MiSeq system technology. An average of

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences, showing the position of strain JC2948T among species of the genus Burkholderia. Numbers at nodes are percentages of bootstrap support (>70%) from 1000 resampled datasets. Filled circles indicate that the corresponding nodes (groupings) were also recovered by the ML and Bayesian tree-making methods. The 16S rRNA gene sequence of Ralstonia pickettii ATCC 27511T was used as an outgroup. Bar, 0.01 nt substitutions per position.
11.446 million paired-end reads with length 360 bp were generated and the reads were assembled into contigs using the CLC Genomics Workbench 6.5 (CLCbio). The resultant draft genome had 30 contigs and the genome size was 7.85 Mb. The ANI value was calculated using the BLAST-based ANI calculation method described previously (Goris et al., 2007; Kim et al., 2014). Strains of species of the genus *Burkholderia* with genome sequences available in the GenBank and GOLD databases were obtained and used for ANI calculations. The ANI values between strain JC2948T and the type strains of *B. sprentiae* LMG 27175T, *B. tuberum* LMG 21444T, *B. acidipaludis* NBRC 101816T and *B. tropica* LMG 22274T, were 89.16 %, 89.72 %, 78.03 % and 77.96 %, respectively (Table S1, available in the online Supplementary Material). These values were below the proposed cut-off ANI values of 95–96 % for bacterial species (Kim et al., 2014). The UPGMA dendrogram based on ANI values (Fig. 2) also distinguished strain JC2948T from other species of the genus *Burkholderia*.

The DNA G+C content of strain JC2948T, calculated from its genome sequence, was 63.78 mol%. This is within the range of values for members of the genus *Burkholderia* (59.0–69.9 mol%; Table 1).

The temperature (4, 10, 20, 25, 30, 37, 45 and 50 °C), pH (4–10 with 1.0 pH unit increments; adjusted with 10 M KOH or HCl after autoclaving media) and NaCl concentration ranges (0–5 % w/v, with 1 %, w/v, unit increments) for growth were assessed on NA over up to 2 weeks. Standard physiological and biochemical tests were carried out on strains of *Burkholderia* species.
Table 2. Major phenotypic characteristics that differentiate strain JC2948T from other species of the genus Burkholderia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4*</th>
<th>5†</th>
<th>6†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Soil</td>
<td>Rn</td>
<td>Rn</td>
<td>Plant</td>
<td>Soil</td>
<td>Rn</td>
</tr>
<tr>
<td>Nitrate reduction to nitrogen</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility on soft agar</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capric acid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Malic acid</td>
<td>w</td>
<td>w</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phenyl acetate</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Mannose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-Arabinose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G + C content</td>
<td>63.8</td>
<td>63.2‡</td>
<td>63.2‡</td>
<td>65.2</td>
<td>64.0</td>
<td>63.0–65.0† (HPLC)</td>
</tr>
</tbody>
</table>

*Data from Aizawa et al. (2010).
†Data from Sheu et al. (2013).
‡Data from Reeve et al. (2013).
§Data from Angus et al. (2014).

out as described previously (Smibert & Krieg, 1994). Cellular morphology was examined after growth on NA at 25 °C for 2 days by transmission electron microscopy (Fig. S1). Cellular motility was determined by observing growth on soft agar (0.4%). Catalase activity was determined by observing the production of oxygen bubbles after the application of 3% (v/v) hydrogen peroxide solution. Further enzyme activities, including oxidase activity, were determined by using API 20NE and API ZYM kits (bioMérieux). The API systems were employed according to the manufacturer’s recommendations and strain JC2948T and three reference strains (B. tropica LMG 22274T, B. tuberum LMG 21444T and B. spreantiae LMG 27175T) were incubated at 30 °C. The results of morphological, biochemical and physiological tests are presented in the species description and in Table 1.

To extract fatty acids, strain JC2948T and B. tropica LMG 22274T were grown on NA for 2 days at 30 °C. The physiological age of the three strains was similar due to harvesting from the third quadrant of the NA plates. Fatty acid methyl esters were extracted by using the standard fatty acid methyl ester method of the Microbial Identification System (MIDI) version 6.0 and analysed by using gas chromatography based on the RTSBA6 database. C17:0cyclo (22.47%) was detected as the major fatty acid of strain JC2948T, followed by C16:0 (22.09%), C19:0 cyclo9c (17.7%) and C18:1ω7c (8.8%) (Table 2).

For polar lipids analysis, strain JC2948T and B. tropica LMG 22274T, grown in NB for 2 days at 30 °C, were prepared. Polar lipids were extracted and analysed by using two-dimensional TLC according to previous studies (Embley & Wait, 1994). The solvent systems were composed of chloroform/methanol/water (65:25:3.8, by vol) for the first dimension and chloroform/methanol/acetic acid/water (40:7.5:6:1, by vol) for the second dimension. Phosphomolybdic acid (Sigma) was used to detect all lipids and ninhydrin reagent (Sigma) was used for lipids containing free amino groups. Molybdenum blue (Sigma) was used to detect phospholipids, α-naphthol reagent (Sigma) for the detection of glycolipids and Dragendorff’s reagent (102035; Merck) for the detection of quaternary nitrogen-containing lipids. The major polar lipids of strain JC2948T were diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) (Fig. S2); these were also observed in B. diazotrophica JPY461T (Sheu et al., 2013).

The isoprenoid quinone composition was analysed by HPLC according to Minnikin et al. (1984) and Collins (1985). The predominant isoprenoid quinone of JC2948T was ubiquinone Q-8, which has also been observed in the reference strain, B. tropica LMG 22274T (Reis et al., 2004).

Table 2. Cellular fatty acid composition (% of total) of strain JC2948T and related strains of species of the genus Burkholderia

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2*</th>
<th>3†</th>
<th>4†</th>
<th>5</th>
<th>6†</th>
</tr>
</thead>
<tbody>
<tr>
<td>C17:0cyclo</td>
<td>22.47</td>
<td>10.60</td>
<td>19.70</td>
<td>16.40</td>
<td>21.26</td>
<td>8.40</td>
</tr>
<tr>
<td>C16:0</td>
<td>22.09</td>
<td>19.90</td>
<td>24.70</td>
<td>27.80</td>
<td>20.17</td>
<td>18.20</td>
</tr>
<tr>
<td>C19:0 cyclo9c</td>
<td>17.70</td>
<td>4.10</td>
<td>17.50</td>
<td>11.30</td>
<td>17.61</td>
<td>8.40</td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>8.88</td>
<td>30.50</td>
<td>10.90</td>
<td>15.60</td>
<td>14.30</td>
<td>32.20</td>
</tr>
<tr>
<td>Summed feature 2‡</td>
<td>6.33</td>
<td>8.50</td>
<td>6.10</td>
<td>6.00</td>
<td>5.29</td>
<td>5.20</td>
</tr>
<tr>
<td>C16:0 3-OH</td>
<td>5.79</td>
<td>6.70</td>
<td>5.40</td>
<td>5.60</td>
<td>5.18</td>
<td>5.20</td>
</tr>
<tr>
<td>C14:0</td>
<td>5.32</td>
<td>4.70</td>
<td>4.10</td>
<td>4.60</td>
<td>4.16</td>
<td>4.50</td>
</tr>
<tr>
<td>C16:0 2-OH</td>
<td>2.61</td>
<td>2.10</td>
<td>2.50</td>
<td>3.00</td>
<td>2.69</td>
<td>3.40</td>
</tr>
<tr>
<td>Summed feature 3‡</td>
<td>1.71</td>
<td>13.10</td>
<td>1.70</td>
<td>2.80</td>
<td>2.30</td>
<td>6.20</td>
</tr>
<tr>
<td>C16:1 2-OH</td>
<td>1.15</td>
<td>3.30</td>
<td>1.20</td>
<td>1.90</td>
<td>1.89</td>
<td>1.90</td>
</tr>
<tr>
<td>C18:1 2-OH</td>
<td>0.98</td>
<td>1.30</td>
<td>–</td>
<td>1.70</td>
<td>1.03</td>
<td>1.20</td>
</tr>
</tbody>
</table>

*Data from De Meyer et al. (2013).
†Data from Sheu et al. (2013).
‡Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 2 comprises C14:0 3-OH and/or C16:1ω7c iso I; summed feature 3 comprises C16:1ω7c and/or C16:1ω6c.
The results of phylogenetic analyses, based on 16S rRNA gene sequences, indicated that strain JC2948\(^T\) belongs to the genus *Burkholderia* and represents an independent phyletic line in the genus. The low DNA–DNA relatedness based on whole genome sequences confirmed that strain JC2948\(^T\) represents a novel species of the genus *Burkholderia*. Furthermore, when compared with phylogenetically related species, strain JC2948\(^T\) showed different phenotypic traits, especially with respect to nitrate reduction, motility on soft agar, oxidase activity and the assimilation of various substrates. For this reason strain JC2948\(^T\) should be seen as constituting a novel species of the genus *Burkholderia* and the name *Burkholderia monticola* sp. nov. is proposed.

**Description of Burkholderia monticola sp. nov.**

*B. monticola* (mon.ti’co.la. L. n. *montis* mountain; -cola from L. *n. incola* an inhabitant; N.L. fem. *monticola* mountain-dwelling)

Ivory/yellow colonies with entire margins (0.1–2 mm in diameter) on NA at 30 °C. Cells are short-rod-shaped and 1.2–1.4 × 0.8–0.9 μm. Aerobic, Gram-stain-negative, non-motile, catalase-positive and oxidase-negative. Growth occurs in the range of 10–37 °C (optimal growth temperature, 25–37 °C); no growth is observed above 45 °C in NA. Growth occurs at pH 5–8 (optimum, pH 5.5) and in the presence of 0–1 % (w/v) NaCl (optimum, 0 %, w/v). Does not reduce nitrate to nitrogen and does not produce indole. In API 20NE strips. Acid is not produced from glucose. Growth occurs at pH 5–8 (optimum, pH 5.5) and in the presence of 0–1 % (w/v) NaCl (optimum, 0 %, w/v). Does not reduce nitrate to nitrogen and does not produce indole in API 20NE strips. Acid is not produced from glucose. Assimilates D-glucose, N-acetylglucosamine and D-mannitol. Weakly assimilates malic acid, potassium gluconate and phenylacetic acid. Negative for β-galactosidase, arginine dihydrolase and urease. Does not hydrolyse aesculin or gelatin. In API ZYM strips, strongly positive for acid phosphatase, alkaline phosphatase and leucine arylamidase and positive for esterase (C14), esterase (C8) and naphthol-β-glucosaminidase, alkaline phosphatase and leucine arylamidase both rRNA- and protein-coding genes. The type strain JC2948\(^T\) (JCM 19904\(^T\)=KACC 17924\(^T\)), was isolated from a soil sample from Gwanak Mountain, Republic of Korea. The genomic DNA G+C content of the type strain is 63.8 mol%.

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**References**


