Burkholderia jiangsuensis sp. nov., a methyl parathion degrading bacterium, isolated from methyl parathion contaminated soil

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A methyl parathion (MP) degrading bacterial strain, designated MP-1T, was isolated from a waste land where pesticides were formerly manufactured in Jiangsu province, China. Polyphasic taxonomic studies showed that MP-1T is a Gram-stain-negative, non-sporo-forming, rod-shaped and motile bacterium. The bacterium could grow at salinities of 0–1 % (w/v) and temperatures of 15–40 °C. Strain MP-1T could reduce nitrate to nitrite, utilize D-glucose and L-arabinose, but not produce indole, or hydrolyze gelatin. Phylogenetic analysis based on 16S rRNA gene sequences demonstrated that MP-1T belongs to the genus Burkholderia, showing highest sequence similarity to Burkholderia grimmiae DSM 25160T (98.5 %), and similar strains including Burkholderia zhejiangensis OP-1T (98.2 %), Burkholderia choica LMG 22940T (97.5 %), Burkholderia glathei DSM 50014T (97.4 %), Burkholderia terrestris LMG 22937T (97.2 %) and Burkholderia telluris LMG 22936T (97.0 %). In addition, the gyrB and recA gene segments of strain MP-1T exhibited less than 89.0 % and 95.1 % similarities with the most highly-related type strains indicated above. The G+C content of strain MP-1T was 62.6 mol%. The major isoprenoid quinine was ubiquinone Q-8. The predominant polar lipids comprised phosphatidyl ethanolamine, phosphatidyl glycerol, aminolipid and phospholipid. The principal fatty acids in strain MP-1T were C18:1ω7c/C18:1ω6c (23.3 %), C16:0 (16.8 %), cyclo-C17:0 (15.0 %), C16:1ω7c/C16:1ω6 (8.5 %), cyclo-C19:0ω8c (8.1 %), C18:1ω iso C14:0-3-OH (6.7 %), C16:0-3-OH (5.6 %) and C16:0-2- OH (5.1 %). The DNA–DNA relatedness values between strain MP-1T and the three type strains (B. grimmiae DSM 25160T, B. zhejiangensis OP-1T and B. glathei DSM 50014T) ranged from 24.6 % to 37.4 %. In accordance with phenotypic and genotypic characteristics, strain MP-1T represents a novel species of the genus Burkholderia, for which the name Burkholderia jiangsuensis sp. nov. is proposed, the type strain is MP-1T (LMG 27927T=MCCC 1K00250T).

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Abbreviations: LMG, Laboratorium voor Microbiologie; MCCC, Marine Culture Collection of China.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strain MP-1T is KJ400396. The gyrB genes of strains MP-1T and Burkholderia grimmiae DSM 25160T are KJ535375 and KJ535376. The recA genes of strains MP-1T and DSM 25160T, Burkholderia zhejiangensis OP-1T, Burkholderia choica LMG 22940T, Burkholderia terrestris LMG 22937T and Burkholderia telluris LMG 22936T are KJ535369, KJ535373, KJ535370, KJ535374, KJ535371 and KJ535372. The GenBank accession numbers for four draft genome sequences of strains MP-1T, DSM 25160T, OP-1T and Burkholderia glathei DSM 50014T are JHF00000000, JFHE00000000, JFHD00000000 and JFHC00000000.

Two supplementary figures and one supplementary table are available with the online version of this paper.

Methyl parathion (MP), a common organophosphorus pesticide, has been applied extensively for crop protection, especially in developing countries. However, it has toxic effects on the water, air and soil (Barton et al., 2004). A variety of MP strains of bacteria have been isolated from soil (Li et al., 2007; Lii et al., 2005).

In order to study the diversity of MP-degrading microbes, MP-degrading strain MP-1T was subjected to taxonomic characterization. On the basis of 16S rRNA gene sequence analysis, phylogenetic trees indicated that strain MP-1T belongs to the genus Burkholderia, with high (>96.9 %) similarity to 16S rRNA sequences of six type strains: B. grimmiae DSM 25160T (Tian et al., 2013), Burkholderia zhejiangensis OP-1T (Lu et al., 2012), Burkholderia choica
LMG 22940\textsuperscript{T} (Vandamme et al., 2013), Burkholderia glathei DSM 50014\textsuperscript{T} (Zolg & Ottow, 1975), Burkholderia terrestris LMG 22937\textsuperscript{T} (Vandamme et al., 2013) and Burkholderia telluris LMG 22936\textsuperscript{T} (Vandamme et al., 2013). Thus, we investigated the characteristics of MP-1\textsuperscript{T} further to determine if it represents a new species of the genus Burkholderia.

The genus Burkholderia, a member of the class Betaproteobacteria, is a group of metabolically versatile Gram-stain-negative bacteria. To date, the genus Burkholderia comprises 83 type strains with validly published names (http://www.bacterio.net/burkholderia.html), which are widely distributed within diverse natural habitats, including water (Ali Khan & Ahmad, 2006; Zhang et al., 2010), fish (Gao et al., 2013) and soil (Yoo et al., 2007). The genus was first created to accommodate seven species from Pseudomonas rRNA group II (Yabuuchi et al., 1992). Species of the genus Burkholderia are responsible for biological degradation, biological control or, as rhizosphere micro-organisms in agriculture, the promotion of plant growth. In the present study, the exact taxonomic position of the newly isolated strain, MP-1\textsuperscript{T}, was determined using phenotypic, genetic and chemotaxonomic analyses.

A conventional enrichment method was employed to isolate MP-degrading strains. About 1 g of sludge was sampled from a pesticide manufacturing company in Jiangsu province, PR China. The soil samples were inoculated into a selective medium mineral salts medium (MSM), containing: 1.0 g\textsuperscript{-1} (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 0.8 g\textsuperscript{-1} K\textsubscript{2}HPO\textsubscript{4}, 0.2 g\textsuperscript{-1} KH\textsubscript{2}PO\textsubscript{4}, 0.2 g\textsuperscript{-1} MgSO\textsubscript{4}, 0.08 g\textsuperscript{-1} CaSO\textsubscript{4}, 0.005 g\textsuperscript{-1} FeSO\textsubscript{4}.7H\textsubscript{2}O, 0.0033 g\textsuperscript{-1} Na\textsubscript{2}MoO\textsubscript{4}.2H\textsubscript{2}O and 25 mg\textsuperscript{-1} MP was supplemented as the sole carbon source. The culture was incubated for about 3 days at 30 °C on a rotary shaker at 180 r.p.m. The enrichment suspension was then serially sub-cultured into fresh MSM containing a gradually increasing concentration of MP up to a maximum of 200 mg\textsuperscript{-1} every 3 days. After a few rounds of enrichment cultivation, the suspension was serially diluted and spread onto a peptone yeast (PY) agar plate (10.0 g\textsuperscript{-1} peptone, 5.0 g\textsuperscript{-1} yeast extract, 5.0 g\textsuperscript{-1} NaCl, 15.0 g\textsuperscript{-1} agar, pH 7.0) supplemented with 200 mg\textsuperscript{-1} MP. Among the isolates, a strain capable of degrading MP was isolated and designated strain MP-1\textsuperscript{T}. It was maintained on PY agar at 4 °C and stored as glycerol suspensions (30 %, v/v) at −80 °C. Biomass for chemotaxonomic studies was prepared by growing the strain in a shaker flask of PY broth at 30 °C for 2 days. Cells were harvested by centrifugation (7690 g) and freeze-dried prior to physiological and biochemical studies. One type strain of a species of the genus Burkholderia (B. zhejiangensis OP-1\textsuperscript{T}) was provided by Nanjing Agricultural University, while the other two strains (B. grimmia DSM 25160\textsuperscript{T} and B. glathei DSM 50014\textsuperscript{T}) were purchased from the DSMZ culture collection centre (Braunschweig, Germany). These were used as reference type strains, and cultured under the same conditions in all analyses except for those of quinone and polar lipids. Routine cultivation of the strains and most phenotypic tests were performed on PY agar plates. The other three strains (B. choica LMG 22940\textsuperscript{T}, B. terrestris LMG 22937\textsuperscript{T} and B. telluris LMG 22936\textsuperscript{T}) reported recently by Vandamme et al. (2013), were purchased from Laboratorium voor Microbiologie (LMG).

Gram staining was conducted as described by Buck (1982). The cell size, morphology and flagellation pattern were observed by transmission electron microscopy (JEM-1230, JEOL) using colonies grown on PY agar plates for 2 days at 30 °C. Spore morphology was examined in cultures grown on PY agar plates for 4 days. Cell motility was observed in vitro according to the hanging-drop method (Robbie, 1945). The optimal temperature for growth was tested over a temperature range of 15–40 °C in PY broth, at 5 °C unit intervals. Growth over a pH range was determined in PY broth adjusted with HCl or NaOH to pH 3–10, at 1 pH unit intervals. Cell growth was very poor at 15 or 40 °C. NaCl tolerance was determined using PY broth supplemented with 0, 0.5, 1, 2, 3, 4, or 5 % (w/v) NaCl. Physiological and biochemical properties were investigated with the API 20NE, API 20E and API ZYM systems (bioMérieux) according to the manufacturer’s instructions. B. grimmia DSM 25160\textsuperscript{T}, B. zhejiangensis OP-1\textsuperscript{T}, B. glathei DSM 50014\textsuperscript{T} and strain MP-1\textsuperscript{T} were all tested under the same conditions.

Cultures were grown in PY broth for 2 days for total DNA preparation using the extraction kit according to the manufacturer’s instructions (TIANamp Bacteria DNA Kit). The G+C content of strain MP-1\textsuperscript{T} was calculated from its draft genome sequence (JFHF00000000, Liu et al., 2014). The 16S rRNA gene sequence was amplified by PCR using a set of universal bacterial primers (Collins et al., 1991). The amplification of the DNA gyrase B subunit (gyrB) was also performed using previously described primers (Tayeb et al., 2008). The recA gene sequence was amplified using the BURI and BUR2 primers (Payne et al., 2005). The PCR product was ligated into vector pMD18-T and sequenced (Majorbio). Sequence similarities between the MP-1\textsuperscript{T} 16S rRNA gene sequence and those of closely related species were determined using the EzTaxon-e server (Feng et al., 2012). Phylogenetic trees were reconstructed using the neighbour-joining method (Saitou & Nei, 1987) in MEGA5.0 software (Tamura et al., 2011). The topology of phylogenetic trees was evaluated using bootstrap values based on 1000 replications.

Fatty acids from whole cells grown on PY plates at 30 °C for 2 days were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analysed by gas chromatography (Agilent Microbial Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System (Steele et al., 1997). The fatty acid profile of strain MP-1\textsuperscript{T}, B. grimmia DSM 25160\textsuperscript{T}, B. zhejiangensis OP-1\textsuperscript{T} and B. glathei DSM 50014\textsuperscript{T} were tested simultaneously. Isoprenoid quinones and polar lipids were analysed by the Identification Service of the MCCC. The quinones were extracted according to a...
method based on one previously described (Tindall, 1990a, b). Isoprenoid quinones fell into different classes (e.g. menaquinones, ubiquinones) as determined by TLC on silica gel, and were then analysed further by HPLC. Polar lipids were extracted from 200 mg freeze-dried cells using a chloroform: methanol: 0.3 % (w/v) aqueous NaCl mixture 1 : 2:0.8 (by vol.) (Bligh & Dyer, 1959). Polar lipids were separated by two-dimensional silica gel thin layer chromatography and then identified according to the method described previously (Tindall et al., 2007).

DNA–DNA hybridization (DDH) estimated values were calculated by the genome-to-genome distance calculator (GGDC2.0) (Auch et al., 2010a, b; Meier-Kolthoff et al., 2013).

Strain MP-1T is a Gram-stain-negative, non-spore-forming, rod-shaped, 0.6–0.9 μm x 1.3–1.6 μm bacterium with motile subpolar flagella (Fig. S1, available with the online Supplementary Material). The diameter of the colonies was 1.5–2.0 mm after incubation on PY agar plates at 30 °C for 2 days. Colonies were circular, yellowish, moist and translucent with a regular margin. The growth of strain MP-1T was observed at 0–1 % NaCl (w/v) (optimum 0.5 %), at 15–45 °C (optimum 30 °C) and at pH 5–9 (optimum pH 7). Other characteristics are described below.

A nearly full-length 16S rRNA gene sequence of strain MP-1T was obtained of 1498 bp. A BLAST search showed it had highest similarity with the sequences of B. grimmiae DSM 25160T (98.5 %), B. zhejiangensis OP-1T (98.2 %), B. choica DSM 22940T (97.5 %), B. glathei DSM 50014T (97.4 %), B. terrestris DSM 22937T (97.2 %) and B. telluris DSM 22936T (97.0 %). A dendrogram based on the neighbour-joining method was constructed, indicating the relationship between strain MP-1T and related species of the genus Burkholderia (Fig. 1). Strain MP-1T with six close relatives constituted a phylogenetic branch. However, it did not form a cluster with any type strain of species of the genus Burkholderia.

In order to analyse the affiliation of strain MP-1T with the most closely related species of the genus Burkholderia, we performed a multilocus sequence analysis using two

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**Fig. 1.** Neighbour-joining phylogenetic tree of strain MP-1T and representatives of other related taxa based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.01 nt substitution rate (K_\text{nuc}) units.
The principal fatty acids in strain MP-1\(^T\) were identified as \(C_{18:1\omega7c}/C_{18:1\omega9c}\) (23.3 %), \(C_{16:0}\) (16.8 %), cyclo-C\(_{17:0}\) (15.0 %), \(C_{16:1\omega7c}/C_{15:0\omega6}\) (8.5 %), cyclo-C\(_{19:0\omega9c}\) (8.1 %), \(C_{16:1}\) isio \(I/C_{14:0}\) 3-OH (5.7 %), \(C_{16:0}\) 3-OH (5.6 %) and \(C_{16:0}\) 2-OH (5.1 %). As shown in Table S1, the major fatty acids of the seven type strains were all composed of \(C_{16:0}\), \(C_{16:0}\) 3-OH, \(C_{16:1}\) isio \(I/C_{14:0}\) 3-OH and \(C_{14:0}\), indicating that the fatty acid profile of strain MP-1\(^T\) was identical to those of the closely related type strains. However, there was a significant difference between \(C_{16:1}\) 2-OH, cyclo-C\(_{17:0}\) and cyclo-C\(_{19:0\omega9c}\) among seven strains. Although a few relevant data (\(C_{18:0}\), \(C_{18:1\omega2}\) 2-OH, \(C_{19:0}\) 10-methyl, \(C_{16:1\omega7c}/C_{16:1\omega9c}\) and \(C_{18:1\omega7c}/C_{18:1\omega9c}\) about \(B.\) \(choica\) LMG 22940\(^T\), \(B.\) \(terrestrial\) LMG 22937\(^T\) and \(B.\) telluris LMG 22936\(^T\) are not indicated in the literature (Vandamme et al., 2013), the different proportions of various fatty acids showed individual differences.

The major isoprenoid quione of strain MP-1\(^T\) was determined to be ubiquinone Q-8. This is consistent with all other members of the genus \(Burkholderia\) (Lu et al., 2012; Tian, et al., 2013). The polar lipid profile of strain MP-1\(^T\) contained phosphatidyl glycerol (PG), phosphatidyl ethanolamine (PE), aminolipid (AL) and phospholipid.
however, two type strains of the genus *Burkholderia* (*B. grimmiae* DSM 25160$^T$, *B. zhejiangensis* OP-1$^T$) contained PG, PE, uncharacterized AL and unknown PL (Lu et al., 2012; Tian et al., 2013). The major profile of MP-1$^T$ was similar to that of closely related strains, except for the absence of glycolipid (GL) compared to *B. grimmiae* DSM 25160$^T$.

The draft genome sequence of the novel strain MP-1$^T$ (JFHF00000000, Liu et al., 2014), along with those of three type strains (*B. grimmiae* DSM 25160$^T$ (JFHE00000000), *B. zhejiangensis* OP-1$^T$ (JFHDF00000000) and *B. glathei* DSM 50014$^T$ (JFHC00000000)), were deposited in GenBank.

Estimated values of DDH between the four type strains were calculated using GGDC2.0 with the BLAST + alignment method. The estimated DDH values between strain MP-1$^T$ and the three type strains were 24.6%—37.4%, which are below the threshold of 70% for the delineation of a species (Wayne et al., 1987). This result confirms that strain MP-1$^T$ represents a novel species of the genus *Burkholderia*.

Phylogenetic, phenotypic and chemotaxonomic analyses assigned strain MP-1$^T$ to the genus *Burkholderia*. Strain MP-1$^T$ could be set apart from closely related species due to some differential phenotypic characteristics, which are listed in Table 1. Owing to the low estimated DDH value (<38%) with closely related species, the isolate MP-1$^T$ should not be assigned to any previously reported species. In conclusion, strain MP-1$^T$ is proposed to represent a novel species of the genus *Burkholderia*, for which the name *Burkholderia jiangsuensis* sp. nov. is suggested.

**Description of Burkholderia jiangsuensis sp. nov.**

*Burkholderia jiangsuensis* (jiang.su.en’sis. N.L. fem. adj. *jiangsuensis* of Jiangsu, a province of the People’s Republic of China, where the type strain was isolated).

Cells are Gram-stain-negative, non-spore-forming, rod-shaped, 0.6–0.9 μm x 1.3–1.6 μm, motile by a subpolar flagella. The diameter of colonies is 1.5–2.0 mm after incubation on PY agar plates at 30 °C for 2 days; colonies are circular, yellowish, moist and translucent with a regular margin. Growth is observable at 0–1% NaCl (w/v) (optimum 0.5%), at 15–45 °C (optimum 30 °C) and at pH 5–9 (optimum pH 7). With the API 20NE kit nitrate is reduced to nitrite and D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, potassium gluconate,

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capric acid, malic acid, trisodium citrate, phenylacetic acid are used and adipic acid weakly used, but not arginine dihydrolase, urease, β-glucosidase, β-galactosidase and maltose. It cannot denitrify, produce indole and hydrolyse gelatin. With the 20E kit, it is positive for citrate utilization and weakly positive for acetoin production (Voges–Proskauer), glucose, rhamnose and arabinose, but negative for lysine decarboxylase, ornithine decarboxylase, H2S production, tryptophan deaminase, gelatinase, inositol, sorbitol, sucrose, melibiose and amygdalin. With API ZYM test strips it is positive for alkaline phosphatase, esterase (C4), lipase (C8), leucine aminopeptidase and acid phosphatase, and weakly positive for valine aminopeptidase and naphthol-AS-Bl-phosphoamidase, but negative for lipase (C14), cystine aminopeptidase, α-chymotrypsin, α-glucosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. It has the ability to degrade MP, a common organophosphorus pesticide, which can be utilized as the sole carbon source for growth. The major fatty acids are C18:1v7c/C18:1v6c, C16:0, cyclo-C17:0, C16:1v7c/C16:1v6c, cyclo-C19:0v8c, C16:1 iso I/C14:0 3-OH, C16:0 3-OH and C16:0 2-OH. The respiratory quinone is Q-8. The polar lipids are comprised of PG, PE, AL and PL.

The type strain, MP-1T (LMG 27927T = MCCC 1K00250T), was isolated from MP-contaminated soil of a pesticide manufacturing company in Jiangsu Province, PR China. The chromosomal DNA G+C content of the type strain is 62.6 mol%.

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Reference


