**Burkholderia sartisoli** sp. nov., isolated from a polycyclic aromatic hydrocarbon-contaminated soil

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A Gram-negative, rod-shaped, aerobic bacterium, designated strain RP007T, was isolated from a polycyclic aromatic hydrocarbon-contaminated soil in New Zealand. Two additional strains were recovered from a compost heap in Belgium (LMG 18808) and from the rhizosphere of maize in the Netherlands (LMG 24204). The three strains had virtually identical 16S rRNA gene sequences and whole-cell protein profiles, and they were identified as members of the genus *Burkholderia*, with *Burkholderia phenazinium* as their closest relative. Strain RP007T had a DNA G+C content of 63.5 mol% and could be distinguished from *B. phenazinium* based on a range of biochemical characteristics. Strain RP007T showed levels of DNA–DNA relatedness towards the type strain of *B. phenazinium* and those of other recognized *Burkholderia* species of less than 30 %. The results of 16S rRNA gene sequence analysis, DNA–DNA hybridization experiments and physiological and biochemical tests allowed the differentiation of strain RP007T from all recognized species of the genus *Burkholderia*. Strains RP007T, LMG 18808 and LMG 24204 are therefore considered to represent a single novel species of the genus *Burkholderia*, for which the name *Burkholderia sartisoli* sp. nov. is proposed. The type strain is RP007T (=LMG 24000T =CCUG 53604T =ICMP 13529T).

Many novel species of the genus *Burkholderia* have been described in recent years. At the time of writing, the genus comprised 45 recognized species, which occupy remarkably diverse ecological niches (Coenye & Vandamme, 2003). These extremely versatile organisms have been isolated as plant and human pathogens. Species of the genus have also found applications as biocontrol agents, in plant growth promotion and in bioremediation (Parke & Gurian-Sherman, 2001).

The pollution of soil and water with crude oil and petroleum products is a problem of increasing concern. Such soil pollution can be reduced via the use of polycyclic aromatic hydrocarbon (PAH)-degrading bacteria (Bogardt & Hemmingsen, 1992; O’Sullivan & Mahenthiralingam, 2005). Strain RP007T was isolated from a PAH-contaminated site in New Zealand in 1995. This organism was shown to be a versatile degrader of low-molecular-mass PAHs, as shown by its ability to grow on naphthalene, phenanthrene and anthracene as sole sources of carbon and energy (Laurie & Lloyd-Jones, 1999). Recently, this strain was used for the design of a bacterial biosensor for the detection of phenanthrene (Tecon et al., 2006). In the course of a long-term survey of the natural biodiversity of the genus *Burkholderia*, we identified two isolates with whole-cell protein profiles that were virtually identical to that of strain RP007T. Strain LMG 18808 was recovered from a compost heap in Belgium and strain LMG 24204 (original strain designation RG6-9 =LMG 22948) was isolated from the rhizosphere soil of maize plants in the Netherlands by Salles et al. (2006). The aim of the present study was to determine the taxonomic position of these three strains by means of a polyphasic approach. The results demonstrate that they represent a single, novel species of the genus *Burkholderia*.

All strains were routinely cultured on trypticase soy agar (TSA) and incubated aerobically at 28 °C for at least 2 days.
unless indicated otherwise. Colonies grown on TSA were cream-coloured, circular, smooth and convex with diameters of 1–2 mm. The subculture of strain RP007T used in the present study is a spontaneous rifampicin-resistant mutant of the original strain (Laurie & Lloyd-Jones, 1999).

Whole-cell protein profiles were determined by SDS-PAGE as described by Pot et al. (1994). Densitometric analysis, normalization and interpolation of the protein profiles and numerical analysis via the Pearson product-moment correlation coefficient were performed with the GelCompar 4.2 software package (Applied Maths). The profiles were compared with those from a database comprising reference strains of all recognized *Burkholderia* species and many unclassified *Burkholderia* strains. Computer-assisted numerical analysis and visual comparison of the whole-cell protein profile of strain RP007T revealed two additional strains (LMG 18808 and LMG 24204) with virtually identical profiles (Fig. 1). The profiles were clearly different from those of recognized *Burkholderia* species (data not shown).

The nearly complete 16S rRNA gene sequence (1347 bp) of strain RP007T was determined by Laurie & Lloyd-Jones (1999). For strains LMG 18808 and LMG 24204, genomic DNA was prepared as described by Pitcher et al. (1989). The nearly complete 16S rRNA genes (corresponding to positions 8–1541 in the *Escherichia coli* numbering system) of strains LMG 18808 (1501 bp) and LMG 24204 (1525 bp) were amplified and sequenced as described by Coenye et al. (2001). 16S rRNA gene sequence comparisons against the GenBank database indicated that these three isolates belonged to the family *Burkholderiaceae*, class *Betaproteobacteria*. Strains LMG 18808 and LMG 24204 showed highest levels of 16S rRNA gene sequence similarity with strain RP007T (99.11 and 98.96 %, respectively). On the basis of 16S rRNA gene sequence similarity comparisons, the closest cultured relatives of strain RP007T were *Burkholderia phenazinium* LMG 2247T (1522 bp, 97.96 % similarity) and several unnamed *Burkholderia* strains. The 16S rRNA gene sequences of *B. phenazinium* LMG 2247T and other related taxa were obtained from the GenBank database. Multiple alignment was performed by using the CLUSTAL_X program (Thompson et al., 1997). The aligned sequences were analysed by using the Bionumerics 4.5 software (Applied Maths). Distances were calculated with the Jukes–Cantor algorithm. Phylogenetic trees were constructed by using the neighbour-joining and maximum-parsimony methods with bootstrap values based on 1000 replications. In the phylogenetic tree (Fig. 2) based on 16S rRNA gene sequences, strains RP007T, LMG 18808 and LMG 24204 formed a single cluster supported by a bootstrap value of 100 %, with *B. phenazinium* LMG 2247T as their nearest neighbour. Strain RP007T also showed >97.0 % 16S rRNA gene sequence similarity to *Burkholderia ginsengisoli* LMG 24044T, *Burkholderia terricola* LMG 20594T and *Burkholderia xenovorans* LMG 21463T, but these latter organisms occupied distinct positions in the phylogenetic tree.

For determination of the G+C content, total DNA was enzymically degraded into nucleosides as described by Mesbah & Whitman (1989). The nucleoside mixture obtained was then separated by using a Waters Breeze HPLC system and XBridge Shield RP18 column thermostabilized at 37 °C. The solvent was 0.02 M NH₄H₂PO₄ (pH 4.0) with 1.5 % (v/v) acetonitrile. Non-methylated lambda phage DNA (Sigma) and *E. coli* LMG 2093 DNA were used as calibration reference and control, respectively. The DNA G+C content of strain RP007T was 63.5 mol%, which is within the range of values reported for members of the genus *Burkholderia* (Coenye et al., 2001).

DNA–DNA hybridization experiments were performed as described by Coenye et al. (2001). Each value is the mean of eight replicate experiments. Based on 16S rRNA gene sequence data, *B. phenazinium* LMG 2247T was selected as a reference strain for DNA–DNA hybridization experiments.

![Fig. 1. Whole-cell protein profiles of *B. phenazinium* LMG 2247T and LMG 18808 and strains RP007T, LMG 18808 and LMG 24204.](http://ijs.sgmjournals.org)

![Fig. 2. Neighbour-joining tree showing the phylogenetic position of strains RP007T, LMG 18808 and LMG 24204 within the genus *Burkholderia*, based on 16S rRNA gene sequence comparisons. *Ralstonia solanacearum* LMG 2299T was used as an outgroup. Bootstrap values (>50 %) based on 1000 replications are shown at nodes of the tree. Bar, 1 % sequence dissimilarity.](http://ijs.sgmjournals.org)
This strain showed a DNA–DNA hybridization value of 31% towards strain RP007T. B. ginsengisol LMG 24044T and B. xenovorans LMG 21463T were also included in the experiments and exhibited DNA–DNA hybridization values of 25 and 20%, respectively, to strain RP007T. These low DNA–DNA hybridization values demonstrate that strain RP007T represents a novel species of the genus Burkholderia.

Determination of the cellular fatty acid profile of strain RP007T was performed as described by Vandamme et al. (1992). The fatty acid profile of strain RP007T consisted of (only components comprising >1% of the total are given) C13:1 (1.3%), C14:0 (5.0%), summed feature 2 (C14:0 3-OH and/or iso-C15:0) I) (5.4%), C16:0 7c (2.8%), C16:0 (22.7%), C17:0 cyclo (28.5%, major component), C16:1 2-OH (2.4%), C16:0 2-OH (3.1%), C16:0 3-OH (3.3%), C18:0 7c/9t/10t (4.2%), C19:0 cyclo 9c (16.8%) and C18:1 2-OH (1.1%).

The presence of C16:0 3-OH is a characteristic feature of the genus Burkholderia (Viallard et al., 1998). The cellular fatty acid profiles of B. phenazinium and other Burkholderia species were available from Kim et al. (2006). Strain RP007T could be differentiated from B. phenazinium based on a high level of C17:0 cyclo but low level of C18:0 7c/9t/10t.

In conclusion, the present study demonstrated that strain RP007T represents a species that can be distinguished from its nearest phylogenetic neighbours by means of whole-cell protein and fatty acid profiles, results of DNA–DNA hybridization experiments and biochemical characterization. Strains LMG 18808 and LMG 24204 had virtually identical whole-cell protein profiles and showed high levels of 16S rRNA gene sequence similarity to strain RP007T. Many polyphasic taxonomic studies of novel Burkholderia species have included both whole-cell protein profiling and DNA–DNA hybridization experiments, and have revealed a clear correlation between the two methods, confirming that bacteria with identical or very similar whole-cell protein patterns possess high levels of gene sequence similarity and thus belong to a single species (Coenye et al., 2001; Vandamme et al., 1996). On this basis, the virtually identical whole-cell protein profiles and high levels of 16S rRNA gene sequence similarity indicate unambiguously that strains RP007T, LMG 18808 and LMG 24204 represent a single novel species. We therefore propose that these three strains represent a novel species of the genus Burkholderia, for which the name Burkholderia sartisoli sp. nov. is proposed.

**Description of Burkholderia sartisoli sp. nov.**

Burkholderia sartisoli (sar.ti.so’li. L. adj. sartus mended, repaired, put in order; L. n. solum soil; N.L. gen. n. sartisoli of cured soil).

Cells are Gram-negative, aerobic rods. Colonies grown on TSA for 2 days are cream-coloured, circular, smooth and convex with diameters of 1–2 mm. Temperature range for growth is 25–30 °C; no growth occurs at 42 °C. Growth occurs in the absence of NaCl and in the presence of 0.5% NaCl (w/v), but not at higher concentrations. Other characteristics that do not differentiate B. sartisoli from B. phenazinium are listed above. The type strain can be differentiated from B. phenazinium LMG 2247T based on production of acid from adonitol but not from D-xylene. Malate, citrate and arabinose are assimilated, but adipate and succrose are not. Catalase- and oxidase-positive. Nitrate is reduced. Aesculin and Tween 80 are hydrolysed. Positive for urease and C4-esterase, but not for amylase or C4-esterase lipase. The predominant fatty acids are C17:0 cyclo and C16:0. The G+C content of the genomic DNA is 63.5 mol%.

*B. sartisoli* strains have been isolated from soil samples. The type strain, RP007T (=LMG 24000T =CCUG 53604T =ICMP 13529T), was isolated from a PAH-contaminated soil in New Zealand. The subculture of strain RP007T used in the present study is a spontaneous rifampicin-resistant mutant of the original strain (Laurie & Lloyd-Jones, 1999). LMG 18808 (=CCUG 54570) and LMG 24204 are two other strains of the species. Strain LMG 24204 is a new deposit of strain RG6-9 (Salles et al., 2006), since LMG 22948 was lost.

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References


