Burkholderia ferrariae sp. nov., isolated from an iron ore in Brazil

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Since Yabuuchi et al. (1992) proposed the genus Burkholderia to include the former rRNA group II pseudomonads, many other bacterial species have been described as belonging to this genus, which at the time of writing includes more than 40 species. Members of the genus Burkholderia have been found within many different ecological niches, but predominantly within the soil and the rhizosphere, from which some of the more recently described species have been isolated, such as Burkholderia sacchari (Brämer et al., 2001), Burkholderia tropica (Reis et al., 2004) and Burkholderia unamae (Caballero-Mellado et al., 2004).

Functionally, Burkholderia is a remarkably diverse genus that includes plant symbionts and both plant and animal pathogens. Some species of the genus are also known as opportunistic pathogens in humans. Certain species of Burkholderia have proved to be very efficient in biocontrol, bioremediation and plant growth promotion (Coene & Vandamme, 2003; O’Sullivan & Mahenthiralingam, 2005).
Many strains of *Burkholderia* species have, among other properties, the ability to solubilize highly insoluble phosphatic minerals and, therefore, are of significant interest to the agricultural sector with regard to their applicability in biofertilization (Artursson et al., 2006; Igual et al., 2001; Peix et al., 2001; Purnomo et al., 2005). Moreover, this property could also be economically useful for emerging industries such as biomining. Many of the current world iron ore resources contain over 0-08% (w/w) phosphorus, a level above the accepted standard for the manufacture of metallic iron and steel (Cheng et al., 1999). Although there are chemical processes to reduce the phosphorus content of iron ores, the historically low prices of this raw material make them non-viable economically. In this context, biotechnology may have a role in overcoming this problem in a cost-effective and environmentally friendly way. In the course of isolating phosphate-solubilizing micro-organisms (PSMs) from a high-phosphorous iron ore from Minas Gerais State, Brazil, we isolated a bacterial strain, designated FeGl01T, that, based on its genotypic and phenotypic characterization, should be classified within a novel species of the genus *Burkholderia*.

Strain FeGl01T was isolated from a suspension of the ore material in sterile distilled water maintained under agitation for 24 h at ambient temperature. The suspension was serially diluted and spread on NBRIP agar plates. The medium NBRIP was described by Nautiyal (1999) for the detection of PSMs, and contains glucose as a carbon source and insoluble tricalcium phosphate as the sole source of phosphorus, allowing the detection of PSMs based on the formation of haloes around their colonies. Cultures used in further studies were purified from a single colony after 12 days incubation at 30°C on NBRIP medium, and subsequently cultivated on YED-P agar plates. On YED-P, colonies of strain FeGl01T were cream-coloured, circular, smooth and convex with diameters of 1–3 mm.

Genomic DNA was extracted as described by Rivas et al. (2001). The 16S rRNA gene of strain FeGl01T was analysed as described by Rivas et al. (2002). The sequence obtained was compared with those from GenBank using the FASTA program (Pearson & Lipman, 1988). Sequences were aligned using CLUSTAL X software (Thompson et al., 1997). Distances were calculated according to Kimura’s two-parameter method (Kimura, 1980). The phylogenetic tree was inferred using the neighbour-joining method (Saitou & Nei, 1987), and bootstrap analysis was based on 1000 resamplings. The MEGA2.1 package (Kumar et al., 2001) was used for all analyses. A neighbour-joining tree showing the phylogenetic position of strain FeGl01T based on its 16S rRNA gene sequence is presented in Fig. 1 (an extended tree is available as Supplementary Fig. S1 in IJSEM Online). The results of the phylogenetic analysis indicate that strain FeGl01T is related to members of the genus *Burkholderia*. The closest relatives to strain FeGl01T among recognized species of the genus *Burkholderia* are *B. sacchari* LMG 19450T, *B. tropica* Ppe8T and *B. unamae* MTI-641T showing, respectively, 16S RNA gene sequence similarities of 97-6, 97-3 and 97-0%. Two recently described *Burkholderia* species are also very closely related to strain FeGl01T: *Burkholderia silvatlantica* SRMrh-20T and *Burkholderia mimosarum* PAS44T show 16S rRNA gene sequence similarities of 97-4 and 97-6%, respectively, to strain FeGl01T. The low similarities found between strain FeGl01T and its closest relatives suggest that it represents a novel species of the genus *Burkholderia*.

According to the results of Payne et al. (2005), *Burkholderia* species can be differentiated by analysis of an internal 385-bp sequence of the recA gene (spanning bases 76 to 461 relative to the *Burkholderia cenocepacia* J2315 genome recA gene). Moreover, Payne et al. (2005) also reported that analysis of this partial recA sequence, obtained with the *Burkholderia*-specific primers BUR3 and BUR4, produced phylogenetic trees with the same topology and discrimination as those derived from analysis of nearly full-length recA gene sequences. Although the recA analysis does not exactly match the phylogeny obtained with 16S rRNA gene sequences, it provides a greater degree of resolution among closely related species within the genus (Payne et al., 2005). Thus, to confirm the phylogenetic position of strain FeGl01T, we amplified and sequenced this partial recA region for strain FeGl01T, *B. tropica* Ppe8T and *B. unamae* MTI-641T as described by Payne et al. (2005), and these sequences were compared with those from GenBank and analysed as described above for the 16S rRNA gene. A phylogenetic tree constructed with these partial recA sequences is shown in Fig. 2. The results roughly confirm the phylogenetic position of strain FeGl01T within the genus *Burkholderia* obtained by analysis of 16S rRNA gene sequences. Although *B. tropica* Ppe8T grouped in a cluster different from that containing strain FeGl01T, *B. sacchari* LMG 19450T and *B. unamae* MTI-641T, a pairwise analysis of the partial recA sequences showed that these three recognized species are the closest relatives to strain FeGl01T, with similarity values of 94-9% (*B. sacchari* LMG 19450T), 93-5% (*B. unamae* MTI-641T) and 92-0% (*B. tropica* LMG 2129T).
Ppe8T). These recA sequence similarity values suggest that strain FeGl01T may belong to a novel species.

For base composition analysis, DNA was prepared according to the method of Chun & Goodfellow (1995). The G + C content of the DNA was determined using the thermal denaturation method (Mandel & Marmur, 1968). The G + C content of strain FeGl01T was 62.7 mol%. DNA–DNA hybridization was performed according to the method of Ezaki et al. (1989), following the recommendations of Willems et al. (2001). Mean levels of DNA–DNA relatedness of 40% were found between strain FeGl01T and both B. sacchari LMG 19450T and B. tropica Ppe8T, and of 24% between strain FeGl01T and B. unamae MTI-64T (mean of four replications). These results indicate that strain FeGl01T does not belong to any of the recognized species of Burkholderia based on the recommended minimum threshold value of 70% DNA–DNA relatedness for the definition of genomic species (Wayne et al., 1987).

Analyses of quinones and of the cellular fatty acid profile of strain FeGl01T were performed at the DSMZ. As in all other species of the genus Burkholderia, ubiquinone Q-8 was detected as the predominant quinone system. The fatty acid profile of strain FeGl01T consisted of (only components comprising >1% of the total are given): C14:0 (4.9%), C16:0 (18.0%), C17:0 cyclo (18.9%), C16:1 2-OH (1.5%), C16:2 2-OH (5.0%), C16:3 3-OH (3.4%), C18:1ω7c (16.7%), C19:0ω8c cyclo (18.8%), C18:1 2-OH (1.5%) and summed features 2 (6.0%) and 3 (1.9%). Summed feature 2 corresponds to C14:0 3-OH, iso-C16:1 I, an unknown fatty acid with equivalent chain length of 10-928, C12:0 ALDE or any combination of these fatty acids, and summed feature 3 corresponds to C16:1ω7c and/or iso-C15:0 2-OH. The components included in summed features 2 and 3 are similar to those reported in other Burkholderia species (Caballero-Mellado et al., 2004; Chen et al., 2006; Coenye et al., 2001; Vandamme et al., 1997). The fatty acid profile of strain FeGl01T shows significant differences from those of the phylogenetically most closely related species, B. sacchari (Bräm et al., 2001), B. unamae (Caballero-Mellado et al., 2004) and B. mimosarum (Chen et al., 2006); the proportions of C17:0 cyclo and C19:0ω8c cyclo are considerably higher and the proportions of C18:1ω7c and summed feature 3 are considerably lower in strain FeGl01T than in these other three Burkholderia species. In comparison with B. silvatlantica (Perin et al., 2006), strain FeGl01T contains relatively high proportions of both C19:0ω8c cyclo and C17:0 cyclo (Table 1).

Phenotypic traits of strain FeGl01T were analysed by using the API 20NE gallery (bioMérieux) as recommended by the manufacturer, and by using the API 50CH galleries (bioMérieux) inoculated with a suspension of cells in 0.7% (w/v) YNB minimal growth medium (Difco) adjusted to pH 7.0. Results are given in the species description below. Strain FeGl01T can be differentiated from B. sacchari, B. tropica, B. unamae, B. silvatlantica and B. mimosarum by its inability to assimilate sorbitol and D-arabinose and from the first four of these species by its ability to assimilate dulcitol.

Fig. 2. Neighbour-joining tree based on partial recA sequences of members of the genus Burkholderia. The phylogenetic tree was rooted using the Neisseria meningitidis MS58 recA gene as the outgroup sequence. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 5 substitutions per 100 nt.
and D-tagatose. Other differences in the assimilation of carbon sources are given in Table 1.

Strain FeGl01T can be differentiated genotypically and phenotypically from recognized species of the genus Burkholderia and we therefore suggest that it represents a novel species, for which the name Burkholderia ferrariae sp. nov. is proposed.

**Description of Burkholderia ferrariae sp. nov.**

Burkholderia ferrariae (fer. ra’ri. ae. L. gen. n. ferrariae of an iron mine).

Cells are Gram-negative, non-sporulating rods. Catalase-and oxidase-positive. Colonies on YED-P medium are cream-coloured, round, smooth and convex with diameters of approximately 1–3 mm. Nitrate is reduced to nitrite. In the API 20NE system, it produces β-galactosidase but does not produce indole, urease, arginine dihydrolase or gelatinase; it does not hydrolyse aesculin. The following substrates are assimilated as carbon sources in the API 20NE and API 50CH systems: glycerol, L-arabinose, ribose, D-xylene, adonitol, galactose, D-glucose, D-fructose, D-mannose, dulcitol, inositol, mannitol, N-acetylglucosamine, cellobiose, trehalose, D-tagatose, L-fucose, D-arabitol, glucose, 2-ketogluconate, malate, citrate, caprate, adipate and phenylacetate. It does not use erythritol, D-arabinose, L-xylene, methyl β-xylolide, L-sorbose, rhamnose, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, salicin, maltose, lactose, melibiose, sucrose, inulin, melezitose, D-rafinose, starch, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-fucose, L-arabitol or 5-ketogluconate as carbon sources. The G+C content is 62.7 mol%.

The type strain, FeGl01T (=LMG 23612T=CECT 7171T=DSM 18251T), was isolated from ore material from the Jangada mine, Minas Gerais State, Brazil.

**References**


**Table 1. Differential phenotypic characteristics of strain FeGl01T and phylogenetically closely related Burkholderia species**

<table>
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<tr>
<th>Characteristic</th>
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<td>Carbon source assimilation:</td>
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<td>D-Arabinose</td>
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<td>Dulcitol</td>
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Fatty acid content (%):

| C<sub>17</sub>:0 cyclo       | 18:9 | 3–7 | 6–6 | ND  | 14–1 | 3–9 |
| C<sub>19</sub>:008c cyclo    | 18:8 | ND  | 3–6 | ND  | 9–4  | 2–0 |
| C<sub>18</sub>:107c         | 16:7 | 34–0| 34–2| ND  | 16–5 | 44–9|
| Summed feature 3*           | 1–9 | 23–4| 15–6| ND  | 7–5  | 12–7|

*Summed feature 3 comprises C<sub>16</sub>:107c and/or iso-C<sub>15</sub>:0 2-OH for strain FeGl01T (B. ferrariae sp. nov.). *B. unamae* (Caballero-Mellado et al., 2004) and B. mimosarum (Chen et al., 2006) C<sub>16</sub>:107c for B. sacchari (Brämer et al., 2001) and C<sub>16</sub>:106c and/or C<sub>16</sub>:107c for B. silvatlantica (Perin et al., 2006).


