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

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A Gram-negative, non-spore-forming bacterial strain with the ability to solubilize highly insoluble phosphatic minerals was isolated from a high-phosphorus iron ore from Minas Gerais State, Brazil. This strain, designated FeGIO1T, was subjected to a polyphasic taxonomic investigation. Comparative 16S rRNA gene sequence analysis indicated that it formed a distinct phylogenetic lineage within the genus Burkholderia together with several other species of the genus, e.g. Burkholderia sacchari, Burkholderia tropica and Burkholderia unamai. Partial nucleotide sequencing and analysis of the recA gene roughly corroborated the phylogenetic position of strain FeGIO1T within the genus Burkholderia. The chemotaxonomic properties of strain FeGIO1T, such as ubiquinone Q-8 as the predominant quinone system and C16:0, C18:1ω7c, C18:1ω9c and C16:0 as the major fatty acids, were also consistent with its classification within the genus Burkholderia. DNA–DNA hybridization experiments between strain FeGIO1T and the type strains of B. unamai, B. sacchari and B. tropica yielded reassociation values of 40% or lower, which, together with qualitative and quantitative differences in fatty acid composition and with differences in several phenotypic traits, support the separation of the new isolate from the phylogenetically most closely related species. Therefore, it is suggested that strain FeGIO1T represents a new species of the genus Burkholderia, for which the name Burkholderia ferrearia sp. nov. is proposed. The type strain is FeGIO1T ( = LMG 23612T = CECT 7171T = DSM 18251T ).

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

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Abbreviation: PSM, phospholipid-solubilizing micro-organisms.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of B. ferrearia FeGIO1T is DQ514597 and the accession numbers for the recA gene sequences of B. ferrearia FeGIO1T, B. unamai MT641T and B. tropica Ppe6 are DQ514538-DQ514540, respectively.

An extended neighbour-joining tree showing the phylogenetic position of strain FeGIO1T based on its 16S rRNA gene sequence is available as supplementary material in USEM Online.

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 unamai (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, bio-remediation and plant growth promotion (Coenye & Vandamme, 2003; O’Sullivan & Mahenthiralingam, 2005).
showing, respectively, 16S rRNA gene sequence similarities of 97.6, 97-3 and 97-0%. Two recently described Burkholderia species are also very closely related to strain FeG011. Burkholderia atmospherae SSM88b [20] and Burkholderia mallei PA541 show 16S rRNA gene sequence similarities of 97-5 and 97-6%, respectively, to strain FeG011. The low similarities found between strain FeG011 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 conspuescens D23522-gene recA). 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 discriminations 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 FeG011, we amplified and sequenced this partial recA region for strain FeG011, B. tropica Ppe8 and B. unamum MT-6412 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 FeG011 within the genus Burkholderia obtained by analysis of 16S rRNA gene sequences. Although B. tropica Ppe8 grouped in a clade different from that containing strain FeG011, B. sacchari LMG 19450 and B. unamum MT-6412, a pairwise analysis of the partial recA sequences showed that these three recognized species are the closest relatives to strain FeG011, with similarity values of 94.9% (B. sacchari LMG 19450), 93.5% (B. unamum MT-6412) and 92.0% (B. tropica)
PepT^1"). These rnsA sequence similarity values suggest that strain FeG01^1 may belong to a novel species.

For base composition analysis, DNA was prepared according to the method of Chun & Goeddelove (1995). The G+C content of the DNA was determined using the thermal densitometry method (Maedel & Marmer, 1968). The G+C content of strain FeG01^1 was 62.7 mol%, DNA–DNA hybridization was performed according to the method of Ezaki et al. (1985), following the recommendations of Willems et al. (2001). Mean levels of DNA–DNA relatedness of 40% were found between strain FeG01^1 and both B. ascarii LMC 1945^1 and B. tropica PepT^2, and of 24% between strain FeG01^1 and B. ascarii MT1^1 (1 of four replications). These results indicate that strain FeG01^1 does not belong to any of the recognized species of Bacillus but is based on the recommended minimum 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 FeG01^1 were performed in the DSMZ. As in all other species of the genus Bacillus, ubiquinone Q-8 was detected as the predominant quinone system. The fatty acid profile of strain FeG01^1 consisted of six components (Table 1). Correlation between these species and any of the members of the genus Bacillus, but belongs to a novel species. The fatty acid profile of strain FeG01^1 shows significant differences from those of the phylogenetically closely related species B. ascarii (Bremer et al., 2001), B. unamae (Cabaneros-Nebreda et al., 2004) and B. minimus (Chen et al., 2006). The proportions of C14:0, C16:0 and C16:0 were considerably higher and the proportions of C17:1ω7c and C15:0 were considerably lower in strain FeG01^1 than in these other Bacillus species. In comparison with B. submarinus (Perth et al., 2006), strain FeG01^1 contains relatively high proportions of both C17:1ω7c and C15:0 cycle (Table 2).

Phenotypic traits of strain FeG01^1 were analyzed by using the API 20NE gallery (BioMérieux) as recommended by the manufacturer, and by using the API 32CH gallery (BioMérieux) in combination 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 FeG01^1 can be differentiated from B. ascarii, B. tropica, B. unamae, B. subtilis and B. minimus by its inability to assimilate sorbitol and d-arabinose and from the first four of these species by its ability to assimilate dulcitol.
Table 1. Differential phenotypic characteristics of strain FCg21 and phylogenetically related Burkholderia species.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Growth on MacConkey medium</td>
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<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>D-Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Succinate</td>
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<td>+</td>
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<td>D-Gluconol</td>
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<td>D-Tartrate</td>
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<td>Fatty acid content (%)</td>
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<td>19-7</td>
<td>14-1</td>
<td>3-9</td>
<td>14-1</td>
<td>3-9</td>
</tr>
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<td>16-0</td>
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<td>2-9</td>
<td>16-0</td>
<td>2-9</td>
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<td>13-4</td>
<td>34-2</td>
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<tr>
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<td>2-4</td>
<td>15-4</td>
<td>14-6</td>
<td>7-5</td>
<td>12-7</td>
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</table>

*Stained feature 5 comprises C14 : 0, C16 : 0 and/or C15 : 0, C14 : 0-3, C15 : 0-3 for strain FCg21 (B. fragilis sp. nov. C16 ; 0 cyclo: Cc, 0.02 for B. sacchari (Brinkman et al., 2001) and Cc, 0.02 cyclo: C16 : 0-3 for B. sacchari (Peri, et al. 2008)."

and t-tagatose. Other differences in the assimilation of carbon sources are given in Table 1.

Strain FCg21 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.ri.ae. L. gen. n. ferrariae of an iron mine).

Cells are Gram-negative, non-spore-forming rods. Catalase- and oxidase-positive. Colonies on YEP-D 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, urea, arginine dihydrolase or gelatinase; it does not hydrolyse casein. The following substrates are assimilated: carbon sources in the API 20NE and API 50CH systems: glycerol, D-arabinose, ribose, D-xylose, adonitol, galactose, D-ribose, D-tartrate, L-mannose, dulcitol, inositol, mannitol, N-acetylglucosamine, cellobiose, trehalose, D-tagatose, D-lactose, D-fructose, gluconate, D-ketogluconate, malate, citrate, caprate, adipate and phylloleucinate. It does not use erythritol, D-arabinose, D-xylose, methyl β-D-xylidose, D-sorbose, ribitol, methyl D-arabinitol, methyl D-z-galactoside, amygdalin, wheat salicin, maltose, lactose, melibiose, sucrose, insulin, melibiose, D-raffinose, starch, glycerol, erythritol, β-galactoside, D-tartarate, D-fructose, L-rhamnose, L-arabinose or 3-kestogluconate as carbon sources. The Q-10 C content is 62.7 mm.

The type strain, FCg21 (=LNC 2962 =CECT 7117 =DMSEC 14257), was isolated from ore material from the jaguari mine, Minas Gerais State, Brazil.

Acknowledgments

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