Burkholderia ferrariae 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-phosphorous iron ore from Minas Gerais State, Brazil. This strain, designated FeGI01^T, 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 unamae*. Partial nucleotide sequencing and analysis of the *recA* gene roughly corroborated the phylogenetic position of strain FeGI01^T within the genus *Burkholderia*. The chemotaxonomic properties of strain FeGI01^T, such as ubiquinone Q-8 as the predominant quinone system and C_{16:0}, C_{17:0} cyclo, C_{18:1ω7c} and C_{19:0ω8c} cyclo as the major fatty acids, were also consistent with its classification within the genus *Burkholderia*. DNA–DNA hybridization experiments between strain FeGI01^T and the type strains of *B. unamae*, *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 FeGI01^T represents a novel species of the genus *Burkholderia*, for which the name *Burkholderia ferrariae* sp. nov. is proposed. The type strain is FeGI01^T (=LMG 23612^T=CECT 7171^T=DSM 18251^T).

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 (Coenye & Vandamme, 2003; O'Sullivan & Mahenthiralingam, 2005).

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Abbreviation: PSM, phosphate-solubilizing micro-organism.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *B. ferrariae* FeGI01^T is DQ514537 and the accession numbers for the *recA* gene sequences of *B. ferrariae* FeGI01^T, *B. unamae* MTI-641^T and *B. tropica* Ppe8^T are DQ514538–DQ514540, respectively.

An extended neighbour-joining tree showing the phylogenetic position of strain FeGI01^T based on its 16S rRNA gene sequence is available as supplementary material in IJSEM Online.

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 environment-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 FeGl01^T, that, based on its genotypic and phenotypic characterization, should be classified within a novel species of the genus *Burkholderia*.

Strain FeGl01^T 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 FeGl01^T 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 FeGl01^T 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 FeGl01^T based on its 16S rRNA gene sequence is presented in Fig. 1 (an extended tree is shown in Supplementary Fig. S1 available in IJSEM Online). The results of the phylogenetic analysis indicate that strain FeGl01^T is related to members of the genus *Burkholderia*. The closest relatives to strain FeGl01^T among recognized species of the genus *Burkholderia* are *B. sacchari* LMG 19450^T, *B. tropica* Ppe8^T and *B. unamae* MTI-641^T

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relatedness among strain FeGl01^T and its closest relatives. The phylogenetic tree was rooted with *B. andropogonis* LMG 2129^T as the outgroup sequence. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 1 substitution per 100 nt. An extended tree is available as Supplementary Fig. S1 in IJSEM Online.

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 FeGl01^T: *Burkholderia silvatlantica* SRMrh-20^T and *Burkholderia mimosarum* PAS44^T show 16S rRNA gene sequence similarities of 97.4 and 97.6 %, respectively, to strain FeGl01^T. The low similarities found between strain FeGl01^T 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 FeGl01^T, we amplified and sequenced this partial *recA* region for strain FeGl01^T, *B. tropica* Ppe8^T and *B. unamae* MTI-641^T 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 FeGl01^T within the genus *Burkholderia* obtained by analysis of 16S rRNA gene sequences. Although *B. tropica* Ppe8^T grouped in a cluster different from that containing strain FeGl01^T, *B. sacchari* LMG 19450^T and *B. unamae* MTI-641^T, a pairwise analysis of the partial *recA* sequences showed that these three recognized species are the closest relatives to strain FeGl01^T, with similarity values of 94.9 % (*B. sacchari* LMG 19450^T), 93.5 % (*B. unamae* MTI-641^T) and 92.0 % (*B. tropica*

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* MC58 *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.

Ppe8^T). These *recA* sequence similarity values suggest that strain FeGI01^T 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 FeGI01^T 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 FeGI01^T and both *B. sacchari* LMG 19450^T and *B. tropica* Ppe8^T, and of 24 % between strain FeGI01^T and *B. unamae* MTI-641^T (mean of four replications). These results indicate that strain FeGI01^T 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 FeGI01^T 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 FeGI01^T consisted of (only components comprising >1 % of the total are given): C_{14:0} (4.9 %), C_{16:0} (18.0 %), C_{17:0} cyclo (18.9 %), C_{16:1} 2-OH (1.5 %), C_{16:0} 2-OH (5.0 %), C_{16:0} 3-OH (3.4 %), C_{18:1} ω7c (16.7 %), C_{19:0} ω8c cyclo (18.8 %), C_{18:1} 2-OH (1.5 %) and summed features 2 (6.0 %) and 3 (1.9 %). Summed

feature 2 corresponds to C_{14:0} 3-OH, iso-C_{16:1} I, an unknown fatty acid with equivalent chain length of 10.928, C_{12:0} ALDE or any combination of these fatty acids, and summed feature 3 corresponds to C_{16:1} ω7c and/or iso-C_{15:0} 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 FeGI01^T shows significant differences from those of the phylogenetically most closely related species, *B. sacchari* (Brämer *et al.*, 2001), *B. unamae* (Caballero-Mellado *et al.*, 2004) and *B. mimosarum* (Chen *et al.*, 2006); the proportions of C_{17:0} cyclo and C_{19:0} ω8c cyclo are considerably higher and the proportions of C_{18:1} ω7c and summed feature 3 are considerably lower in strain FeGI01^T than in these other three *Burkholderia* species. In comparison with *B. silvatlantica* (Perin *et al.*, 2006), strain FeGI01^T contains relatively high proportions of both C_{19:0} ω8c cyclo and C_{17:0} cyclo (Table 1).

Phenotypic traits of strain FeGI01^T 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 FeGI01^T 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

Table 1. Differential phenotypic characteristics of strain FeGl01^T and phylogenetically closely related *Burkholderia* species

Taxa: 1, strain FeGl01^T; 2, *B. sacchari* (data taken from Brämer *et al.*, 2001); 3, *B. unamae* (Caballero-Mellado *et al.*, 2004); 4, *B. tropica* (Reis *et al.*, 2004); 5, *B. silvatlantica* (Perin *et al.*, 2006); 6, *B. mimosarum* (Chen *et al.*, 2006). +, Good growth; ±, poor growth; –, no growth; V, variable; ND, no data available.

Characteristic	1	2	3	4	5	6
Growth on MacConkey medium at 29 °C	–	–	±	+	–	ND
Carbon source assimilation:						
D-Arabinose	–	+	+	+	+	+
Sorbitol	–	+	+	+	+	+
Dulcitol	+	–	–	–	–	ND
D-Tagatose	+	–	–	–	–	ND
Ribose	+	–	–	+	+	ND
Rhamnose	–	–	+	+	+	–
Salicin	–	–	–	V	–	ND
Cellobiose	+	–	+	+	+	–
Lactose	–	–	–	V	–	–
Sucrose	–	+	–	–	+	–
Trehalose	+	–	+	V	–	–
D-Raffinose	–	+	–	–	–	–
β-Gentiobiose	–	–	–	+	–	–
D-Lyxose	–	+	–	V	–	ND
D-Fucose	–	+	–	+	–	+
Fatty acid content (%):						
C _{17:0} cyclo	18.9	3.7	6.6	ND	14.1	3.9
C _{19:0} ω8c cyclo	18.8	ND	3.6	ND	9.4	2.0
C _{18:1} ω7c	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_{16:1}ω7c and/or iso-C_{15:0} 2-OH for strain FeGl01^T (*B. ferrariae* sp. nov.), *B. unamae* (Caballero-Mellado *et al.*, 2004) and *B. mimosarum* (Chen *et al.*, 2006), C_{16:1}ω7c for *B. sacchari* (Brämer *et al.*, 2001) and C_{16:1}ω6c and/or C_{16:1}ω7c for *B. silvatlantica* (Perin *et al.*, 2006).

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

Strain FeGl01^T 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.æ. 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-xylose, adonitol, galactose, D-glucose, D-fructose, D-mannose, dulcitol, inositol, mannitol, N-acetylglucosamine, cellobiose, trehalose, D-tagatose, L-fucose, D-arabitol, gluconate, 2-ketogluconate, malate, citrate, caprate, adipate and phenylacetate. It does not use erythritol, D-arabinose, L-xylose, methyl β-xyloside, L-sorbose, rhamnose, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, salicin, maltose, lactose, melibiose, sucrose, inulin, melezitose, D-raffinose, 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, FeGl01^T (=LMG 23612^T=CECT 7171^T=DSM 18251^T), was isolated from ore material from the Jangada mine, Minas Gerais State, Brazil.

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