IJSEM 64498 (revised version 2)

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

Angel Valverde†[1], Pedro Delvasto[2], Alvaro Peix[1], Encarna Velázquez[3], Ignacio Santa-Regina[1], Antonio Ballester[2], Claudino Rodríguez-Barrueco[1], Camino García-Balboa[4] and José M. Igual[1*]

1: Instituto de Recursos Naturales y Agrobiología de Salamanca. Consejo Superior de Investigaciones Científicas. Salamanca. Spain.
2: Biohydrometallurgy Research Group, Department of Materials Science and Metallurgical Engineering. Universidad Complutense de Madrid. 28040 Madrid. Spain.
3: Departamento de Microbiología y Genética, Universidad de Salamanca. 37007 Salamanca. Spain.
4: Department of Industrial Technology, Universidad Alfonso X "El Sabio". Av. de la Universidad 1. 28691 Villanueva de la Cañada. Madrid. Spain.
† Present address: Department of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel.

[*]**Corresponding author:** José M. Igual. Instituto de Recursos Naturales y Agrobiología de Salamanca. CSIC. Cordel de Merinas, 40-52. Apdo. 257. 37071 Salamanca. Spain. Phone: +34 923 219606. Fax: +34 923 219609. E-mail: igual@usal.es

Running title: *Burkholderia ferrariae* sp. nov.

Subject Category: New Taxa (*Proteobacteria*)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *B. ferrariae* FeGl01^T is DQ514537 and the accession numbers for the *recA* gene sequences of *B. ferrariae* FeGl01^T, *B. unamae* MTI-641^T and *B. tropica* Ppe8^T are DQ514538, DQ514539 and DQ514540, respectively.

Summary

A Gram-negative, non-spore-forming bacterial strain with the ability to solubilize hardly soluble phosphatic minerals was isolated from a high-phosphorous iron ore located in the Brazilian region of Minas Gerais. It was designated as strain FeGl01^T and subjected to taxonomic investigation. In a comparison of 16S rDNA sequences, strain FeGl01^T is included in a distinct phylogenetic lineage within the genus *Burkholderia* together with several other species of this genus such as *B. sacchari*, *B. tropica* and *B. unamae*. Partial nucleotide sequencing and analysis of the *recA* gene roughly corroborated the phylogenetic position of strain FeGl01^T within the *Burkholderia* genus. Chemotaxonomic properties of the strain FeGl01^T, such as the ubiquinone Q-8 as the predominant quinone system and $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}\omega 7c$, $C_{19:0}\omega 8c$ cyclo as the major fatty acids, were also consistent with classification in the genus *Burkholderia*. DNA–DNA hybridization experiments between strain FeGl01^T and the type strains of *B. unamae*, *B. sacchari* and *B. tropica* yielded reassociation value of 40% or lower that, together with qualitative and quantitave differences in fatty acid composition and with several differences in phenotipic traits, support the separation of the new isolate from closely related species. Therefore, it is proposed that strain FeGl01^T (=LMG 23612^T =CECT 7171^T) be recognized as a novel species, for which the name *Burkholderia ferrariae* sp. nov. is proposed.

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 currently includes more than 40 species. Members of the genus *Burkholderia* are present at many different ecological niches, the predominant being the soil and the rhizosphere, from which some of the more recently described species have been isolated, such as *B. sacchari* (Brämer *et al.*, 2001), *B. tropica* (Reis *et al.*, 2004) and *B. unamae* (Caballero-Mellado *et al.*, 2004).

Functionally, *Burkholderia* is a remarkably diverse genus that include plant symbiotic, and both plant and animal pathogenic species. Some of its species are also known as opportunistic pathogens in humans. Certain burkholderias have proved to be very efficient in biocontrol, bioremediation and plant growth promotion (Coenye & Vandamme, 2003; O'Sullivan & Mahenthiralingam, 2005).

Many *Burkholderia* strains have, among other properties, the ability to solubilize hardly soluble phosphatic minerals and, therefore, are of significant interest to the agriculture sector due to its applicability in biofertilization (Artursson *et al*., 2006; Igual *et al*., 2001; Peix *et al*., 2001; Purnomo *et al*., 2005). Moreover, such a feature could also be economically useful for emerging industries like biomining. Many of the current world iron ore resources contain clearly over 0.08 % (w/w) of P, making this material out-of-standard for the manufacture of metallic iron and steel (Cheng *et al*., 1999). Although there exist chemical processes to reduce the P content of iron ores, the historical low prices of this raw material make all this attempts unworthy. In this context, biotechnology can play a role in overcoming this problem in a cost effective and environmental friendly way. In the course of isolating phosphate-solubilizing microorganisms from a high-phosphorous iron ore located in the Brazilian region of Minas Gerais, we isolated a bacterial strain, termed FeGl01^T, that, based on their

genotypic and phenotypic characterization, should be classified as a novel species of the genus *Burkholderia*, for which we propose the name *Burkholderia ferrariae* sp. nov.

Strain FeGl01^T was isolated from a suspension of the ore material in sterile distilled water maintained in 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 detection of phosphate-solubilizing microorganisms (PSM), which contains glucose as carbon source and insoluble tricalcium phosphate as the sole source of phosphorus, allowing the detection of PSM by the formation of halos around their colonies. The cultures used in further studies were purified from a single colony after 12 days of incubation at 30 °C on NBRIP medium, and subsequently cultivated on YED-P agar plates. On YED-P, colonies of strain FeGl01^T are cream-coloured, round, smooth and convex with approximate diameters of 1-3 mm.

Genomic DNA was extracted as described by Rivas *et al*. (2001). The 16S rRNA gene of the strain FeGl01^T was analysed as described by Rivas *et al.* (2002). The sequence obtained was compared with those from the GenBank using the FASTA program (Pearson & Lipman, 1988). Sequences were aligned using the Clustal X software (Thompson *et al*., 1997). The 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). Bootstrap analysis was based on 1000 resamplings. The MEGA2.1 package (Kumar *et al*., 2001) was used for all analyses. The resulting neighbour joining tree corresponding to 16S rRNA gene sequences is shown in Fig. 1 (a extended tree is available at http://ijs.sgmjournals.org data in IJSEM online). The results of the phylogenetic analysis indicate that strain FeGl01^T is related to members of the genus *Burkholderia*. According to the 16S rRNA gene sequences, the closest relatives to strain FeGl01^T (accession number DQ514537) among the described

101 species of *Burkholderia* are *B. sacchari* LMG 19450^T, *B. tropica* Ppe8^T and *B. unamae*
102 MTI-641^T showing, respectively, a similarity of 97.6%, 97.3% and 97.0%. There exist
103 two novel *Burkholderia* strains, unavailable at the time of writing the present article but
104 included in forthcoming descriptions of novel taxa in the IJSEM, which are also very
105 closely related with strain FeGl01^T. They are *B. silvatlantica* SRMrh-20^T and *B.*
106 *mimosarum* PAS44^T, which show percentages of 16S rRNA gene sequence similarities
107 with respect to the strain FeGl01^T of 97.4% and 97.6%, respectively. The low
108 similarities found between strain FeGl01^T and their closest relatives suggest that it
109 belong to a new species of genus *Burkholderia.*

110 According to the results of Payne *et al*. (2005), *Burkholderia* species can be
111 discriminated by analysis of an internal 385-bp sequence of the *recA* gene (spanning
112 bases 76 to 461 relative to the *B. cenocepacia* J2315 genome *recA* gene). Moreover, in
113 their work, these authors report that analysis of this partial *recA* sequence, obtained with
114 the *Burkholderia* specific primers BUR3 and BUR4, produced phylogenetic trees with
115 the same topology and discrimination as the trees derived from analysis of nearly full-
116 length *recA* sequences. Although the *recA* analysis does not exactly match the
117 phylogeny obtained with 16S rRNA gene sequences, it provides a greater degree of
118 resolution among closely related species within the *Burkholderia* genus (Payne *et al*.,
119 2005). Thus, to confirm the phylogenetic position of strain FeGl01^T, we amplified and
120 sequenced this partial *recA* region of strain FeGl01^T (accession number DQ514538), *B.*
121 *tropica* Ppe8^T (accession number DQ514540) and *B. unamae* MTI-641^T (accession
122 number DQ514539) as described by Payne *et al*. (2005), which were compared with
123 those from the GenBank and analysed as described above for the 16S rRNA gene. A
124 phylogenetic tree constructed with these partial *recA* sequences from *Burkholderia*
125 species is shown in Fig. 2. The results roughly confirm the phylogenetic position of

126 strain FeGl01^T within the genus *Burkholderia* obtained by analysis of 16S rRNA gene
127 sequences. Although *B. tropica* Ppe8^T grouped in a cluster different from that in which
128 strain FeGl01^T, *B. sacchari* LMG 19450^T and *B. unamae* MTI-641^T clustered, a
129 pairwise analysis of the partial *recA* sequences showed that these three species are the
130 closest relatives to strain FeGl01^T, with similarity values of 94.9% (*B. sacchari* LMG
131 19450^T), 93.5% (*B. unamae* MTI-641^T) and 92.0% (*B. tropica* Ppe8^T). These *recA*
132 sequence similarity values with respect to its closely related recognized species suggest
133 that the FeGl01^T strain may belong to a new species.

134 For base composition analysis, DNA was prepared according to Chun &
135 Goodfellow (1995). The mol% G+C content of DNA was determined using the thermal
136 denaturation method (Mandel & Marmur, 1968). The G+C content of the strain FeGl01^T
137 was 62.7 mol%. DNA-DNA hybridization was performed by using the method of Ezaki
138 *et al*. (1989), following the recommendations of Willems *et al*. (2001). The results of
139 DNA-DNA hybridization showed mean values of similarity of 40% between strain
140 FeGl01^T and both *B. sacchari* LMG 19450^T and *B. tropica* Ppe8^T, and of 24% between
141 strain FeGl01^T and *B. unamae* MTI-641^T (mean of four replications). These results
142 indicate that strain FeGl01^T does not belong to any of the known species of
143 *Burkholderia* when the recommendation of a threshold value of 70% DNA-DNA
144 relatedness for definition of species is considered (Wayne *et al.*, 1987).

145 The analysis of quinones and cellular fatty acid profile of strain FeGl01^T were
146 performed at the DSMZ. As in all other species of the genus *Burkholderia*, ubiquinone
147 Q-8 was detected as the predominant quinone system in strain FeGl01^T. Its fatty acid
148 profile consisted of (only values higher than 1% are given): 14:0 (4.9%), 16:0 (18.0%),
149 17:0 cyclo (18.9%), 16:1 2-OH (1.5%), 16:0 2-OH (5.0%), 16:0 3-OH (3.4%), 18:1ω7*c*
150 (16.7%), 19:0ω8*c* cyclo (18.8%), 18:1 2-OH (1.5%) and summed features 2 (6.0%) and

3 (1.9%). Summed feature 2 could correspond to 14:0 3-OH, iso-16:1 I, an unknown fatty acid with equivalent chain-length of 10.928, 12:0 ALDE or any combination of these fatty acids; and summed feature 3 could correspond to 16:1ω7*c* or iso-15:0 2-OH or both, similar to those fatty acids 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 FeGl01^T shows significant differences with respect to those of its phylogenetically close 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 $C_{19:0}\omega 8c$ cyclo, and of $C_{18:1}\omega 7c$ and summed feature 3 in strain FeGl01^T are, respectively, considerably higher and lower than in these other three *Burkolderia* species. In comparison to *B. silvatlantica* (Perin *et al*., 2006), strain FeGl01^T contains relatively high amounts of both $C_{19:0}\omega 8c$ cyclo and $C_{17:0}$ cyclo (Table 1).

Phenotypic traits of strain FeGl01^T were analysed with API 20NE (bioMérieux) as recommended by the manufacturer, and with 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. *Burkholderia ferrariae* FeGl01^T can be differentiated from its closely related species *B. sacchari*, *B. tropica, B. unamae*, *B. silvatlantica* and *B. mimosarum* by its inability to assimilate sorbitol and D-arabinose, and from the four first species by its ability to assimilate dulcitol and D-tagatose. Other differences in the assimilation of carbon sources are shown in Table 1.

Therefore, the strain FeGl01^T can be genotypically and phenotypically differentiated from previously described species and we therefore propose to name it as *Burkholderia ferrariae* sp. nov.

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-sporulated rods. Catalase and oxidase positive. Colonies of strain FeGl01^T on YED-P medium are cream-coloured, round, smooth and convex with approximate diameters of 1-3 mm. Nitrate is reduced to nitrite. In API 20NE system, strain FeGl01^T produces β-galactosidase but does not produce indol, urease, arginine dehydrolase or gelatinase; it does not hydrolyse esculin. The following substrates are assimilated as carbon sources in 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-glucosido, amygdalin, arbutin, salicin, maltose, lactose, melibiose, sucrose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-fucose, L-arabitol and 5-ketogluconate as carbon source. The G+C content of the strain FeGl01^T is 62.7 mol%. The type strain FeGl01^T (=LMG 23612^T =CECT 7171^T) was isolated from ore material from the Jangada mine, State of Minas Gerais, Brazil.

Acknowledgements

The authors wish to thank Dr. Jesús Caballero-Mellado (UNAM; Cuernavaca, México) for kindly providing the type strains of *B. unamae* and *B. tropica*. Angel Valverde is indebted to the Ministry of Education and Science of Spain for a postdoctoral fellowship at The Hebrew University of Jerusalem. Pedro Delvasto acknowledges the

201 financial support, in the form of Doctoral Scholarship, of the Venezuelan Foundation
202 for Science, Technology and Innovation (FONACIT) and the Simón Bolívar University
203 of Venezuela.
204

204 **References**

205

206 **Artursson, V., Finlay, R. D. & Jansson, J. K. (2006).** Interactions between arbuscular
207 mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environ*
208 *Microbiol* **8**, 1-10.

209

210 **Brämer, C. O., Vandamme, P., da Silva, L. F., Gomez, J. G. C. & Steinbüchel, A.**
211 **(2001).** *Burkholderia sacchari* sp. nov., a polyhydroxyalkanoate-accumulating
212 bacterium isolated from soil of a sugar-cane plantation in Brazil. *Int J Syst Evol*
213 *Microbiol* **51,** 1709-1713.

214

215 **Caballero-Mellado, J., Martínez-Aguilar, L., Paredes-Valdez, G. & Estrada-de los**
216 **Santos, P. (2004).** *Burkholderia unamae* sp. nov., an N_2-fixing rhizospheric and
217 endophytic species. *Int J Syst Evol Microbiol* **54,** 1165-1172.

218

219 **Chen, W. -M., James, E. K., Coenye, T., Chou, J. -H., Barrios, E., de Faria, S. M.,**
220 **Elliott, G. N., Sheu, S. -Y., Sprent, J. I. & Vandamme, P. (2006).** *Burkholderia*
221 *mimosarum* sp. nov., isolated from root nodules of *Mimosa* spp. from Taiwan and South
222 America. *Int J Syst Evol Microbiol* (in press).

223

224 **Cheng, C., Misra, V., Clough, J. & Mun, R. (1999).** Dephosphorisation of western
225 Australian iron ore by hydrometallurgical process. *Miner Eng* **12,** 1083-1092.

226

227 **Chun, J. & Goodfellow, M. (1995).** A phylogenetic analysis of the genus *Nocardia*
228 with 16S rRNA sequences. *Int J Syst Bacteriol* **45,** 240-245.

Coenye, T., Mahenthiralingam, E., Henry, D., LiPuma, J. J., Laevens, S., Gillis, M., Speert, D. P. & Vandamme, P. (2001). *Burkholderia ambifaria* sp.nov. a novel member of the *Burkholderia cepacia* complex including biocontrol and cystic fibrosis-related isolates. *Int J Syst Evol Microbiol* **51,** 1481-1490.

Coenye, T. & Vandamme, P. (2003). Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environ Microbiol* **5,** 719-729.

Ezaki, T., Hashimoto, Y. & Yabuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224-229.

Igual, J. M., Valverde, A., Cervantes, E. & Velázquez, E. (2001). Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. *Agronomie* **21,** 561-568.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16,** 111-120.

Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). Molecular Evolutionary Genetics Analysis software. Arizona State University. Tempe, Arizona. USA.

Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B,** 195-206.

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol Lett* **170,** 265-270.

O'Sullivan, L. A. & Mahenthiralingam, E. (2005). Biotechnological potential within the genus *Burkholderia*. *Lett Appl Microbiol* **41,** 8-11.

Payne, G. W., Vandamme, P., Morgan, S. H., LiPuma, J. J., Coenye, T., Weightman, A. J., Jones, T. H. & Mahenthiralingam, E. (2005). Development of a *recA* gene-based identification approach for the entire *Burkholderia* genus. *Appl Environ Microbiol* **71,** 3917-3927.

Pearson, W. R. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci USA* **85,** 2444-2448.

Peix, A., Mateos, P. F., Rodríguez-Barrueco, C., Martínez-Molina, E. & Velázquez, E. (2001). Growth promotion of common bean (*Phaseolus vulgaris* L.) by a strain of *Burkholderia cepacia* under growth chamber conditions. *Soil Biol Biochem* **33**, 1927-1935.

Perin, L., Martínez-Aguilar, L., Paredes-Valdez, G., Baldani, J. I., Estrada- de los Santos, P. & Caballero-Mellado, J. (2006). *Burkholderia silvatlantica* sp. nov., a

novel diazotrophic bacterium associated with sugarcane and maize. *Int J Syst Evol Microbiol* (in press).

Purnomo, E., Mursyid, A., Syarwani, M., Jumberi, A., Hashidoko, Y., Hasegawa, T., Honma, S. & Osaki, M. (2005). Phosphorus solubilizing microorganisms in the rhizosphere of local rice varieties grown without fertilizer on acid sulfate soils. *Soil Sci Plant Nutr* **51**, 679-681.

Reis, V. M., Estrada-de los Santos, P., Tenorio-Salgado, S., Vogel, J., Stoffels, M., Guyon, S., Mavingui, P., Baldani, V. L. D., Schmid, M., Baldani, J. I., Balandreau, J., Hartmann, A. & Caballero-Mellado, J. (2004). *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant-associated bacterium. *Int J Syst Evol Microbiol* **54,** 2155-2162.

Rivas, R., Velázquez, E., Valverde, A., Mateos, P.F. & Martínez-Molina, E. (2001). A two primers random amplified polymorphic DNA procedure to obtain polymerase chain reaction fingerprints of bacterial species. *Electrophoresis* **22,** 1086-1089.

Rivas, R., Velázquez, E., Willems, A., Vizcaíno, N., Subba-Rao, N. S., Mateos, P. F., Gillis, M., Dazzo, F. B. & Martínez-Molina, E. (2002). A new species of *Devosia* that forms a unique nitrogen-fixing root-nodule symbiosis with the aquatic legume *Neptunia natans* (L. f.) Druce. *Appl Environ Microbiol* **68,** 5217-5222.

Saitou, N. & Nei, M. (1987). A neighbour-joining method: a new method for reconstructing phylogenetics trees. *Mol Biol Evol* **44,** 406-425.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The clustalX windows interface: flexible strategies for multiple sequence alignement aided by quality analysis tools. *Nucleic Acid Res* **24,** 4876-4882.

Vandamme, P., Holmes, B., Vancanneyt, M. & 8 other authors (1997). Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. *Int J Syst Bacteriol* **47**, 1188-1200.

Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E., Starr, M.P. & Trüper, H.G. (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37,** 463-464.

Willems, A., Doignon-Bourcier, F., Goris, J., Coopman, R., De Lajudie, P. & Gillis, M. (2001). DNA-DNA hybridization study of *Bradyrhizobium* strains. *Int J Syst Evol Microbiol* **51,** 1315-1322.

Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T. & Arakawa, M. (1992). Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiol Immunol* **36,** 1251-1275.

Table 1. Differential phenotypic characteristics of *B. ferrariae* and phylogenetically closely related *Burkholderia* species.

Species: 1, *B. ferrariae*; 2, *B. sacchari* ; 3, *B. unamae*; 4, *B. tropica*; 5, *B. silvatlantica*; 6, *B. mimosarum*. +, Good growth; ±, poor growth; -, no growth; V, variable; ND, no data available.

Characteristic	**1**	**2**[*a]	**3**[b]	**4**[c]	**5**[d]	**6**[e]
Growth on:						
MacConkey medium at 29 ºC	-	-	±	+	-	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
$C_{19:0}\omega 8c$ cyclo	18.8	ND	3.6	ND	9.4	2.0
$C_{18:1}\omega 7c$	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

*Data taken from: a, Brämer *et al*. (2001); b, Caballero-Mellado *et al.* (2004); c, Reis *et al.* (2004); d, Perin *et al*. (2006); e, Chen *et al*. (2006).

†Summed feature 3 comprises 16:1ω7*c* or iso-15:0 2-OH or both for *B. ferrariae*, *B. unamae* (Caballero-Mellado *et al.*, 2004) and *B. mimosarum* (Chen *et al*., 2006); 16:1ω7*c* for *B. sacchari* (Brämer *et al*., 2001); and comprises 16:1ω6*c* or 16:1ω7*c* or both for *B. silvatlantica* (Perin *et al*., 2006).

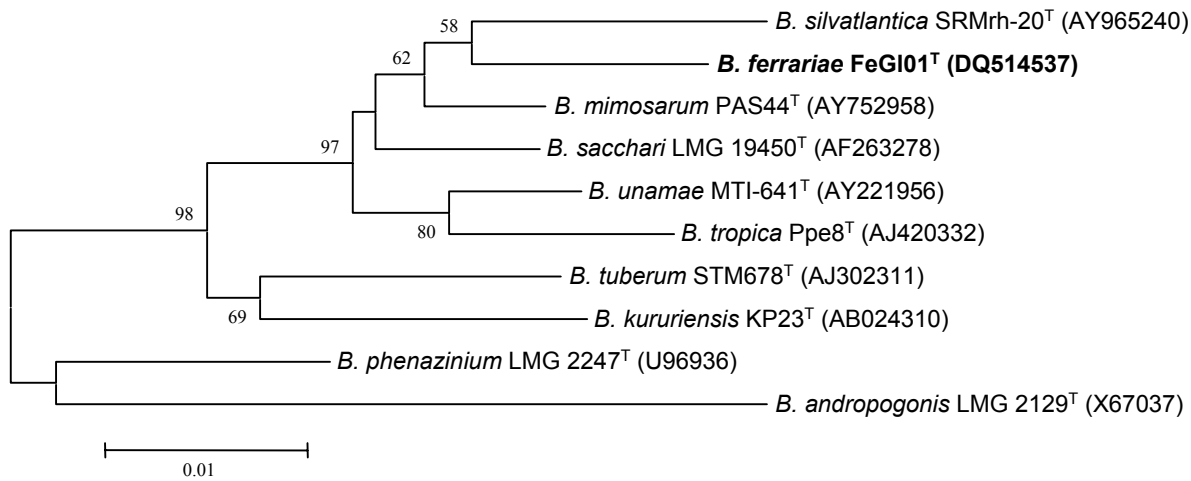

Figure 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relatedness among *Burkholderia ferrariae* sp. nov. and the nearest *Burkholderia* species. The phylogenetic tree was rooted with *B. andropogonis* LMG 2129^T. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 1 nt substitutions per 100 nt.

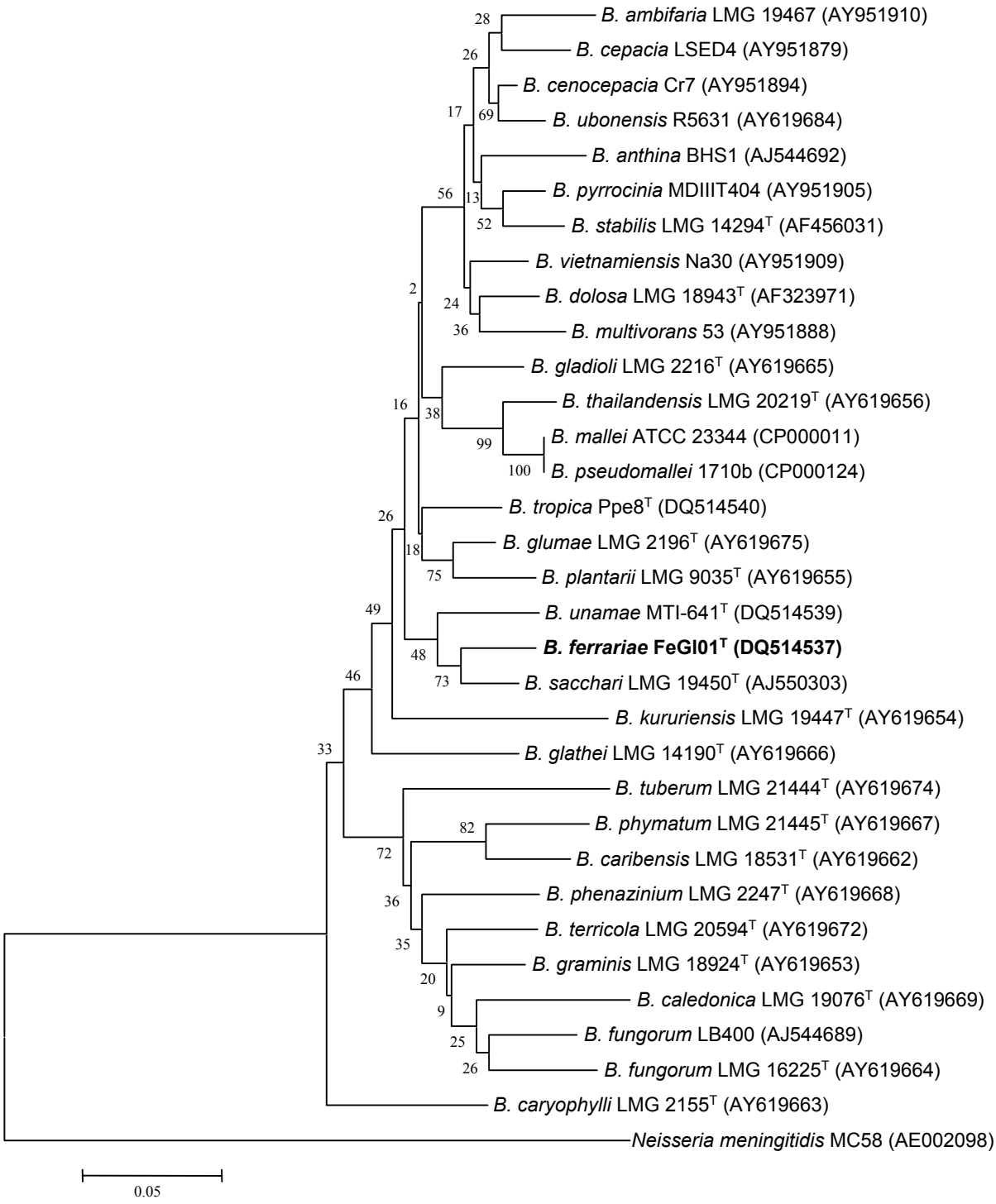

Figure 2. Neighbour-joining tree based on partial *Burkholderia recA* sequences. The phylogenetic tree was rooted using *Neisseria meningitidis* MC58 *recA* gene as the outgroup sequence. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 1 nt substitutions per 100 nt.

Figure S1 (for supplementary data system). Neighbour-joining tree based on nearly complete 16S rRNA gene sequences of *Burkholderia ferrariae* sp. nov. and other *Burkholderia* species. The phylogenetic tree was rooted using *Neisseria meningitidis* MC58 as outgroup. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 1 nt substitutions per 100 nt.