Pseudogulbenkiania subflava gen. nov., sp. nov., isolated from a cold spring

Mei-Chun Lin,¹ Jui-Hsing Chou,² A. B. Arun,² Chiu-Chung Young² and Wen-Ming Chen¹

Correspondence

Wen-Ming Chen

p62365@ms28.hinet.net

¹Laboratory of Microbiology, Department of Seafood Science, National Kaohsiung Marine University, No. 142 Hai-Chuan Road, Nan-Tzu, Kaohsiung City 811, Taiwan, ROC

²Department of Soil Environmental Science, College of Agriculture and Natural Resources, National Chung Hsing University, Taichung, Taiwan, ROC

A yellow-coloured bacterial strain, designated BP-5^T, was isolated from a water sample of a cold spring located in the Hsinchu area of northern Taiwan. Cells of the strain were Gram-negative, non-spore-forming rods with a polar flagellum. The isolate was able to grow at 15–42 °C, 0–1 % NaCl and pH 6–8. The predominant fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and C_{18:1}ω7c. The major respiratory quinone was ubiquinone 8. The DNA G + C content was 63.2 mol%. 16S rRNA gene sequence analysis indicated that strain BP-5^T belonged to the class *Betaproteobacteria* and was a member of the family *Neisseriaceae*. Its closest phylogenetic neighbours were *Gulbenkiania mobilis* E4FC31^T (95.0 % 16S rRNA gene sequence similarity), *Chromobacterium subtsugae* PRAA4-1^T (95.0 %), *Chromobacterium violaceum* ATCC 12472^T (94.3 %), *Vogesella indigofera* ATCC 19706^T (93.8 %) and *Aquitalea magnusonii* TRO-001DR8^T (93.7 %). On the basis of the evidence from this polyphasic study, strain BP-5^T is considered to represent a novel species of a new genus, for which the name *Pseudogulbenkiania subflava* gen. nov., sp. nov. is proposed. The type strain of *Pseudogulbenkiania subflava* is BP-5^T (=BCRC 17727^T =LMG 24211^T).

During the characterization of micro-organisms in water samples collected from a cold spring located in Hsinchu County, Taiwan, a pale-yellow-coloured bacterial strain, designated BP-5^T, was isolated on nutrient agar (BD Difco) and maintained on R2A agar (BD Difco) plates after incubation at 25 °C for 3 days. Subcultivation was performed on R2A agar at 25 °C for between 48 and 72 h. On this medium, strain BP-5^T was able to grow at 15–42 °C but not at 10 or 45 °C.

Cells were observed by phase-contrast microscopy (DM 2000; Leica) in lag, exponential and stationary phases of growth. Motility was tested by the hanging-drop method. A Spot Test flagella stain kit (BD Difco) was used for flagella staining. A Gram stain set S kit (BD Difco) and the Ryu non-staining KOH method (Powers, 1995) were used to assess the Gram staining reaction. Accumulation of poly-β-hydroxybutyrate granules was observed under light microscopy after staining the cells with Sudan black. Colony morphology was observed on R2A agar by using a stereoscopic microscope (SMZ 800; Nikon). Details of cell morphology are given in the species description.

The pH range for growth was determined by measuring the optical densities (wavelength 600 nm) of the culture grown on nutrient broth (BD Difco), which was adjusted, prior to sterilization, to various pH values (pH 4–10 at intervals of 1.0 pH units) by using appropriate biological buffers (Chung *et al.*, 1995). To investigate the tolerance to NaCl, nutrient broth was prepared according to the formula of the BD Difco medium, while NaCl concentrations were varied according to requirements (0, 0.5, 1.0 and 2 %, w/v). Anaerobic growth was checked by using an Oxoid AnaeroGen system (Miller *et al.*, 1995). Strain BP-5^T was examined for a broad range of phenotypic properties. The presence of catalase, oxidase, DNase, urease and lipase and hydrolysis of starch, casein and Tweens 20, 40, 60 and 80 were determined by using standard methods (Gerhardt *et al.*, 1994; Lányi, 1987; MacFaddin, 2000). Phenotypic characteristics, biochemical tests, carbon source utilization (Biolog GN2) and API ZYM and API 20NE (both bioMérieux) reaction profiles were investigated.

The 16S rRNA gene sequence was analysed as described by Chen *et al.* (2001). Analysis of the sequence data was performed by using the software package BioEdit (Hall, 1999) and MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignments of the data by CLUSTAL_X (Thompson *et al.*, 1997). A distance matrix method (distance options

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BP-5^T is EF626692.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain BP-5^T and some members of the class *Betaproteobacteria*. Bootstrap values based on 1000 replications are listed as percentages at branch points. Asterisks indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.01 nucleotide substitutions per nucleotide position.

according to Kimura's two-parameter model; Kimura, 1983), including clustering by the neighbour-joining (Saitou & Nei, 1987) (Fig. 1), maximum-likelihood and discrete character-based maximum-parsimony (Kluge & Farris, 1969) methods, was used. In each case, bootstrap values were calculated based on 1000 replications. The partial 16S rRNA gene sequence of strain BP-5^T was a continuous stretch of 1421 bp. 16S rRNA gene sequence analysis indicated that strain BP-5^T belonged to the class *Betaproteobacteria* and was a member of the family *Neisseriaceae*. Sequence similarity calculations with the BioEdit software (over 1350 bp) indicated that strain BP-5^T was related most closely to *Gulbenkiania mobilis* E4FC31^T (95.0% 16S rRNA gene sequence similarity), *Chromobacterium subtsugae* PRAA4-1^T (95.0%), *Chromobacterium violaceum* ATCC 12472^T (94.3%), *Vogesella indigofera* ATCC 19706^T (93.8%) and *Aquitalea magnusonii* TRO-001DR8^T (93.7%). Lower levels of sequence similarity (<93.0%) were found between strain BP-5^T and representative members of the other genera shown in Fig. 1. The overall topologies of the phylogenetic trees obtained with the neighbour-joining, maximum-likelihood and maximum-parsimony methods were similar (data not shown).

Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (Microbial ID; Sasser, 1990). The predominant fatty acid constituents of strain BP-5^T were summed feature 3 (*C*_{16:1}ω7*c* and/or iso-*C*_{15:0} 2-OH), *C*_{16:0} and *C*_{18:1}ω7*c*. The fatty acid profile of strain BP-5^T was generally comparable with those of the type strains of *G. mobilis*, *C. subtsugae*, *C. violaceum*, *V. indigofera* and *A. magnusonii* (Table 1).

The DNA G + C content of strain BP-5^T was estimated as described by Mesbah *et al.* (1989) in duplicate experiments. The nucleoside mixture was separated by means of HPLC.

Table 1. Fatty acid compositions of strain BP-5^T and members of phylogenetically related genera

Strains: 1, BP-5^T; 2, *G. mobilis* E4FC31^T (data from this study); 3, *C. violaceum* ATCC 12472^T; 4, *C. subtsugae* PRAA4-1^T; 5, *A. magnusonii* TRO-001DR8^T (data in columns 3–5 from Lau *et al.*, 2006; Martin *et al.*, 2007); 6, *V. indigofera* ATCC 19706^T (data from the MIDI database). All strains were grown on trypticase soy broth agar. Fatty acids comprising <1% of the total are not listed or are indicated by a dash.

Fatty acid	1	2	3	4	5	6
<i>C</i> _{10:0}	—	2.1	—	—	—	—
<i>C</i> _{10:0} 3-OH	2.8	2.4	5.2	4.3	1.5	5.3
<i>C</i> _{12:0}	6.0	3.1	5.6	5.0	5.0	2.9
<i>C</i> _{12:0} 2-OH	—	—	3.0	2.9	—	—
<i>C</i> _{12:0} 3-OH	1.9	2.8	5.0	4.0	1.2	—
<i>C</i> _{14:0}	—	1.0	1.7	3.2	2.8	—
<i>C</i> _{15:0}	—	—	—	1.3	2.0	—
<i>C</i> _{16:0}	33.6	28.3	23.9	25.0	21.7	26.6
<i>C</i> _{17:0} cyclo	—	—	1.7	—	—	—
<i>C</i> _{17:1} ω6 <i>c</i>	—	—	—	—	1.2	—
<i>C</i> _{18:1} ω7 <i>c</i>	9.1	13.0	15.0	10.6	8.0	3.9
<i>C</i> _{18:0}	—	1.1	—	—	—	—
Summed feature 3*	43.8	46.2	35.8	41.9	52.0	56.9

*Summed feature 3 comprises *C*_{16:1}ω7*c* and/or iso-*C*_{15:0} 2-OH.

The DNA G+C content of strain BP-5^T was 63.2 ± 1.0 mol%. Isoprenoid quinones were purified by the methods outlined in Minnikin *et al.* (1984) and were analysed by HPLC as described by Collins (1985). The major isoprenoid quinone of strain BP-5^T was ubiquinone Q-8.

DNA–DNA hybridization experiments were performed with strain BP-5^T and the type strains of *G. mobilis*, *C. subtsugae* and *C. violaceum* by using the method described by Ezaki *et al.* (1989). DNA–DNA hybridization results indicated low levels of relatedness between strain BP-5^T and its closest phylogenetic neighbours *G. mobilis* E4FC31^T (18.5 ± 5.0 %), *C. subtsugae* PRAA4-1^T (12.3 ± 2.0 %) and *C. violaceum* ATCC 12472^T (10.9 ± 3.2 %). These values are clearly below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987).

Sensitivity to antibiotics was tested after spreading cells (0.5 McFarland standard) on Mueller–Hinton agar (BD Difco) plates. The tested antibiotic discs (Oxoid) contained:

ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), rifampicin (5 µg), penicillin G (10 U), streptomycin (10 µg), sulfamethoxazole (23.75 µg) plus trimethoprim (1.25 µg) or tetracycline (30 µg). The effect of antibiotics on cell growth was assessed after 3 days.

Detailed results of the physiological characterization are given in the genus and species descriptions and in Table 2. Strain BP-5^T was not capable of producing acid from various carbohydrates, including amygdalin, L-arabinose, α-D-glucose, glycogen, myo-inositol, α-D-lactose, maltose, D-mannitol, melibiose, L-rhamnose, ribose, D-sorbitol, sucrose and xylose. However, carbon substrate utilization tests with various sugars and organic acids as substrates revealed only a few positive reactions. In API 20NE tests, strain BP-5^T was positive for reduction of nitrates to nitrites and assimilation of α-D-glucose, maltose, D-gluconate, caprate, malate and citrate. It was negative for indole production, acid production from glucose, activities

Table 2. Genotypic and phenotypic characteristics that can be used to distinguish strain BP-5^T from closely related phylogenetic neighbours

Strains: 1, strain BP-5^T; 2, *G. mobilis* E4FC31^T; 3, *C. violaceum* ATCC 12472^T; 4, *C. subtsugae* PRAA4-1^T; 5, *A. magnusonii* TRO-001DR8^T; 6, *V. indigofera* ATCC 19706^T. Data in columns 3–6 were taken from Grimes *et al.* (1997), Gillis & Logan (2005), Lau *et al.* (2006) and Martin *et al.* (2007). Data for isolation source and G+C content for *G. mobilis* E4FC31^T were taken from Vaz-Moreira *et al.* (2007); all other data were obtained in the present study. ND, No data available; +, positive; –, negative.

Characteristic	1	2	3	4	5	6
Isolation source	Cold spring water	Municipal wastewater	Soil	Soil	Humic lake	Pond sediment
Colony colour	Pale yellow	Cream	Violet	Violet	Tan	Blue
Growth at 45 °C	–	+	–	–	–	–
Glucose fermentation	–	–	+	+	+	–
Nitrite reduction to N ₂	–	–	–	+	–	+
Indole production	–	+	–	ND	+	+
Arginine dihydrolase	–	+	+	ND	+	–
Oxidation of (Biolog):						
Citric acid	–	–	–	–	+	–
α-D-Glucose 1-phosphate	+	–	+	+	–	–
α-D-Glucose 6-phosphate	+	–	+	+	–	–
DL-α-Glycerol phosphate	+	–	+	–	–	–
cis-Aconitic acid	+	–	–	–	+	–
γ-Aminobutyric acid	–	+	–	–	+	+
p-Hydroxyphenylacetic acid	+	–	–	–	+	–
α-Ketoglutaric acid	+	+	–	–	+	+
2-Aminoethanol	+	–	+	–	–	–
2,3-Butanediol	+	–	+	–	–	–
L-Leucine	+	+	–	–	+	+
L-Phenylalanine	+	–	+	–	–	+
L-Threonine	+	+	+	+	–	+
D-Serine	+	+	+	+	–	–
L-Alaninamide	+	+	+	+	–	+
Inosine	+	–	+	+	–	–
Thymidine	+	–	+	–	–	–
Uridine	+	–	+	–	–	–
DNA G+C content (mol%)	63	63	65	65	59	65

of arginine dihydrolase, urease, β -glucosidase, protease and β -galactosidase and assimilation of arabinose, mannose, mannitol, *N*-acetylglucosamine, adipate and phenylacetate. Strain BP-5^T showed positive test results (API ZYM) for alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase. It gave negative results for C14 lipase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

Strain BP-5^T showed low levels of 16S rRNA gene sequence similarity (<95 %) to all phylogenetically related members of the genera *Gulbenkiania*, *Chromobacterium*, *Aquitalea* and *Vogesella*, with the exception of *Chromobacterium* sp. strain 2002 (GenBank accession no. AY609199), which showed a similarity of 99.3 %. However, *Chromobacterium* sp. strain 2002 has not been formally described based on a polyphasic taxonomic approach and this strain is strictly anaerobic, lithoautotrophic, nitrate-dependent and Fe (II)-oxidizing (Weber *et al.*, 2006). Strain BP-5^T could be distinguished clearly from *G. mobilis* E4FC31^T based on several characteristics, especially with regard to utilization of sugars as carbon sources, growth at 45 °C and assimilation of a variety of carbon substrates. Unlike *C. violaceum* ATCC 12472^T, *C. subtsugae* PRAA4-1^T and *V. indigofera*, strain BP-5^T was unable to produce characteristic violet- or blue-pigmented colonies on peptone agar and showed a negative reaction for glucose fermentation. Strain BP-5^T could be differentiated from *A. magnusonii* TRO-001DR8^T based on fermentation of glucose and oxidization of various carbon substrates.

On the basis of these results, we suggest that strain BP-5^T represents a novel species of a new genus, for which the name *Pseudogulbenkiania subflava* gen. nov., sp. nov. is proposed.

Description of *Pseudogulbenkiania* gen. nov.

Pseudogulbenkiania (Pseu.do.gul.ben'ki.a.ni.a. Gr. adj. *pseudēs* false; N.L. fem. n. *Gulbenkiania* a bacterial generic name; N.L. fem. n. *Pseudogulbenkiania* false *Gulbenkiania*).

Cells are Gram-negative, non-spore-forming rods occurring either singly or in pairs. Cells are aerobic, motile by means of a single polar flagellum, oxidase-positive and catalase-negative. Chemo-organotrophic and able to utilize a large spectrum of carbon sources. The predominant fatty acids are summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and C_{18:1}ω7c. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The major respiratory quinone is ubiquinone Q-8. The type species is *Pseudogulbenkiania subflava*.

Description of *Pseudogulbenkiania subflava* sp. nov.

Pseudogulbenkiania subflava (sub.fl'a'va. L. fem. adj. *subflava* yellowish).

Accumulation of poly- β -hydroxybutyrate is observed. After 48 h growth on R2A agar at 25 °C, mean cell size is about 0.2–0.5 μ m (width) by 1.0–1.6 μ m (length). Colonies are round, entire, convex and pale-yellow-pigmented. Colonies are approximately 0.8–1.0 mm in diameter on R2A agar after 48 h incubation at 25 °C. Grows at 15, 25, 30, 37 and 42 °C, in the presence of 0–1 % NaCl and at pH 6–8. Optimal growth occurs at 25–35 °C, with 0 % NaCl and at pH 7–8. The DNA G+C content of the type strain is 63.2 mol%. Additional phenotypic properties are given in Table 2. The following compounds are utilized as sole carbon sources in the Biolog GN2 test system: Tween 80, α -D-glucose, maltose, sucrose, trehalose, turanose, pyruvic acid methyl ester, succinic acid monomethyl ester, formic acid, D-gluconic acid, DL-lactic acid, quinic acid, succinic acid, bromosuccinic acid, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, DL-carnitine, γ -aminobutyric acid and urocanic acid. Unable to oxidize α -cyclodextrin, dextrin, glycogen, Tween 40, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, *myo*-inositol, α -D-lactose, lactulose, D-mannitol, D-mannose, melibiose, methyl β -D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, xylitol, acetic acid, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, malonic acid, propionic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanyl glycine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-phenylalanine, L-pyrogutamic acid, D-serine, L-serine, L-threonine, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate, or D-glucose 6-phosphate. Sensitive to tetracycline, kanamycin, rifampicin, gentamicin, penicillin G, chloramphenicol, nalidixic acid, novobiocin, streptomycin, ampicillin and sulfamethoxazole plus trimethoprim.

The type strain, BP-5^T (=BCRC 17727^T =LMG 24211^T), was isolated from a cold spring from northern Taiwan.

Acknowledgements

W.-M. C. was supported by grants from the National Science Council, Taipei, Taiwan, ROC (NSC 95-2320-B-022-001-MY2 and 96-2313-B-022-001-MY3).

References

- Chen, W. M., Laevens, S., Lee, T. M., Coenye, T., de Vos, P., Mergeay, M. & Vandamme, P. (2001). *Ralstonia taiwanensis* sp. nov., isolated from root nodules of *Mimosa* species and sputum of a cystic fibrosis patient. *Int J Syst Evol Microbiol* **51**, 1729–1735.

- Chung, Y. C., Kobayashi, T., Kanai, H., Akiba, T. & Kudo, T. (1995). Purification and properties of extracellular amylase from the hyperthermophilic archaeon *Thermococcus profundus* DT5432. *Appl Environ Microbiol* **61**, 1502–1506.
- Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in micro-dilution 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.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Gillis, M. & Logan, N. A. (2005). Genus IV. *Chromobacterium* Bergonzini 1881, 153^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 824–827. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Grimes, D. J., Woese, C. R., MacDonell, M. T. & Colwell, R. R. (1997). Systematic study of the genus *Vogesella* gen. nov. and its type species, *Vogesella indigofera* comb. nov. *Int J Syst Bacteriol* **47**, 19–27.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Lau, H.-T., Faryna, J. & Triplett, E. W. (2006). *Aquitalea magnusonii* gen. nov., sp. nov., a novel Gram-negative bacterium isolated from a humic lake. *Int J Syst Evol Microbiol* **56**, 867–871.
- MacFaddin, J. F. (2000). *Biochemical Tests for the Identification of Medical Bacteria*, 3rd edn. Baltimore: Williams & Wilkins.
- Martin, P. A. W., Gundersen-Rindal, D., Blackburn, M. & Buyer, J. (2007). *Chromobacterium subtsugae* sp. nov., a betaproteobacterium toxic to Colorado potato beetle and other insect pests. *Int J Syst Evol Microbiol* **57**, 993–999.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Miller, P. H., Wiggs, L. S. & Miller, J. M. (1995). Evaluation of AnaeroGen system for growth of anaerobic bacteria. *J Clin Microbiol* **33**, 2388–2391.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Powers, E. M. (1995). Efficacy of the Ryu nonstaining KOH technique for rapidly determining gram reactions of food-borne and waterborne bacteria and yeasts. *Appl Environ Microbiol* **61**, 3756–3758.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for constructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Vaz-Moreira, I., Nobre, M. F., Nunes, O. C. & Manaia, C. M. (2007). *Gulbenkiania mobilis* gen. nov., sp. nov., isolated from treated municipal wastewater. *Int J Syst Evol Microbiol* **57**, 1108–1112.
- 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. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Weber, K. A., Pollock, J., Cole, K. A., O'Connor, S. M., Achenbach, L. A. & Coates, J. D. (2006). Anaerobic nitrate-dependent iron(II) bio-oxidation by a novel lithoautotrophic betaproteobacterium, strain 2002. *Appl Environ Microbiol* **72**, 686–694.