Pseudogulbenkiania - (gbm01834)

1. CONTRIBUTORS DETAILS

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2. KEYWORDS

spring water; soil; rice paddy soil; iron and nitrogen biogeochemical cycle; *Chromobacteriaceae*

3. ABSTRACT

Rods, non-spore forming, Gram-stain-negative. Motile by a polar flagellum. **Aerobic** with chemo-organotrophic respiratory metabolism. **Mesophilic**, growing between 15 and 42 °C. React positively to cytochrome *c* oxidase and negatively to catalase tests. Sole carbon sources include sugars, organic acids, and amino acids. The DNA G+C content ranges between 63 and 66 mol%. Major fatty acids are $C_{16:1}\ \omega 7c$ and $C_{16:0}$. Phylogenetically, belong to the family *Chromobacteriaceae*. The type species is *Pseudogulbenkiania subflava* and the genus includes also the species *Pseudogulbenkiania gefcensis*. *"Pseudogulbenkiania ferrooxidans"* is a non-validly published name.

4. DEFINING PUBLICATION

Pseudogulbenkiania, Lin, Chou, Arun, Young and Chen 2008, 2387$^{\mathrm{VP}}$.

5. ETYMOLOGY

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

6. GENERIC DEFINITION

Rods, non-spore forming, Gram-stain-negative. Motile by a polar flagellum. **Aerobic** with chemo-organotrophic respiratory metabolism. **Mesophilic**, growing between 15 and 42 °C. React positively to cytochrome *c* oxidase and negatively to catalase tests. Sole carbon sources include sugars, organic acids, and amino acids. The DNA G+C content ranges between 63 and

66 mol%. Major fatty acids are $C_{16:1}$ *ω7c* and $C_{16:0}$. Phylogenetically, belong to the family *Chromobacteriaceae*. The type species is *Pseudogulbenkiania subflava* and the genus includes also the species *Pseudogulbenkiania gefcensis. "Pseudogulbenkiania ferrooxidans"* is a non-validly published name.

The DNA G+C content (mol%) is 63.2-65.9 (HPLC) and 63.4 (WGS).

Type species: *Pseudogulbenkiania subflava*, Lin, Chou, Arun, Young and Chen 2008, 2387^{VP}.

Number of species with validly published names: 2.

7. FAMILY CLASSIFICATION

Chromobacteriaceae

8. FURTHER DESCRIPTIVE INFORMATION

8.1. Cell and colony morphology and culture conditions

Two species have validly published names within the genus *Pseudogulbenkiania*: *Pseudogulbenkiania subflava* and *Pseudogulbenkiania gefcensis*, each described based on a single strain, *P. subflava* BP-5^T and *P. gefcensis* yH16^T (Lee et al., 2013, Lin et al., 2008). *P. subflava* BP-5^T forms round, entire, convex, and pale-yellow-pigmented colonies with approximately 0.8–1.0 mm in diameter on Reasoner's agar (R2A, g/L: 0.5 of each - yeast extract, proteose peptone, casamino acids, dextrose, soluble starch; 0.3 of sodium pyruvate and dipotassium phosphate; 0.05 of magnesium sulfate; and 15.0 agar) after 48 h incubation at 25 ºC. The cells of strain BP-5^T have sizes that range 0.2–0.5 µm (width) and 1.0–1.6 µm (length) and have a single polar flagellum (Lin et al., 2008). *P. gefcensis* yH16^T forms round, entire, convex, and white colonies with approximately 1.0–1.3 mm in diameter, after 48 h incubation at 32 ºC on nutrient agar (NA, g /L: 3.0 of beef extract; 5.0 of peptone and sodium chloride; and 15.0 of agar). The cells of strain yH16^T have sizes that range 0.3–0.5 µm (width) and 0.6–0.8 µm (length) and have a single polar flagellum (Lee et al., 2013).

Pseudogulbenkiania spp. can be routinely cultured on R2A or NA for 48 h at 25-32 ºC (Lee et al., 2013, Lin et al., 2008). *P. subflava* BP-5^T is reported as being able to grow in the interval of temperature of 15-42 ºC (no growth at 10 ºC or 45 ºC), of NaCl 0-1% (w/v) and pH 6–8, with optimal growth at 25–35 ºC, 0% NaCl and pH 7–8 (Lin et al., 2008). *P. gefcensis* yH16^T is reported as being able to grow in the interval of temperature of 25-40 ºC, NaCl of 0.3-2% (w/v) and pH 6–8, with optimal growth at 32 ºC, 0.7 % NaCl, and pH 6 (Lee et al., 2013).

8.2. Nutrition and metabolism

The *Pseudogulbenkiania* spp. with validly published names are represented by bacteria described as being aerobic chemo-organotrophic, whose heterotrophic growth may occur at the expense of sugars, organic acids, and amino acids. Tested with the Biolog GN2 system, both type strains (BP-5^T and yH16^T) were able to oxidize dextrin, glycogen, tween-80, D-fructose, and gamma-aminobutyric acid (Lee et al., 2013). Both strains were also reported as able to use succinic acid monomethyl ester, formic 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-ornithine, L-proline, gamma-aminobutyric acid, and urocanic acid as sole carbon sources, tested with the Biolog GN2 system (Lee et al., 2013, Lin et al., 2008). In addition, *P. subflava* BP-5^T was able to utilize alpha-D-glucose, maltose, sucrose, trehalose, turanose, pyruvic acid methyl ester, D-gluconic acid, L-leucine, and DL-carnitine (Lin et al.,

2008) and *P. gefcensis* yH16^{T} to use beta-hydroxybutyric acid, p-hydroxyphenylacetic acid, D-saccharic acid, L-ornithine, and L-phenylalanine (Lee et al., 2013). Neither type strains were reported as capable of anaerobic growth. Nitrate (NO_3^-) reduction to nitrite (NO_2^-), but not to nitrogen (N_2), was reported for *P. gefcensis* yH16^{T}, a property not observed in *P. subflava* BP-5^{T} (Lee et al., 2013, Lin et al., 2008). However, these descriptions contrast with other reports of closely related bacteria, which were not focused on taxonomic characterization. Strain 2002 (Weber et al., 2006) that, based on the 16S rRNA gene sequence analysis (99.3% sequence identity) (Byrne-Bailey et al., 2012), is closely related to *P. subflava* BP-5^{T}, can reduce nitrate (NO_3^-) to nitrite (NO_2^-) anaerobically. Strain 2002 was proposed as a member of "*Pseudogulbenkiania ferrooxidans*", a non-validly published name (Byrne-Bailey et al., 2012). This strain and other *Pseudogulbenkiania* spp. (e.g., strain NH8B) are described as being lithotrophic bacteria capable of anaerobic and nitrate-dependent Fe(II) oxidation (NDFO) (Han et al., 2022, Ishii et al., 2016, Jokai et al., 2021, Price et al., 2022), although the authors (Ishii et al., 2016) noted that the use of this pathway to generate energy to promote growth might be strain-dependent. Strains NH8B and 2002 are also described as capable of simultaneously remove nitrate and heavy metals (iron and zinc) (Jokai et al., 2021) or to decrease nitrate soil content, with possible implications on soil fertility (Sun et al., 2022).

Both type strains BP-5^{T} and yH16^{T} are described as being susceptible to (disc diffusion method, µg) kanamycin (30), tetracycline (30), rifampicin (5), sulfamethoxazole plus trimethoprim (25), ampicillin (10 for BP-5^{T} and 25 for yH16^{T}), streptomycin (10 for BP-5^{T} and 25 for yH16^{T}), gentamicin (10 for BP-5^{T} and 30 for yH16^{T}), chloramphenicol (30) and novobiocin (30) (Lee et al., 2013, Lin et al., 2008). In addition, strain BP-5^{T} was also reported as being sensitive to penicillin G (10 U) and nalidixic acid (30 µg) (Lin et al., 2008).

8.3. Chemotaxonomic characteristics

The major respiratory quinone, reported for the type strains *P. subflava* BP-5^T and *P. gefcensis* yH16^T is ubiquinone 8 (Lee et al., 2013, Lin et al., 2008). The major polar lipids described in *P. subflava* BP-5^T were diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine (Lin et al., 2008). The analysis of the fatty acid composition revealed a profile similar for both type strains, where summed feature 3 ($C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH) and $C_{16:0}$ predominated (>25%). Respectively, in *P. gefcensis* yH16^T and *P. subflava* BP-5^T: $C_{10:0}$ 3-OH (2.4 *vs.* 2.9%); $C_{12:0}$ (6.2 *vs.* 8.6%); $C_{12:0}$ 3-OH (2.2% in both); $C_{14:0}$ (1.5 *vs.* 1.6%); $C_{15:0}$ (1.0 *vs.* 2.1%); $C_{16:0}$ (38.0 *vs.* 25.1%); $C_{17:0}$ cyclo (2.8% *vs.* not detected); $C_{17:1}$ *ω6c* (not detected *vs*. 1.2%); $C_{18:1}$ *ω7c* (6.9 *vs.* 5.9%); Summed feature 3 (37.2 *vs*. 47.7%) (Lee et al., 2013).

8.4. Genome features

At least five whole genome sequences of bacteria identified as *Pseudogulbenkiania* spp. are available in public databases (Table 1). Only one of these refers to a type strain, *P. subflava* DSM 22618^T (=BP-5^T), while the others are representative of environmental isolates with the capability to promote nitrate-dependent Fe(II) oxidation. Among these, it is included strain 2002 named as "*Pseudogulbenkiania ferrooxidans*" (Byrne-Bailey et al., 2012). The five genomes of *Pseudogulbenkiania* spp. available share average nucleotide and amino acid identity (ANI and AAI) values between 80.2-97.2 and 67.6-97.1%, respectively, and 80.1-93.7 and 67.8-94.8% with *P. subflava* BP-5^T (Table 2). These values are consistent with the inclusion of the strains 2002, NH8B and MAI-1 in the genus *Pseudogulbenkiania*, although none in the species *P. subflava* (Konstantinidis et al., 2017, Rodriguez-R and Konstantinidis, 2014). Accordingly, *in silico* DDH hybridization values range 21.8% to 54.2% between *P. subflava* BP-5^T and *Pseudogulbenkiania* spp. and 21.8% to 76.8% amongst the latter (Table 2).

Pseudogulbenkiania spp. and *Paludibacterium* spp. strains share ANI, AAI and DDH values ranging 77.6-79.4%, 63.1-66.6%, and 19.5-20.9%, respectively (Table 2).

<Table 1 near here>

<Table 2 near here>

8.5. Ecology and Habitat

P. subflava BP-5^{T} was isolated from a cold spring in Hsinchu County in Taiwan (Lin et al., 2008). *P. gefcensis* yH16^{T} was isolated from soil collected at KyungHee University, Suwon City in the Republic of Korea. The soil had a pH of 5.1, moisture of 50.6 % and about 5 log-units colony forming units per gram of aerobic culturable bacteria (Lee et al., 2013). Strain 2002 was isolated from a sediment core of a small freshwater lake, Campus Lake, at Southern Illinois University, Carbondale, in the United States of America (Weber et al., 2006). Numerous bacteria closely related with members of the genus *Pseudogulbenkiania* have been isolated or their 16S rRNA gene sequence was cloned from rice paddy soil and rice-soybean rotation fields (e.g. clones N52 or JH-WH6, Figure 1) (He et al., 2008, Yoshida et al., 2012), or related environments in Japan (Ishii et al., 2016, Tago et al., 2011) and China (e.g., 16S rRNA acc. No. KY287282; EF492895.1), and from a lake in northern India (Puranik et al., 2013).

Although the valid publication of the two species *P. subflava* and *P. gefcensis* report a chemoorganotrophic metabolism, the literature has shown consistently that members of this genus are able to oxidize Fe(II) while reducing nitrate (Ishii et al., 2016). In a study involving 70 isolates of *Pseudogulbenkiania* spp., including the type strains BP-5^{T}and yH16^{T} it was demonstrated that all strains were capable of oxidizing Fe(II) under anaerobic conditions using nitrate as electron acceptor (Weber et al., 2006; Ishii et al., 2016). According to these authors, who did not find the involvement of specific enzymes in the process, nitrate reduction triggers Fe(II) oxidation. The comparison of the genes nitrite reductase (*nir*), nitric oxide reductase (*nor*), and nitrous oxide reductase (*nos*) in the strains NH8B and 2002 showed high sequence identity between both strains, although the genes presented distinct organization and patterns of expression regulation, suggesting an independent acquisition history (Ishii et al., 2016). Moreover, this process does not support autotrophic growth, as expected due to the low energy yield. These evidences suggest that *Pseudogulbenkiania* spp. may actively participate in the nitrogen and iron biogeochemical cycles (Ishii et al., 2016, Weber et al., 2006), mainly in anoxic environments.

9. ENRICHMENT/ISOLATION AND MAINTENANCE PROCEDURES

P. subflava BP-5^T was isolated on NA (BD Difco) after incubation at 25 ºC for 3 days. Subcultivation and maintenance were made on R2A agar (BD Difco) at the same temperature with incubation periods of 2-3 days (Lin et al., 2008). This strain is available as freeze dried biomass in different culture collections (DSM 22618^T, BCRC 17727^T, LMG 24211^T). *P. gefcensis* yH16^T was isolated and maintained on NA after incubation for 36-48 h at 32 ºC. This strain is available in different culture collections (KCCM 90100^T, JCM 17850^T); JCM 17850 can be supplied as a freeze dried or active culture. Strain 2002 was isolated from a lake by adding 1 g of sediment into 9 ml of anoxic (80:20 N_2:CO_2 headspace) bicarbonate-buffered (pH 6.8) freshwater basal medium (per L: 0.25 g NH_4Cl; 1.03 g $NaClO_3$; 1.36 g CH_3COONa; 0.60 g NaH_2PO_4; 0.1 g KCl; 2.5 g $NaHCO_3$; 0.02 mg biotin; 0.02 mg folic acid; 0.1 mg pyridoxine HCl; 0.05 mg riboflavin; 0.05 mg thiamine; 0.05 mg nicotinic acid; 0.05 mg pantothenic acid; 0.001 mg vitamin B12; 0.05 mg p-aminobenzoic acid; 0.05 mg thioctic acid; 15 mg nitrilotriacetic acid; 30 mg $MgSO_4$; 5 mg $MnSO_4 \cdot H_2O$; 10 mg NaCl; 1 mg $FeSO_4 \cdot 7H_2O$; 1 mg $CaCl_2 \cdot 2H_2O$; 1 mg $CoCl_2 \cdot 6H_2O$; 1.3 mg ZnCl; 0.1 mg $CuSO_4$; 0.1 mg $AlK(SO_4)_2 \cdot 12H_2O$;

0.1 mg H_3BO_2; 0.25 mg Na_2MoO_4; 0.24 mg $NiCl_2 \cdot 6H_2O$; 0.25 mg $Na_2WO_4 \cdot 2H_2O$) (Bruce et al., 1999), supplemented with 5 mM of nitrate as the electron acceptor and 0.1 mM acetate as the carbon source (Weber et al., 2006). Iron (II) was supplied as electron donor, at a final concentration of 10 mM Fe(II) chloride salt, under anoxic (100% N_2 atmosphere) conditions. After 8 weeks of incubation in the dark at 30 °C, cultures indicating iron oxidation, through the presence of a brownish-red or -green precipitate, were subcultured on R2A amended with 10 mM nitrate and incubated for 120 h under anaerobic conditions (95:5 N_2:H_2 atmosphere) at 30 °C. After that period, an overlay with a 2 mM $FeCl_2$ solution and incubation under anoxic conditions, would reveal Fe(II) oxidizing bacteria, further cultured in the presence of the same electron acceptors and donors, and successively with acetate or CO_2 as carbon source (Weber et al., 2006).

Pseudogulbenkiania spp. strains were also isolated from rice and soybean culturing soils, through inoculation with 1 g of dry soil in 15-mL sterile water for 1 week at 30 °C. After this period, the excess water (~ 2 mL) was removed and 0.1 mg N-nitrate and 0.5 mg C-succinate were added to each vial and incubated for one day at 30 ºC under an atmosphere of Ar-C_2H_2 (90:10) gas, to stimulate denitrifying activity (Ishii et al., 2010, Saito et al., 2008). The functional single-cell isolation method was used by injecting a single cell into a vial containing 100-fold diluted nutrient broth medium supplemented with 3.0 mM nitrate and 4.4 mM succinate (DNB-NS medium) that was incubated for one week at 30 ºC anaerobically. The resultant cultures were streaked on DNB-NS medium and incubated at the same conditions for 2 weeks. This procedure led to the isolation of 110 cultures with the capacity of denitrification, 67 of which were identified as *Pseudogulbenkiania* spp. (Tago et al., 2011).

10. DIFFERENTIATION OF THE GENUS *PSEUDOGULBENKIANIA* FROM OTHER GENERA

The genus *Pseudogulbenkiania* is a member of the family *Chromobacteriaceae* that also includes the genera *Chromobacterium*, *Aquitalea*, *Craterilacuibacter*, *Crenobacter*, *Gulbenkiania*, *Paludibacterium*, and *Vogesella* (Chen et al., 2021). Members of this family are motile rods (except *Vogesella*), have a DNA G + C content (mol%) ranging between 59.2 and 68.8% and genomes with 3-4 Mb (Chen et al., 2021). All members have an aerobic metabolism and some species are facultatively anaerobic (Chen et al., 2021). Within this family, at the moment of writing, the type strains of the validly named type species of other genera with closest proximity to *P. subflava* BP-5^{T}, the type species of the genus *Pseudogulbenkiania*, are *Paludibacterium purpuratum* CECT 8976^{T} (16S rRNA gene sequence identity of 95.99%, ANI of 78.4%, and AAI of 65.1%) and *Paludibacterium yongneupense* DSM 18731^{T} (16S rRNA gene sequence identity of 95.20%, ANI of 77.7%, and AAI of 65.9%) (Table 2), while others (Figure 1) share a 16S rRNA gene sequence identity lower than 95% (based on EzBiocloud). The phenotypic comparison shows a reduced set of distinctive features between *Paludibacterium* and *Pseudogulbenkiania* species. Nevertheless, the negative catalase reaction, as well as the inability to produce arginine dihydrolase, or ferment glucose in *Pseudogulbenkiania* isolates, allows their distinction from the type strains of the three *Paludibacterium* species (*Pal. yongneupense*, *Pal. paludis* and *Pal. purpuratum*) (Kang et al., 2016, Lee et al., 2013, Lin et al., 2008). The ability of *Pseudogulbenkiania* spp. to utilize formic acid also contrasts with the incapacity of *Paludibacterium* spp. to use this sole carbon source (Kang et al., 2016, Lee et al., 2013, Lin et al., 2008). In addition, the low relative abundance of summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) in *Pseudogulbenkiania* isolates when compared with *Pal. paludis* and *Pal. purpuratum* allow the distinction of these species (Durán-Viseras et al., 2021, Kang et al., 2016).

11. TAXONOMIC COMMENTS

Based on the 16S rRNA gene sequence phylogenetic analysis, the type strain of the type species of the genus *P. subflava* BP-5^{T} shares 96.3% sequence identity with *P. gefcensis* yH16^{T}. The 16S rRNA gene-based phylogenetic analysis suggests that both type strains represent distinct lineages within the genus (Figure 1). The 16S rRNA gene sequence similarity, ANI, AAI and DDH values show that *Pseudogulbenkiania subflava* BP-5^{T} is closely related with strains NH8B and 2002 (Figure 1, Table 2), characterized by the anaerobic and nitrate-dependent Fe(II) oxidation (Ishii et al., 2016). According to Ishii et al. (2016) this property is also shared by all members of the genus, including the two known type strains. However, the fact that there is no genome sequence available for *P. gefcensis* yH16^{T} limits a further in-depth discussion about this relationship as well as the possible evidence of horizontal gene transfer of denitrification related genes.

<Figure 1 near here>

12. LIST OF SPECIES OF THE GENUS *PSEUDOGULBENKIANIA*

1. *Pseudogulbenkiania gefcensis* Lee, Im, Kang, Yun, Park, Hyun, Hwang 2013, 190^{VP}

gefcensis [gef.cen'sis. N.L. fem. adj. gefcensis pertaining to GFC, an arbitrary adjective formed from the acronym for Green Flower Cosmetics (GFC)].

In addition to the genus description, the species is described as able to assimilate glucose, maltose, gluconate and malate, but not for arabinose, mannose, mannitol, N-acetylglucosamine, caprate, adipate, citrate, and phenylacetate. Able to oxidize dextrin, glycogen, Tween-80, D-fructose, succinic acid monomethyl ester, formic acid, beta-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, DL-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, L-phenylalanine, L-proline, gamma-aminobutyric acid and urocanic acid. Unable to oxidize alpha-cyclodextrin, Tween-40, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, i-erythritol, L-fucose, D-galactose, gentiobiose, *myo*-inositol, alpha-D-lactose, lactulose, D-mannitol, D-mannose, D-melibiose, methyl beta-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, acetic acid, cis-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, alpha-hydroxybutyric acid, gamma-hydroxybutyric acid, itaconic acid, alpha-ketobutyric acid, alpha-ketoglutaric acid, alpha-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-leucine, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-alpha-glycerol phosphate, alpha-D-glucose 1-phosphate or D-glucose 6-phosphate. The species tests negative for indole production, acid production from glucose, and activities of arginine dihydrolase, urease, beta-glucosidase, protease, and beta-galactosidase.

The DNA G+C content (mol %) is 65.9 (HPLC method)

Type strain: yH16 (=KCCM 90100 =JCM 17850).

GenBank accession number (16S rRNA): JF728876.

2. *Pseudogulbenkiania subflava*, Lin, Chou, Arun, Young, Chen 2008, 2387[VP]

subflava (sub.fla'va. L. fem. adj. subflava yellowish).

In addition to the genus description, the species is described as able to assimilate alpha-D-glucose, maltose, D-gluconate, caprate, malate, and citrate, but not arabinose, mannose, mannitol, N-acetylglucosamine, adipate, and phenylacetate. Able to oxidize Tween 80, alpha-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, gamma-aminobutyric acid and urocanic acid. Unable to oxidize alpha-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, alpha-D-lactose, lactulose, D-mannitol, D-mannose, melibiose, methyl beta-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, alpha-hydroxybutyric acid, beta-hydroxybutyric acid, gamma-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, alpha-ketobutyric acid, alpha-ketoglutaric acid, alpha-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-pyroglutamic acid, D-serine, L-serine, L-threonine, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-alpha-glycerol phosphate, alpha-D-glucose 1-phosphate, or D-glucose 6-phosphate.

Tests negative for indole production, and acid production from amygdalin, L-arabinose, alpha-D-glucose, glycogen, myo-inositol, alpha-D-lactose, maltose, D-mannitol, melibiose, L-rhamnose, ribose, D-sorbitol, sucrose, and xylose. Tests positive for the production of alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and alpha-glucosidase, and negative for the production of arginine dihydrolase, urease, protease, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, alpha-chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase and alpha-fucosidase.

The DNA G+C content (mol %) is 63.2 (HPLC method) - 63.4 (whole genome sequencing).
Type strain: BP-5 (=DSM 22618 =LMG 24211 =BCRC 17727).

GenBank accession number (16S rRNA): EF626692.

GenBank accession number (genome): GCA_900177275.1

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Table 1. Characteristics of *Pseudogulbenkiania* spp. genomes available in public databases.

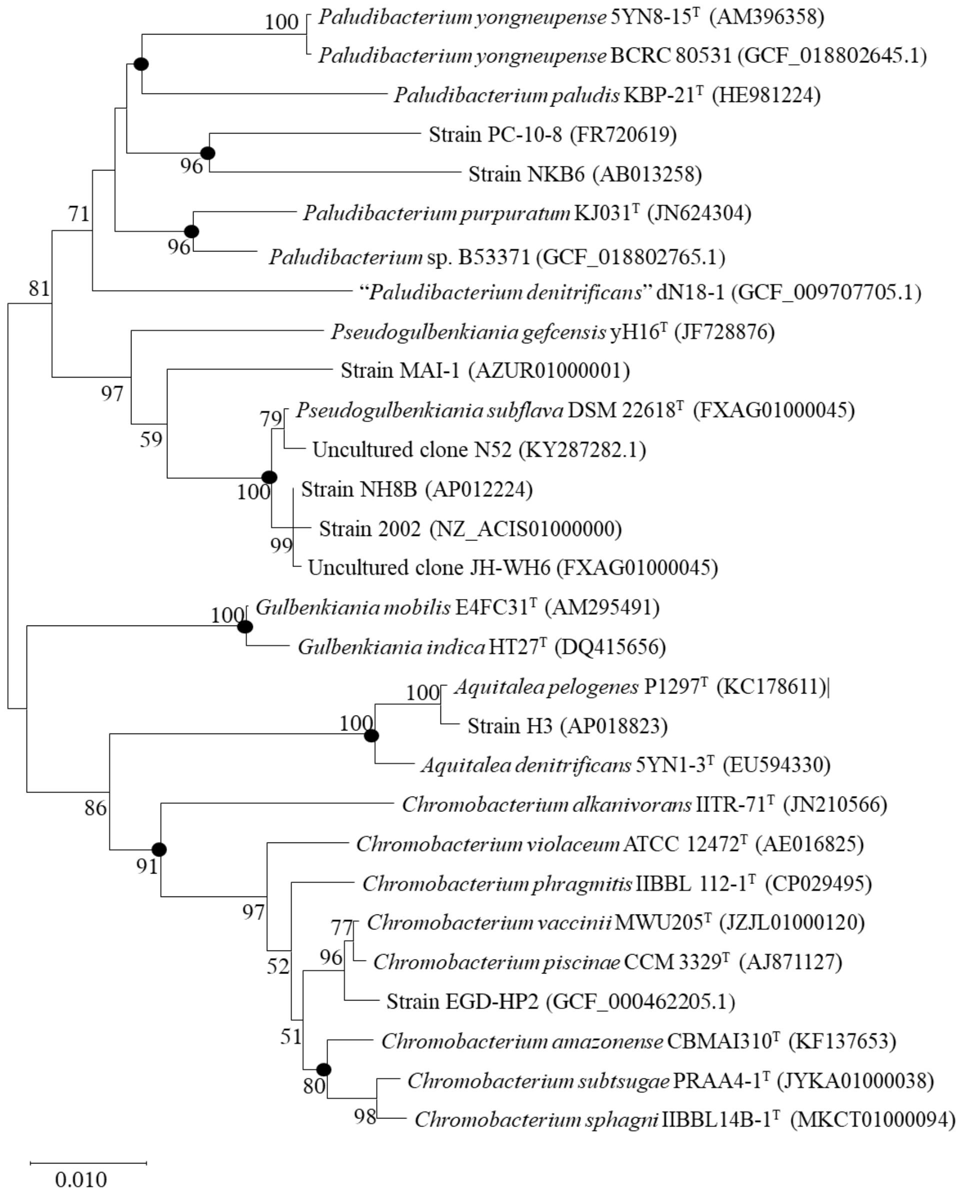
	P. subflava DSM 22618^{T}	"*P. ferrooxidans*" strain 2002	"*P. ferrooxidans*" strain EGD-HP2	*Pseudogulbenkiania* sp. strain NH8B	*Pseudogulbenkiania* sp. strain MAI-1
Bioproject	PRJEB20430	PRJNA30761	PRJNA215707	PRJDA68325	PRJNA82805
NCBI assembly accession number	GCF_900177275.1	GCF_000174355.1	GCF_000462205.1	GCF_000283535.1	GCF_000527175.1
Genome size (bp)	4 367 405	4 228 787	4 801 407	4 332 995	4 339 789
GC content (%)	63.4	64.7	64.2	64.4	64.2
No. scaffolds	45	20	301	1	1
Scaffold N50	156 093 (scaffold)	395 779 (scaffold)	48 594 (scaffold)	4 332 995 (complete)	4 339 789 (complete)
Scaffold L50	9	5	31	1	1
Genes	4 142	3 914	4 771	3 946	3 946
Protein	3 972	3 925	4 499	4 015	n.p.
Sequencing technology / Coverage	Illumina MiSeq / 403x	454 GS FLX / n.a.	Illumina MiSeq / 33x	Sanger; Roche 454 / 22x	Illumina / n.p.

n.p., not provided

Table 2. Average nucleotide identity (ANI), average amino acid identity (AAI) and DNA-DNA hybridization (DDH) values shared by *Pseudogulbenkiania* and *Paludibacterium* strains with a genome available. Data obtained at http://enve-omics.ce.gatech.edu/at (Rodriguez-R and Konstantinidis, 2016) and http://ggdc.dsmz.de/ggdc.php# (Meier-Kolthoff et al., 2022).

ANI/AAI/DDH	DSM 22618^{T}	2002	EGD-HP2	NH8B	MAI-1	DSM 18731^{T}	BCRC 80531	CECT 8976^{T}	dN18-1	KCTC 32182	BCRC 80514^{T}	B53371
P. subflava DSM 22618^{T}	100 / 100 / 100											
"P. ferrooxidans" 2002	93.6 / 94.7 / 53.7	100 / 100 / 100										
"*P. ferrooxidans*" EGD-HP2	80.0 / 67.7 / 21.8	80.1 / 67.6 / 21.8	100 / 100 / 100									
Pseudogulbenkiania sp. NH8B	93.6 / 94.7 / 54.2	97.2 / 97.0 / 76.8	80.2 / 67.7 / 21.7	100 / 100 / 100								
Pseudogulbenkiania sp. MAI-1	88.3 / 88.5 / 36.2	88.0 / 87.9 / 35.4	80.4 / 68.0 / 21.9	88.1 / 88.0 / 36.0	100 / 100 / 100							
Pal. yongneupense DSM 18731^{T} (GCF_000422925.1)	77.7 / 65.9 / 19.9	77.8 / 65.5 / 19.9	77.5 / 64.3 / 19.5	77.9 / 65.7 / 20.0	77.8 / 66.0 / 19.8	100 / 100 / 100						
Pal. yongneupense BCRC 80531 (GCF_018802645.1)	77.9 / 65.9 / 20.1	78.0 / 65.5 / 20.3	77.6 / 64.4 / 19.9	78.4 / 65.7 / 20.8	78.2 / 66.0 / 20.4	99.9 / 99.9 / 99.9	100 / 100 / 100					
Pal. purpuratum CECT 8976^{T} (GCF_004363805.1)	78.4 / 65.0 / 20.2	78.5 / 65.2 / 20.1	78.2 / 64.6 / 20.0	78.5 / 65.2 / 20.5	78.5 / 65.2 / 20.2	77.4 / 65.9 / 19.1	77.8 / 66.0 / 19.6	100 / 100 / 100				

"Pal. denitrificans" dN18-1 (GCF_009707705.1)	78.3 / 64.6 / 20.4	78.5 / 64.7 / 20.7	78.4 / 63.1 / 20.7	79.0 / 64.5 / 20.8	79.0 / 64.4 / 20.4	76.6 / 59.8 / 19.0	77.7 / 59.7 / 20.3	77.1 / 59.4 / 19.6	100 / 100 / 100			
Pal. paludis KCTC 32182 (GCF_014652495.1)	78.6 / 66.5 / 20.2	78.6 / 66.4 / 20.3	78.2 / 64.9 / 20.0	78.7 / 66.1 / 20.5	78.9 / 66.4 / 20.4	78.1 / 65.5 / 19.6	78.3 / 65.6 / 20.1	78.5 / 65.1 / 19.9	77.0 / 60.3 / 19.4	100 / 100 / 100		
Pal. paludis BCRC 80514^T (GCF_018802605.1)	78.7 / 66.5 / 20.4	78.7 / 66.5 / 20.6	78.2 / 64.9 / 20.3	79.1 / 66.1 / 20.8	79.3 / 66.4 / 20.8	78.3 / 65.5 / 19.8	79.0 / 65.7 / 20.7	78.4 / 65.3 / 20.4	77.6 / 60.4 / 20.0	99.9 / 99.9 / 100	100 / 100 / 100	
Paludibacterium sp. B53371 (GCF_018802765.1)	78.6 / 65.9 / 20.6	78.9 / 65.6 / 20.9	78.2 / 65.1 / 20.5	79.3 / 65.8 / 21.4	79.2 / 65.9 / 21.0	78.2 / 66.4 / 19.9	78.8 / 66.4 / 20.8	81.8 / 80.5 / 23.1	78.1 / 59.9 / 20.5	78.7 / 65.5 / 20.8	79.0 / 65.6 / 21.3	100 / 100 / 100



Paludibacterium yongneupense 5YN8-15T (AM396358)
Paludibacterium yongneupense BCRC 80531 (GCF_018802645.1)
Paludibacterium paludis KBP-21T (HE981224)
Strain PC-10-8 (FR720619)
Strain NKB6 (AB013258)
Paludibacterium purpuratum KJ031T (JN624304)
Paludibacterium sp. B53371 (GCF_018802765.1)
"Paludibacterium denitrificans" dN18-1 (GCF_009707705.1)
Pseudogulbenkiania gefcensis yH16T (JF728876)
Strain MAI-1 (AZUR01000001)
Pseudogulbenkiania subflava DSM 22618T (FXAG01000045)
Uncultured clone N52 (KY287282.1)
Strain NH8B (AP012224)
Strain 2002 (NZ_ACIS01000000)
Uncultured clone JH-WH6 (FXAG01000045)
Gulbenkiania mobilis E4FC31T (AM295491)
Gulbenkiania indica HT27T (DQ415656)
Aquitalea pelogenes P1297T (KC178611)|
Strain H3 (AP018823)
Aquitalea denitrificans 5YN1-3T (EU594330)
Chromobacterium alkanivorans IITR-71T (JN210566)
Chromobacterium violaceum ATCC 12472T (AE016825)
Chromobacterium phragmitis IIBBL 112-1T (CP029495)
Chromobacterium vaccinii MWU205T (JZJL01000120)
Chromobacterium piscinae CCM 3329T (AJ871127)
Strain EGD-HP2 (GCF_000462205.1)
Chromobacterium amazonense CBMAI310T (KF137653)
Chromobacterium subtsugae PRAA4-1T (JYKA01000038)
Chromobacterium sphagni IIBBL14B-1T (MKCT01000094)
100
96
71
96
81
97
59
79
100
99
100
100
100
86
91
97
52
77
96
51
80
98
0.010

Figure 1. Dendrogram based on 16S rRNA gene sequences, showing the position of the *Pseudogulbenkiania* species in relation to the type strains of species with which any of the two *Pseudogulbenkiania* type strains share ≥94% sequence identity. The dendrogram was generated by the Neighbour-joining method. Bootstrap values, generated from 1000 re-samplings, are indicated at branch points (≥60%). Filled circles indicate branches on the tree that were also recovered in the tree generated using the Maximum-likelihood and Minimum Evolution algorithms. Bar, 1 substitution per 100 nucleotide positions.