Gulbenkiania - (gbm01832)

1. CONTRIBUTORS DETAILS

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KEYWORDS: *Gulbenkiania*; urban treated wastewater; hot springs; *Chromobacteriaceae*;

2. ABSTRACT

Rods, non-spore forming, Gram-staining-negative. Motile by a single polar flagellum. **Aerobic** or facultative aerobic chemo-organotrophic respiratory metabolism. **Mesophilic**, growing between 15 and 45 °C. Shows positive reaction in the catalase- and cytochrome *c* oxidase tests. Sole carbon sources include amino acids and organic acids. Sugars are not assimilated nor fermented. Anaerobic growth in the presence of nitrate is a variable trait. The DNA G+C content is 63 mol%. Major fatty acids are $C_{16:1}$ *ω7c*, $C_{16:0}$, and $C_{18:1}$ *ω7c*. Phylogenetically, belongs to the family *Chromobacteriaceae*. The type species is *Gulbenkiania mobilis* and the genus includes also *Gulbenkiania indica*.

3. DEFINING PUBLICATION

Gulbenkiania, Vaz-Moreira, Nobre, Nunes and Manaia 2007, 1110^{VP}.

4. ETYMOLOGY

Gulbenkiania [Gul.ben.ki.a'ni.a. N.L. fem. n. *Gulbenkiania* in honour of Calouste Gulbenkian (1869–1955), a protector of the arts and sciences in Portugal, and founder of the Fundação Calouste Gulbenkian]

5. GENERIC DEFINITION

Rods, non-spore forming, Gram-staining-negative. Motile by a single polar flagellum. **Aerobic** or facultative aerobic chemo-organotrophic respiratory metabolism. **Mesophilic**, growing between 15 and 45 °C. Shows positive reaction in the catalase- and cytochrome *c* oxidase tests. Sole carbon sources include amino acids and organic acids. Sugars are not assimilated nor

fermented. Anaerobic growth in the presence of nitrate is a variable trait. The DNA G+C content is 63 mol%. Major fatty acids are $C_{16:1}$ *ω7c*, $C_{16:0}$, and $C_{18:1}$ *ω7c*. Phylogenetically, belongs to the family *Chromobacteriaceae*. The type species is *Gulbenkiania mobilis* and the genus includes also *Gulbenkiania indica*.

The DNA G+C content (mol %) is 63-63.6 (HPLC), 63.6 (whole genome analysis).

Type species: *Gulbenkiania mobilis*, Vaz-Moreira, Nobre, Nunes and Manaia 2007, 1111^{VP}

Number of species with validated names: 2.

6. FAMILY CLASSIFICATION

Chromobacteriaceae

7. FURTHER DESCRIPTIVE INFORMATION

7.1. Cell and colony morphology and culture conditions

Currently, two species are validly named within the genus *Gulbenkiania*: *Gulbenkiania mobilis* and *Gulbenkiania indica*, each described based on a single strain, *G. mobilis* $E4FC31^T$ and *G. indica* $HT27^T$ (Jyoti et al., 2010, Vaz-Moreira et al., 2007). *G. mobilis* $E4FC31^T$ forms non-pigmented, regular and convex colonies with 1-2 mm diameter after 24 h incubation at 30 °C on Plate Count Agar (PCA) or modified Luria–Bertani (MLB, containing per liter: 5.0 g tryptone, 2.5 g yeast extract, 1.0 g NaCl, 15 g agar). Under the same incubation conditions, *G. indica* $HT27^T$ forms cream, smooth, mucoid, round colonies with 1.5-2 mm on nutrient agar (NA) (Jyoti et al., 2010, Vaz-Moreira et al., 2007).

Gulbenkiania spp. cells are non-spore forming Gram-negative staining rods, motile through a single polar flagellum. The *G. mobilis* E4FC31^T cells are curved with 0.95±0.17 µm in length and 0.38±0.11 µm wide, whereas *G. indica* HT27^T cells are straight, 2.3 µm long and 1.1 µm wide (Jyoti et al., 2010, Vaz-Moreira et al., 2007).

Gulbenkiania spp. can be routinely cultured on PCA or NA for 24 h at 30 ºC (Jyoti et al., 2010, Vaz-Moreira et al., 2007). *G. mobilis* E4FC31^T does not require growth factors such as vitamins, amino acids or nucleosides, since it is able to grow in mineral medium with malic acid as the sole source of carbon and energy (Vaz-Moreira et al., 2007). *G. mobilis* E4FC31^T also grows on the nutritive medium Columbia agar with 5% sheep blood and on the selective/differential media Eosin Methylene Blue agar (EMB) and Membrane Fecal Coliform agar (m-FC), where it forms grey, dark-red and greenish colonies, respectively; in addition, *G. mobilis* E4FC31^T grows in culture media such as *Escherichia coli* (EC) and glucose broths, although without gas production from lactose and glucose, respectively (Vaz-Moreira et al., 2007). The ability to grow on MacConkey Agar is a distinctive trait between both *Gulbenkiania* type strains, since only *G. indica* HT27^T is able to grow on that culture medium (Jyoti et al., 2010, Vaz-Moreira et al., 2007).

The type strains of the two *Gulbenkiania* species share the same temperature growth range (15 to 45° C, optimum between 30-37 °C) and salt tolerance (up to 1% (w/v) NaCl) (Jyoti et al., 2010, Vaz-Moreira et al., 2007). *G. indica* HT27^T grows in a wider pH range (pH 5.5 - 11.0) than *G. mobilis* E4FC31^T (pH 5.5 - 9.0) (Jyoti et al., 2010, Vaz-Moreira et al., 2007), although the highest growth yield of *G. indica* HT27^T is at pH 7.5-8.0 (Jyoti et al., 2010).

7.2. Nutrition and metabolism

Gulbenkiania spp. are aerobic chemoorganotrophic heterotrophic bacteria. Anaerobic growth is only reported for the type strain of the species *G. mobilis* that reduces nitrate (NO_3^-) to nitrite (NO_2^-) as a possible alternative electron acceptor (Jyoti et al., 2010, Vaz-Moreira et al., 2007).

Organic acids and amino acids can be used as sole carbon sources, although these are variable traits within the genus. For example, succinate, malate, fumarate, glutamic acid, leucine, and proline are assimilated by the type strain of *G. mobilis*, but not by the type strain of *G. indica*, being the opposite observed for the assimilation of 4-aminobutyric acid, tyrosine, threonine, lysine, cysteine, inositol, and sodium acetate (Jyoti et al., 2010). Traits shared by both type strains include the production of catalase and cytochrome *c* oxidase, and the inability to assimilate sugars such as glucose and mannose or to hydrolyse gelatin (Jyoti et al., 2010, Vaz-Moreira et al., 2007).

G. mobilis E4FC31^T is susceptible to amoxicillin (25 µg), gentamicin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), sulfamethoxazole (25 µg), sulfamethoxazole/trimethoprim (23.75/1.25 µg), cephalothin (30 µg), streptomycin (10 µg), ticarcillin (75 µg), ceftazidime (30 µg), meropenem (10 µg) and colistin sulphate (50 µg), whereas *G. indica* HT27^T is susceptible to kanamycin (50 µg ml^{-1}), neomycin (30 µg ml^{-1}), chloramphenicol (30 µg ml^{-1}), tetracycline (15 µg ml^{-1}), nalidixic acid (20 µg µl^{-1}), rifampicin (20 µg ml^{-1}) and ampicillin (40 µg ml^{-1}), and resistant to streptomycin (10 µg ml^{-1}) (Jyoti et al., 2010, Vaz-Moreira et al., 2007).

7.3. Chemotaxonomic characteristics

The major respiratory quinone, reported for the type strain *G. mobilis* E4FC31^T, is ubiquinone 8, whereas ubiquinone 9 is a minor component (Vaz-Moreira et al., 2007). According to Jyoti et al. (2010), *G. mobilis* E4FC31^T and *G. indica* HT27^T share a similar fatty acids profile, with $C_{16:1}$ ω*7c* (39.0% and 44.2%, respectively), $C_{16:0}$ (24.9% and 27.7%, respectively) and $C_{18:1}$ ω*7c*

(15.7% and 14.0%, respectively) predominating. Other minor fatty acids (< 4%) shared by these type strains include $C_{10:0}$, $C_{10:0}$ 3-OH, $C_{12:0}$, $C_{12:0}$ 3-OH, $C_{14:0}$, $C_{17:0}$, and $C_{18:0}$. Only fatty acids found at trace amounts (< 1.2%) distinguish these type strains, with $C_{11:0}$ iso 3-OH and $C_{14:1}$ ω*5c* occurring in *G. mobilis* E4FC31^T, and $C_{15:1}$ ω*6c*, $C_{15:0}$, $C_{16:1}$ ω*5c*, $C_{17:1}$ ω*8c*, $C_{17:1}$ ω*6c* and $C_{18:0}$ 10-methyl in *G. indica* HT27^T (Jyoti et al., 2010).

7.4. Genome features

The whole genome raw data of strain *G. mobilis* DSM 18507 is available in the GenBank under the accession number GCA_004346645.1 (Bioproject reference PRJNA520311). Although available at the EzBioCloud (https://www.ezbiocloud.net/genome/explore?puid=198498), the assembly of this genome is not available at the NCBI database because the taxonomy check status is described as "inconclusive", according to the information available, it is not possible to confirm the source of the organism. This whole genome sequence shares 99.2% average nucleotide identity (ANI) with *G. indica* 17901^T (https://www.ncbi.nlm.nih.gov/assembly/GCA_004346645.1/#/qa).

The assembly of the whole genome of strain *G. indica* HT27^T (Badhai et al., 2016), obtained based on Illumina HiSeq, is available in the GenBank under the accession number GCA_001418035.1 (Bioproject reference PRJNA289009). *G. indica* HT27^T genome sequence, with a coverage of 506.0x, presents a total length of 2,848,002 bp, with 23 contigs, a contig N50 value of 286,056 bp and L50 value of 23. The genes sum 2792, corresponding to 2699 proteins, 59 tRNAs, and 14 corresponding rRNAs; the DNA G+C content (mol %) is determined to be 63.6 (and 63.0% based on HPLC method, Jyoti et al., 2010).

The draft genome of strain MB1 is also available (assembly accession number GCA_001302325.1, Bioproject PRJNA293922). The genome sequence was obtained with the

Illumina NextSeq 500 platform (Saxena et al., 2015). It has a total length of 3,420,264 bp, with 614 contigs, a contig N50 value of 106,255 bp, 3,531 encoding regions, with 65 and 5 corresponding to tRNAs and rRNAs, respectively, and an estimated DNA G+C content of 62.0 mol%. Strain MB1 shares 99.18% ANI and 99.02% average amino acid identity (AAI) with *G. indica* HT27^T. Therefore, the taxonomy check status of the draft genome of strain MB1 is also described as "inconclusive" (https://www.ncbi.nlm.nih.gov/assembly/GCF_001302325.1#/qa)

7.5. Ecology and Habitat

Gulbenkiania spp. were isolated from both human-impacted and natural environments. The type strain of the species *G. mobilis* was isolated in northern Portugal from treated urban wastewater on m-FC agar medium, during a survey of antibiotic resistance patterns of members of the *Enterobacteriaceae* family (Ferreira da Silva et al., 2007, Vaz-Moreira et al., 2007). *G. indica* HT27^T was isolated from the sediment of a sulfur spring in Athamallik, Orissa, India, during a study aiming to assess its bacterial diversity; the surface temperature and pH of the sediment from which strain HT27^T was recovered were 43 °C and 7.4, respectively (Jyoti et al., 2010). Accordingly, strain MB1 was isolated from samples collected at Chhoti Anhoni, a hot spring located near the hill station Pachmarhi (Madhya Pradesh, India). The on-site temperature, pH, and total dissolved solids concentration of the hot spring were 43.5 °C, 7.8, and 590 parts per million, respectively (Saxena et al., 2015). Also *Gulbenkiania* sp. strain 27M, which shares 100% partial (640 bp) 16S rRNA gene sequence similarity with both *G. mobilis* E4FC31^T and *G. indica* HT27^T, was isolated from water of the Mphephu hot spring in Limpopo, South Africa, with a temperature of 42.4 °C, which has been used as a resort for human recreational purposes (Jardine et al., 2017). In another study, the strain IARI-MB-18 was isolated from the Manikaran thermal springs located in Himachal Pradesh, India (Verma

et al., 2015), which shares 100% partial 16S rRNA gene sequence similarity with *G. mobilis* E4FC31^T.

Interestingly, culture independent methods (16S rRNA gene Illumina MiSeq sequencing) suggest the presence of bacteria affiliated to the genus *Gulbenkiania* in the human microbiota (Bayal et al., 2021, Engen et al., 2021). According to Bayal et al. (2021), organisms belonging to genera *Gulbenkiania* and *Staphylococcus* constitute the core population of the skin microbiota of healthy humans, but not of leprosy patients. In another study, Engen et al. (2021) reported an increased prevalence of bacteria belonging to the genus *Gulbenkiania* in the nasopharyngeal microbiota of COVID-19 positive patients when compared to that of non-infected human controls.

8. ENRICHMENT/ISOLATION PROCEDURES

Strain E4FC31^T was the first *Gulbenkiania* sp. representative whose isolation was reported in the literature (Vaz-Moreira et al., 2007). This type strain was isolated from the treated effluent of an urban wastewater treatment plant (Ferreira da Silva et al., 2007, Vaz-Moreira et al., 2007). Briefly, the effluent of the secondary clarifier was serially diluted in sterile saline solution (0.85% (w/v) NaCl) and 1 ml samples were filtered through cellulose nitrate membranes (0.45 µm pore size, 47 mm diameter); the membranes were placed onto m-FC agar, and the cultures incubated at 35 °C for 24 h. *G. mobilis* E4FC31^T, which formed an atypical greenish colony in this faecal coliforms selective medium, was purified by subculturing on PCA (Ferreira da Silva et al., 2006, Ferreira da Silva et al., 2007, Vaz-Moreira et al., 2007). *G. indica* HT27^T was isolated from the sediment of a sulfur spring. Briefly, 10 g (wet weight) of sediment was suspended in 50 ml of Nutrient Broth and incubated overnight at 37 ºC, 200 rpm. After incubation, the suspension was serially diluted and plated onto NA. *G. indica* HT27^T, which

formed a cream-coloured colony, that was further preserved and characterized (Jyoti et al., 2010).

9. MAINTENANCE PROCEDURES

Gulbenkiania spp. can be routinely grown on PCA or NA agar at 30 ºC. For long-term preservation, cultures can be stored suspended in a nutritive broth supplemented with 15% (v/v) glycerol at -80 ºC. The type strains of the species of the genus *Gulbenkiania* are supplied as freeze dried cultures by different culture collections (e.g., DSMZ, *G. mobilis* E4FC31^T and JCM, *G. indica* HT27^T).

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

Based on the 16S rRNA gene sequence, at the moment of writing, the closest related species to *Gulbenkiania* include members of the genera *Paludibacterium* (*P. purpuratum*) and *Pseudogulbenkiania* (*Ps. subflava*), with which *G. mobilis* E4FC31^T and *G. indica* HT27^T share pairwise 16S rRNA gene sequence similarity values of 95.8-95.3% and 95.5-94.4%, respectively. Other related neighbours include *Ps. gefcensis* and *P. yongneupense* with which *G. mobilis* E4FC31^T and *G. indica* HT27^T share pairwise 16S rRNA gene sequence similarity values of 94.7-94.5% and 93.8-94.1%, respectively. *Gulbenkiania* spp. can be distinguished from members of *Paludibacterium* and *Pseudogulbenkiania* due to their lower temperature and narrower pH range for growth (15/20 °C - 37/42 °C and pH 6/7 - 8 for *Paludibacterium*, 15/25 °C - 40/42 °C and pH 5.5/6 - 7/8 for *Pseudogulbenkiania*) (Kang et al., 2016, Lee et al., 2013, Lin et al., 2008). In addition, the inability of *Gulbenkiania* spp. to assimilate D-glucose and D-fructose as sole carbon sources allows their distinction from *Paludibacterium* spp. and

Pseudogulbenkiania spp., respectively (Kang et al., 2016, Lee et al., 2013). Also the absence of catalase activity in *Pseudogulbenkiania* spp. contributes to their distinction from *Gulbenkiania* spp. (Lee et al., 2013, Lin et al., 2008).

Although not supported by phenotypic testing, the positioning of the genus *Gulbenkiania* within the order *Neisseriales* was recently reviewed (Chen et al., 2021). According to this study based the phylogenomic and comparative analyses of deduced protein sequences from 110 *Neisseriales* genomes and 3 *Chitinimonas* genomes, the genus *Gulbenkiania* integrates the clade *Chromobacteriaceae*, together with the genera *Paludibacterium*, *Chromobacterium*, *Aquitalea*, *Vogesella*, *Pseudogulbenkiania* and *Crenobacter*. In their study, Chen et al. (2021) incorrectly identify the type strain of *G. mobilis* as strain MB1, which means that only the species *G. indica* might have been considered in this study. However, that fact is not supposed to affect the conclusion about the positioning of the genus.

11. TAXONOMIC COMMENTS

Based on 16S rRNA gene sequence phylogenetic analysis, the type strain of the species *Gulbenkiania indica* shares 99.2% sequence identity with strain E4FC31^T, the type strain of the type species of the genus *Gulbenkiania*. According to Jyoti et al. (2010), the level of DNA–DNA relatedness between strains HT27^T and E4FC31^T (30%) was well below the 70% threshold value proposed for separation of bacterial strains at the species level (Wayne et al., 1987). Based on the low DNA–DNA relatedness together with differentiating phenotypic traits, such as the ability of strain HT27^T to grow on MacConkey agar and in a wider pH range, its inability to grow anaerobically in the presence of nitrate, to grow in the presence of L-glutamic acid, L-leucine, L-proline or sodium salts of succinate, malate and fumarate, or to produce

237 indole and acetoin (Voges–Proskauer), supported the proposal of Jyoti et al. (2010) that strain
238 HT27^T represents a novel species, *G. indica*.
239 At the time of the description of the *Gulbenkiania* species, *G. mobilis* and *G. indica* were
240 integrated into the family *Neisseriaceae* (Jyoti et al., 2010, Vaz-Moreira et al., 2007). However,
241 based on phylogenetic and comparative analyses of 27 genome sequenced species of the order
242 *Neisseriales*, Adeolu and Gupta (2013) proposed the integration of genus *Gulbenkiania* in a
243 new family, *Chromobacteriaceae* fam. nov., together with *Andreprevotia*, *Aquaspirillum*,
244 *Aquitalea*, *Chitinibacter*, *Chitinilyticum*, *Chitiniphilus*, *Chromobacterium*, *Deefgea*,
245 *Formivibrio*, *Iodobacter*, *Jeongeupia*, *Laribacter*, *Leeia*, *Microvirgula*, *Paludibacterium*,
246 *Pseudogulbenkiania*, *Silvimonas* and *Vogesella*, also formely allocated to family
247 *Neisseriaceae.*
248 The type strain of *G. indica* HT27^T and the closest neighbours, members of the genera
249 *Paludibacterium*, *Pseudogulbenkiania*, *Chromobacterium* and *Aquitalea*, share ANI values
250 ranging from 77.12% to 78.83%, below the threshold proposed for the delineation of species
251 (95%) (Richter et al., 2016). However, the AAI values (66.07%-70.85%) are in the radiation
252 or above the cut-off value proposed for the genus boundary (65%) (Konstantinidis et al., 2017)
253 (Table 1). These observations suggest the high relatedness of these bacterial groups supporting
254 their assembly in the same family. According to the 16S rRNA gene sequence based
255 phylogenetic tree (Figure 1), the *Gulbenkiania* species validly named at the time of writing
256 form a distinct clade within the radiation of these closest neighbours. The availability of a
257 trustful whole genome assembly of *G. mobilis* E4FC31^T will permit to further confirm these
258 relationships in the future.
259
260 <Table 1 near here>
261

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12. LIST OF SPECIES OF THE GENUS *GULBENKIANIA*

1. *Gulbenkiania indica* Jyoti, Narayan, Das 2010, 1054[VP]

indica (in'di.ca. L. fem. adj. *indica* of India, the geographical origin of the type strain).

In addition to the genus description, the species is described as comprising Gram-negative rods, generally occurring singly, and motile through a single polar flagellum. Grows at 15-45 °C and pH 5.0-11.0. Growth occurs in the presence of 1% (w/v) NaCl but not with 2%.

Positive for catalase and cytochrome *c* oxidase tests. Assimilate sodium acetate, inositol, 4-aminobutyric acid, L-tyrosine, L-threonine, L-lysine, and L-cysteine. Do not assimilate D-glucose, D-mannose, D-fructose, D-xylose, D-rhamnose, D-arabinose, D-ribose, D-sorbose, D-mannitol, maltose, lactose, sucrose, raffinose, trehalose sodium succinate, sodium malate, sodium fumarate, L-glutamic acid, L-leucine, L-glycine, L-alanine, L-proline, L-aspartic acid, L-tryptophan, L-phenylalanine, L-methionine, L-serine, L-valine, L-isoleucine, L-histidine, and L-asparagine. Negative for methyl red test, Voges-Proskauer reaction, indole production, urease, H_2S production, citrate utilization, anaerobic growth in the presence of nitrate, gelatin, and starch hydrolysis.

The DNA G+C content (mol %) is 63.0 (HPLC method) - 63.6 (whole genome sequencing).

Type strain: HT27 (=DSM 17901 =JCM 15969).

GenBank accession number (16S rRNA): DQ415656.

GenBank accession number (genome): GCA_001418035.1

2. *Gulbenkiania mobilis*, Vaz-Moreira, Nobre, Nunes and Manaia 2007, 1111^{VP}

mobilis (mo'bi.lis. L. fem. adj. *mobilis* movable, motile).

In addition to the genus description, the species is described as comprising Gram-negative short curved rods, motile through a single polar flagellum. Do not form a capsule. Growth occurs between 15 and 45 °C, between pH 5.5 and 9.0, and in the presence of 1% (w/v) NaCl but not with 3%. Anaerobic growth occurs in the presence of nitrate, which is reduced to nitrite. Positive for catalase and cytochrome *c* oxidase tests. Produce indole and acetoin (Voges–Proskauer) and arginine dihydrolase. No specific organic growth factors are required. Malate, caprate, fumarate, lactic acid, succinate, glutamic acid, L-leucine, and L-proline support growth as sole carbon sources. None of the API 50CH carbon sources supports growth or acid production. Unable to assimilate D-glucose, D-mannose, D-galactose, D-fructose, D-ribose, D- and L-arabinose, D- and L-xylose, L-sorbose, L-rhamnose, methyl alpha-D-mannopyranoside, methyl alpha-D-glucopyranoside, methyl beta-D-xylopyranoside, amygdalin, arbutin, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, beta-gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D-adonitol, dulcitol, inositol, D-mannitol, D-sorbitol, D- and L-arabitol, xylitol, glycerol, erythritol, N-acetylglucosamine, L-asparagine, L-aspartic acid, L-histidine, L-serine, L-phenylalanine, L-hydroxyproline, glycine, L-alanine, L-arginine, potassium gluconate, citric acid, propionic acid, 2-ketogluconate, 5-ketogluconate, adipate, phenylacetate, gluconic acid, and citric acid. Do not ferment/oxidise D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, D-melibiose, amygdalin, and L-arabinose. Test negative for production of amylase, beta-galactosidase, urease, lysine and ornithine decarboxylases, hydrolysis of aesculin, gelatin, Tween 80, and H_2S production.

The DNA G+C content (mol %) is 63 (HPLC method)

Type strain: E4FC31 (=DSM 18507 =LMG 23770).

GenBank accession number (16S rRNA): AM295491.

RELATED ARTICLES

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gbm01833

BIBLIOGRAPHY

Adeolu, M. & Gupta, R. S. (2013) Phylogenomics and molecular signatures for the order *Neisseriales*: proposal for division of the order *Neisseriales* into the emended family *Neisseriaceae* and *Chromobacteriaceae* fam. nov. *Antonie Van Leeuwenhoek* 104: 1-24.

Bayal, N., Nagpal, S., Haque, M. M., Patole, M. S., Shouche, Y., Mande, S. C. & Mande, S. S. (2021) Structural aspects of lesional and non-lesional skin microbiota reveal key community changes in leprosy patients from India. *Scientific reports* 11: 1-17.

Chen, S., Rudra, B. & Gupta, R. S. (2021) Phylogenomics and molecular signatures support division of the order Neisseriales into emended families Neisseriaceae and Chromobacteriaceae and three new families Aquaspirillaceae fam. nov., Chitinibacteraceae fam. nov., and Leeiaceae fam. nov. *Syst Appl Microbiol* 44: 126251.

Engen, P. A., Naqib, A., Jennings, C., Green, S. J., Landay, A., Keshavarzian, A. & Voigt, R. M. (2021) Nasopharyngeal Microbiota in SARS-CoV-2 Positive and Negative Patients. *Biol Proced Online* 23: 10.

335 Ferreira Da Silva, M., Tiago, I., Verissimo, A., Boaventura, R. A., Nunes, O. C. & Manaia,
336 C. M. (2006) Antibiotic resistance of enterococci and related bacteria in an urban
337 wastewater treatment plant. *FEMS Microbiol Ecol* 55: 322-9.
338 Ferreira Da Silva, M., Vaz-Moreira, I., Gonzalez-Pajuelo, M., Nunes, O. C. & Manaia, C. M.
339 (2007) Antimicrobial resistance patterns in *Enterobacteriaceae* isolated from an urban
340 wastewater treatment plant. *FEMS Microbiol Ecol* 60: 166-76.
341 Jardine, J. L., Abia, A. L. K., Mavumengwana, V. & Ubomba-Jaswa, E. (2017) Phylogenetic
342 analysis and antimicrobial profiles of cultured emerging opportunistic pathogens
343 (Phyla *Actinobacteria* and *Proteobacteria*) identified in Hot Springs. *Int J Environ*
344 *Res Public Health* 14.
345 Jyoti, V., Narayan, K. D. & Das, S. K. (2010) *Gulbenkiania indica* sp. nov., isolated from a
346 sulfur spring. *Int J Syst Evol Microbiol* 60: 1052-1055.
347 Kang, H., Kim, H., Joung, Y., Kim, K.-J. & Joh, K. (2016) *Paludibacterium purpuratum* sp.
348 nov., isolated from wetland soil. *Int J Syst Evol Microbiol* 66: 2711-2716.
349 Konstantinidis, K. T., Rossello-Mora, R. & Amann, R. (2017) Uncultivated microbes in need
350 of their own taxonomy. *ISME J* 11: 2399-2406.
351 Lee, D. G., Im, D. M., Kang, H., Yun, P., Park, S. K., Hyun, S. S. & Hwang, D. Y. (2013)
352 *Pseudogulbenkiania gefcensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol*
353 63: 187-191.
354 Lin, M. C., Chou, J. H., Arun, A. B., Young, C. C. & Chen, W. M. (2008)
355 *Pseudogulbenkiania subflava* gen. nov., sp. nov., isolated from a cold spring. *Int J*
356 *Syst Evol Microbiol* 58: 2384-8.
357 Richter, M., Rossello-Mora, R., Oliver Glockner, F. & Peplies, J. (2016) JSpeciesWS: a web
358 server for prokaryotic species circumscription based on pairwise genome comparison.
359 *Bioinformatics* 32: 929-31.

Rodriguez-R, L. & Konstantinidis, K. (2016) The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ Preprints* 4: e1900v1.

Saxena, R., Chaudhary, N., Dhakan, D. B. & Sharma, V. K. (2015) Draft genome sequence of *Gulbenkiania mobilis* strain MB1, a sulfur-metabolizing thermophile isolated from a Hot Spring in Central India. *Genome Announc* 3.

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.

Verma, P., Yadav, A. N., Shukla, L., Saxena, A. K. & Suman, A. (2015) Hydrolytic enzymes production by thermotolerant *Bacillus altitudinis* IARI-MB-9 and *Gulbenkiania mobilis* IARI-MB-18 isolated from Manikaran hot springs. *Int J Adv Res* 3: 1241-1250.

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. & Authors, O. (1987) International Committee on Systematic Bacteriology. Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 37: 463–464.

379 Table 1. Average nucleotide (ANI) and amino acid (AAI) identity values shared by *G. indica*
380 DSM 17901^T and the type strains of the closest relatives for which a whole genome assembly
381 is available. Data obtained at http://enve-omics.ce.gatech.edu/at (Rodriguez-R and
382 Konstantinidis, 2016)

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	ANI (%)	AAI (%)
P. yongneupense DSM 18731^T	77.30	66.07
P. purpuratum CECT 8976^T	77.12	66.28
Ps. subflava DSM 22618^T	78.69	70.85
C. amazonense DSM 26508^T	78.55	68.66
C. paludis IIBBL 257-1^T	78.83	68.80
C. phragmitis IIBBL112-1^T	78.70	68.35
C. alkalinivorans IITR-71^T	78.59	69.39
A. denitrificans 5YN1-3^T	78.33	69.19
A. pelogenes CCM 7557^T	78.37	69.59

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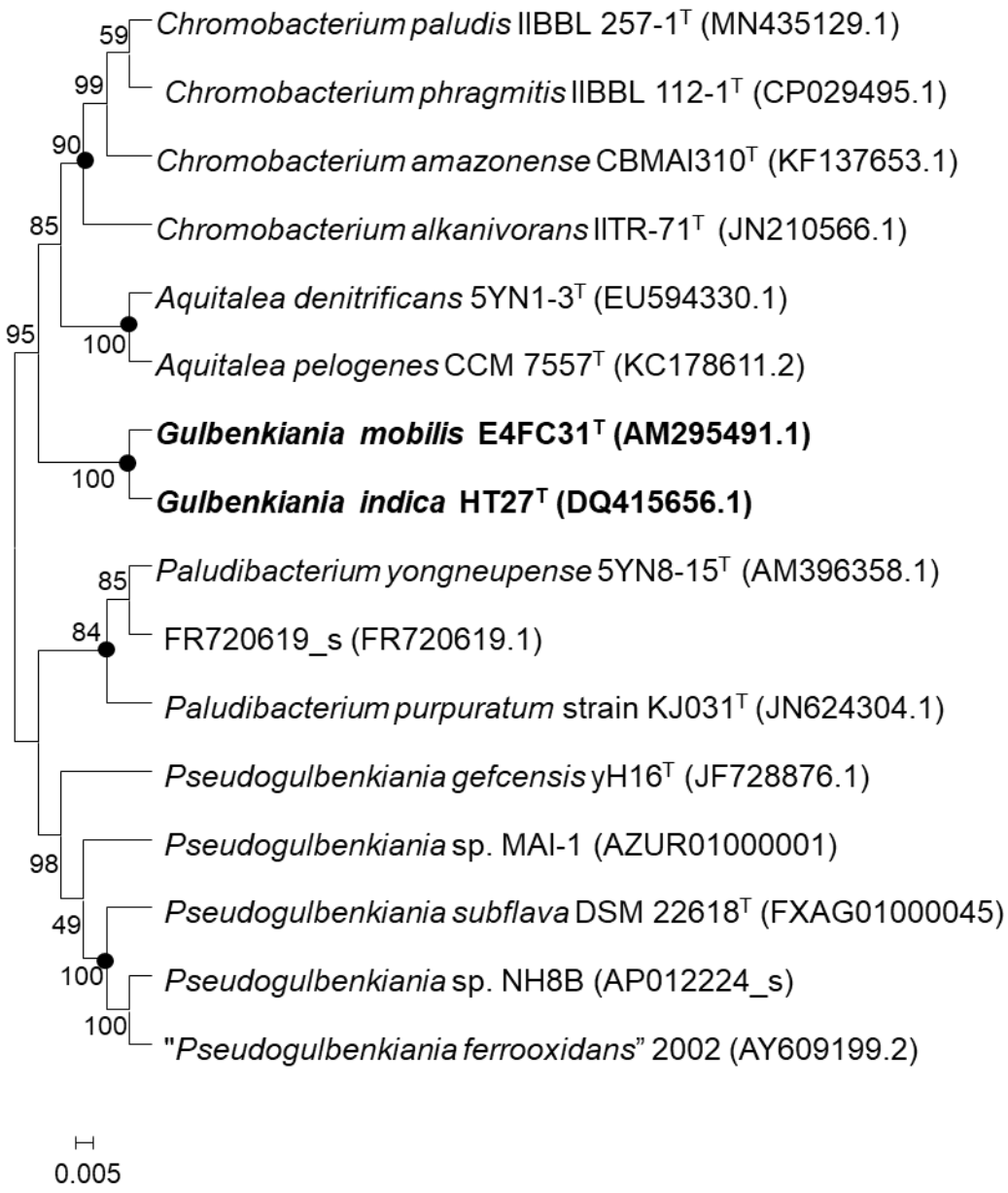

Figure 1. Dendrogram based on 16S rRNA gene sequences, showing the position of the *Gulbenkiania* species in relation to the type strains of species with which any of the two *Gulbenkiania* types strains share ≥ 94% sequence identity. The dendrogram was generated by the Neighbor-Joining method. Bootstrap values, generated from 1000 re-samplings, are indicated at branch points. Filled circles indicate branches on the tree that were also recovered in the tree generated using the maximum-likelihood and Maximum Parsimony algorithms. Bar, 1 substitution per 200 nucleotide positions.