# *Gulbenkiania mobilis* gen. nov., sp. nov., isolated from treated municipal wastewater

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A bacterial strain (E4FC31$^T$) isolated from treated municipal wastewater was characterized phenotypically and phylogenetically. Cells were Gram-negative, curved rods with a polar flagellum. The isolate was catalase-, oxidase- and arginine dihydrolase-positive, and able to grow between 15 and 45 °C and between pH 5.5 and 9.0. The predominant fatty acids were $C_{16:1}$/iso-$C_{15:0}$ 2-OH and $C_{16:0}$, the major respiratory quinone was ubiquinone 8 and the G+C content of the genomic DNA was 63 mol%. 16S rRNA gene sequence analysis indicated that strain E4FC31$^T$ belonged to the class *Betaproteobacteria* and was a member of the family *Neisseriaceae*. Its closest phylogenetic neighbours were *Aquitalea magnusonii* and *Chromobacterium violaceum* (<94 % 16S rRNA gene sequence similarity). Phylogenetic analysis and phenotypic characteristics of strain E4FC31$^T$ suggest that it represents a novel species of a new genus, for which the name *Gulbenkiania mobilis* gen. nov., sp. nov. is proposed. The type strain of *Gulbenkiania mobilis* is E4FC31$^T$ (=DSM 18507$^T$=LMG 23770$^T$).

At the time of writing, the family *Neisseriaceae* comprises more than 20 genera (Euzéby, 1997; http://www.bacterio.cict.fr/classifgenerafamilies.html#Neisseriaceae). Members of these genera have been isolated from human and animal clinical specimens, and from soil and water (Patureau *et al.*, 1998; Yuen *et al.*, 2001; Tønjum, 2005; Vela *et al.*, 2005; Lau *et al.*, 2006). Major distinguishing features among genera of the family *Neisseriaceae* are nutritional pattern, biochemical characteristics, such as the production of indole or arginine dihydrolase, and G+C content of the genomic DNA, supported by low 16S rRNA gene sequence similarities (Grimes *et al.*, 1997; Yuen *et al.*, 2001; Chern *et al.*, 2004; Tønjum, 2005; Lau *et al.*, 2006). Members of some of these genera, such as *Microvirgula* and *Laribacter* (Patureau *et al.*, 1998; Yuen *et al.*, 2001), do not have the ability to oxidize, ferment or assimilate sugars and have been referred to as asaccharolytic (Yuen *et al.*, 2001).

This paper describes a member of the family *Neisseriaceae*, strain E4FC31$^T$, that was isolated from treated wastewater on m-faecal coliform (m-FC) agar medium, during a survey of antibiotic resistance patterns of members of the *Enterobacteriaceae* (Ferreira da Silva *et al.*, 2007). The isolate was purified by subculturing, and was maintained on plate count agar (PCA). Long-term preservation was in modified Luria–Bertani (MLB) medium (Tiago *et al.*, 2004) supplemented with 15 % (v/v) glycerol, at −80 °C. Preliminary characterization, based on analysis of colony and cell morphology, Gram-staining, presence of endospores, poly-$\beta$-hydroxybutyrate granules, presence of a capsule, motility, number and position of flagella, cytochrome *c* oxidase and catalase, was based on the methodologies of Murray *et al.* (1994) and Smibert & Krieg (1994) as described by Manaia *et al.* (2003). Unless stated otherwise, all biochemical and physiological tests were performed in MLB medium, incubated at 30 °C. The pH range for growth was examined in culture medium containing 12 mM MES (Sigma) to adjust the pH to 5.5 and 12 mM CAPS (Sigma) to adjust the pH to 9.0 or 10.0. NaCl tolerance and temperature range for growth were assayed, respectively, in culture medium supplemented with 1 and 3 % NaCl (w/v) or incubated at 15, 22, 30, 40, 45 and 50 °C. The production of extracellular amylases, gelatinases and tweenases (Tween 80) was tested as described by Tiago *et al.* (2004).

The ability to grow anaerobically was tested in culture medium supplemented with 0.1 % $KNO_3$ (w/v), under a $N_2$-saturated atmosphere. Growth in minimal medium was tested in mineral medium B (Barreiros *et al.*, 2003) supplemented with 4 mM $(NH_4)_2SO_4$ and 5 mM malic acid, in

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain E4FC31$^T$ is AM295491.

100-ml screw-capped Erlenmeyer flasks containing 20 ml medium, with shaking at 120 r.p.m. Other biochemical and nutritional tests were performed by using the API 20E, API 20NE and API 50CH galleries (bioMérieux) following the manufacturer's instructions. The API 50CH gallery was inoculated with three different culture media: the medium recommended to test acid production (50 CHB/E; bioMérieux), mineral medium B with $(NH_4)_2SO_4$ and bioMérieux AUX medium recommended for the inoculation of API 20NE galleries. The ability to use other amino acids and organic acids was assayed in mineral medium B with $(NH_4)_2SO_4$ supplemented with 20 mM (amino acids) or 5 mM (organic acids) of the test substrate. Growth and colony morphology on selective/differential media were tested on m-FC, eosin methyl blue (EMB) agar, MacConkey agar, glucose broth and Columbia agar with 5 % sheep blood. Antibiotic susceptibility was assayed as described by Ferreira da Silva *et al.* (2006), following the interpretation criteria proposed by the Comité de l'Antibiogramme de la Société Française de Microbiologie (1998).

For the determination of the DNA G+C content, genomic DNA was extracted based on the method of Cashion *et al.* (1977) and G+C ratios were estimated by using the HPLC method as described by Mesbah *et al.* (1989). For analysis of respiratory quinones, freeze-dried cells were extracted according to Tindall (1989) and extracts were analysed by UV HPLC (Knauer) at 260 nm with a Lichrosphere 5-μm RP-18 column (Merck). A mixture of methanol/hexane (75 : 25, v/v) was used as the mobile phase at a flow rate of 1 ml $min^{-1}$. Retention times were compared with known standards. Fatty acid methyl esters (FAMEs) were analysed in 24-h cultures on trypticase casein soy agar. Cell harvesting and preparation of FAMEs were performed as described by Kuykendall *et al.* (1988). Separation, identification and quantification of individual FAMEs were achieved with the Sherlock version 4.6 microbial identification system (MIS-MIDI). FAMEs were extracted and analysed twice.

The nucleic acid sequence of the 16S rRNA gene was determined after PCR amplification of total DNA extracts as described by Rainey *et al.* (1996). The 16S rRNA gene sequence was compared with others available in the GenBank/EMBL/DDBJ database via BLASTN from NCBI and aligned with reference sequences included in those databases. The phylogenetic analysis was conducted by using MEGA software, version 3.1 (Kumar *et al.*, 2004). Sequence similarity was estimated based on the model of Jukes & Cantor (1969) and a phylogenetic tree was constructed with the neighbour-joining method. Other methods, namely maximum-parsimony, minimum-evolution and unweighted pair group analysis with arithmetic means, were used to assess tree stability. A total of 1335 nucleotide positions in each 16S rRNA gene were included in the analysis. Non-homologous and ambiguous nucleotide positions were excluded from the calculations.

Strain E4FC31$^T$ was isolated on m-FC agar medium. Whereas faecal coliform bacteria produce typical blue colonies on this selective and differential medium, strain E4FC31$^T$ produced atypical, greenish colonies. Subsequent DNA typing of the m-FC isolates revealed that strain E4FC31$^T$ presented a unique genotype, distinct from those obtained for the other isolates, subsequently identified as members of the family *Enterobacteriaceae* (Ferreira da Silva *et al.*, 2007). 16S rRNA gene sequence analysis showed that strain E4FC31$^T$ represented a member of the class *Betaproteobacteria*, branching within the family *Neisseriaceae*. The low levels of 16S rRNA gene sequence similarity (<94 %) with other members of the *Neisseriaceae* and the distinctive phenotypic characteristics of strain E4FC31$^T$ suggested its placement within a new genus (Table 1, Fig. 1). The closest phylogenetic neighbours of strain E4FC31$^T$ were *Aquitalea magnusonii* TRO-001DR8$^T$ (93.9 % 16S rRNA gene sequence similarity) and *Chromobacterium violaceum* ATCC 12472$^T$ (93.5 % similarity).

Although strain E4FC31$^T$ was isolated on m-FC agar, the isolate showed only poor and occasional growth on this culture medium. On EMB agar and MacConkey agar, also recommended for the isolation of enterobacteria, strain E4FC31$^T$ showed good growth with dark-red colonies and absence of growth, respectively. EC and glucose broths supported growth, although without gas production, from lactose and glucose, respectively. Similar results were observed for *A. magnusonii* LMG 23054$^T$ and *C. violaceum* LMG 1267$^T$. On blood agar, no haemolysis was observed and grey colonies were formed. On nutritive media, such as PCA or MLB agar, strain E4FC31$^T$ produced non-pigmented, regular and convex colonies 1–2 mm in size.

Strain E4FC31$^T$ was able to grow, after subculturing, in mineral medium with malic acid as the only source of carbon and energy, indicating that this organism has no specific growth requirements, e.g. vitamins, amino acids or nucleosides. Strain E4FC31$^T$ did not assimilate any of the API 50CH carbon sources when tested in mineral medium or in bioMérieux AUX medium. Similarly, strain E4FC31$^T$ did not produce acid from any of the carbon sources when tested in API 50CHB/E medium. Acid was not produced in this same culture medium, even when sodium malate was added as the single carbon source. Strain E4FC31$^T$ did not ferment or oxidize any of the API 20E carbon sources. In the carbon source assimilation tests with the API 20NE system, strain E4FC31$^T$ was able to use malate and caprate. Other carbon sources assimilated were organic acids and amino acids, namely fumarate, lactate, succinate, glutamic acid, L-leucine and L-proline. The nutritional pattern observed for isolate E4FC31$^T$ was consistent with the asaccharolytic profile described for *Laribacter hongkongensis* and *Microvirgula denitrificans*, but contrasts with that of its close phylogenetic neighbour *C. violaceum* (Patureau *et al.*, 1998; Yuen *et al.*, 2001; Gillis & Logan, 2005). Although *A. magnusonii* can also be considered to be asaccharolytic, its ability to use glucose distinguishes it from strain E4FC31$^T$ (Lau *et al.*, 2006). With regard to the key diagnostic tests of the API 20E gallery, strain E4FC31$^T$ displayed positive

**Table 1.** Differential characteristics between strain E4FC31$^T$ and the type strains of *A. magnusonii* and *C. violaceum*

Data were obtained in this study unless indicated. Strain E4FC31$^T$ was also negative for assimilation of glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, 2-ketogluconate, 5-ketogluconate, adipate, phenylacetate, L-hydroxyproline, glycine, L-alanine, L-arginine, gluconic acid, L-phenylalanine and citric acid, fermentation/oxidation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, D-melibiose, amygdalin and L-arabinose, production of amylase, β-galactosidase, urease, lysine and ornithine decarboxylases, aesculin hydrolysis and $H_2S$ and growth at 50 °C, pH 10 and 3 % NaCl.

| Characteristic | E4FC31$^T$ | *A. magnusonii* LMG 23054$^T$ | *C. violaceum* LMG 1267$^T$ |
|---|---|---|---|
| pH range for growth | 5.5–9.0 | 5.0–8.0[a]* | 4.0–8.0[b] |
| Growth at 45 °C | + | − | − |
| Sugars as carbon sources | − | + | + |
| Assimilation of: | | | |
| *N*-Acetylglucosamine | − | + | + |
| L-Asparagine | − | + | + |
| L-Aspartic acid | − | + | + |
| L-Histidine | − | + | + |
| L-Leucine | + | + | − |
| L-Serine | − | + | + |
| Potassium gluconate | − | + | + |
| Citric acid | − | + | +† |
| Propionic acid | − | + | + |
| D-Glucose | − | + | + |
| D-Mannose | − | − | + |
| Hydrolysis of: | | | |
| Gelatin | − | − | + |
| Tween 80 | − | − | + |
| Indole production | + | + | − |
| Acetoin production (Voges–Proskauer) | + | + | − |
| DNA G+C content (mol%) | 63 | 59[a] | 64[a] |
| Isolation source | Municipal wastewater | Humic lake | Soil and water |

*Data from: *a*, Lau *et al.* (2006); *b*, Gillis & Logan (2005).
†Negative for citrate assimilation in the API 20NE test.

reactions for arginine dihydrolase, indole production and the Voges–Proskauer reaction. It showed low 16S rRNA gene sequence similarity (<94 %) with its closest neighbours *A. magnusonii* LMG 23054$^T$ and *C. violaceum* LMG 1267$^T$, while the latter two type strains shared a sequence similarity of about 95 %. The chemotaxonomic characterization of strain E4FC31$^T$, namely FAME profile (Table 2) and the presence of ubiquinone 8 as the major respiratory quinone, confirmed its affiliation to the family *Neisseriaceae* (Wilkinson, 1988; Chern *et al.*, 2004; Yang *et al.*, 2005; Lau *et al.*, 2006). However, the low levels of 16S rRNA gene sequence similarity with recognized taxa in this family and the identification of distinctive phenotype characteristics support the proposal of a new genus to accommodate this organism. Characteristics that differentiate strain E4FC31$^T$ from *A. magnusonii* LMG 23054$^T$ include the inability to assimilate glucose, serine, histidine, asparagine, aspartic acid, propionic acid and citric acid, the ability to grow at 45 °C and a higher G+C DNA content (Table 1).

On the basis of the data presented, strain E4FC31$^T$ is considered to represent a novel species of a new genus within the family *Neisseriaceae*, for which the name *Gulbenkiania mobilis* gen. nov., sp. nov. is proposed.

## Description of *Gulbenkiania* gen. nov.

*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].

Cells are non-spore-forming, Gram-negative, motile, curved rods. Catalase-, oxidase- and arginine dihydrolase-positive. Mesophilic. Chemoorganoheterotrophic with aerobic respiratory metabolism. No specific organic growth factors are required. Nitrate is reduced to nitrite. Amino acids and organic acids, but not sugars, are used as single carbon sources. The major respiratory quinone is ubiquinone 8;

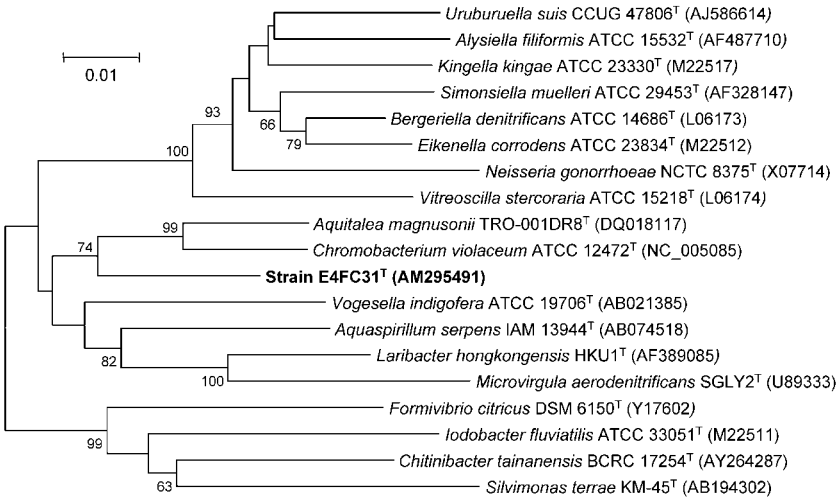


**Fig. 1.** Phylogenetic tree generated by the neighbour-joining method based on 16S rRNA gene sequences showing the nearest neighbours of strain E4FC31$^T$. Bootstrap values were generated from 10 000 resamplings; only values greater than 60 % are shown. Bar, 1 substitution per 100 nucleotide positions. The 16S rRNA gene has the gene ID 2548502 within the deposited genome sequence of *Chromobacterium violaceum* ATCC 12472$^T$.

ubiquinone 9 is a minor component. The DNA G+C content of the type strain of the type species is 63 mol%. $C_{16:1}$/iso-$C_{15:0}$ 2-OH and $C_{16:0}$ are the predominant fatty acids. Phylogenetically, the genus belongs to the family *Neisseriaceae*. The type species is *Gulbenkiania mobilis*.

## Description of *Gulbenkiania mobilis* sp. nov.

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

Cells are short rods (0.95±0.17 μm in length and 0.38±0.11 μm in width), motile by means of a polar flagellum. No capsule is seen. Produces indole and acetoin. Growth occurs between 15 and 45 °C and between pH 5.5 and 9. Growth occurs in the presence of 1 % NaCl but not with 3 %. Anaerobic growth occurs in the presence of nitrate. None of the API 50CH carbon sources supports growth or acid production. Malate, caprate, fumarate, lactic acid, succinate, glutamic acid, L-leucine and L-proline support growth as single carbon sources. Susceptible to amoxicillin (25 μg), gentamicin (10 μg), ciprofloxacin (5 μg), tetracycline (30 μg), SXT (sulfamethoxazole/trimethoprim; 23.75/1.25 μg), sulfamethoxazole (25 μg), cephalothin (30 μg), streptomycin (10 μg), ticarcillin (75 μg), ceftazidime (30 μg), meropenem (10 μg) and colistin sulphate (50 μg).

**Table 2.** Fatty acid composition of strain E4FC31$^T$ and the type strains of *A. magnusonii* and *C. violaceum*

Data were obtained in this study. Components comprising less than 1 % of the total are summed as 'Other' and included iso-$C_{11:0}$, $C_{11:0}$, iso-$C_{11:0}$ 3-OH, $C_{14:1}\omega5c$, $C_{15:0}$, iso-$C_{15:0}$, $C_{15:1}$, $C_{16:1}$, iso-$C_{17:1}$, anteiso-$C_{17:1}$, iso-$C_{17:0}$, $C_{17:0}$ and iso-$C_{20:0}$. −, Not detected.

| Fatty acid methyl ester | E4FC31$^T$ | *A. magnusonii* LMG 23054$^T$ | *C. violaceum* LMG 1267$^T$ |
|---|---|---|---|
| $C_{10:0}$ 3-OH | 2.8 | 2.6 | 6.3 |
| $C_{10:0}$ | 2.8 | 0.2 | 0.8 |
| $C_{12:1}$ 3-OH | − | − | 1.1 |
| $C_{12:0}$ 2-OH | 0.1 | 0.1 | 3.5 |
| $C_{12:0}$ 3-OH | 2.7 | 1.7 | 6.1 |
| $C_{12:0}$ | 4.4 | 7.6 | 7.4 |
| $C_{14:0}$ | 0.9 | 2.4 | 1.8 |
| $C_{16:1}\omega7c$/$C_{16:1}\omega6c$/iso-$C_{15:0}$ 2-OH | 46.8 | 48.5 | 32.7 |
| $C_{16:0}$ | 24.4 | 27.4 | 22.9 |
| $C_{17:0}$ cyclo | − | − | 4.5 |
| $C_{18:1}\omega7c$ | 9.1 | 6.9 | 11.3 |
| $C_{18:0}$ | 1.2 | 0.6 | 0.4 |
| Other | 4.8 | 2.0 | 1.2 |

The type strain, E4FC31$^T$ (=DSM 18507$^T$=LMG 23770$^T$), was isolated from treated municipal wastewater.

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