Caenimicrobium

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2. KEYWORDS: *Caenimicrobium hargitense*; *Alcaligenaceae*; activated sludge; gellan-gum-solidified oligotrophic medium

3. ABSTRACT:

Gram-negative staining **non-motile** short rods forming beige colonies on nutrient medium. **Mesophilic**, **aerobic**, catalase- and cytochrome *c* oxidase positive. **Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an unidentified phospholipid, and major cellular fatty acids are $C_{16:1}$ ω7c, $C_{16:0}$, cyclo $C_{17:0}$ and $C_{18:1}$ ω7c. Ubiquinone 8** is the major respiratory quinone. The type and unique species is *Caenimicrobium hargitense*; represented by the type strain CGII-59m2^T isolated from activated sludge. *Caenimicrobium hargitense* strain CGII-59m2^T shares 16S rRNA gene sequence similarity >97% with members of the genera *Bordetella*, *Candidimonas*, *Paracandidimonas* and *Parapusillimonas*.

4. DEFINING PUBLICATION:

Caenimicrobium, Felföldi, Schumann, Mentes, Kéki, Máthé and Tóth, 2017, 630^{VP}.

5. ETYMOLOGY:

Caenimicrobium [Cae.ni.mi.cro'bi.um, L. neut. n. *caenum* mud, referring to the isolation of the type strain from activated sludge; N.L. neut. n. *microbium* microbe (from Gr. adj. *mikros* small and Gr. n. *bios* life); N.L. neut. n. *Caenimicrobium* mud (-inhabiting) microbe].

6. GENERIC DEFINITION:

Gram-negative staining **non-motile** short rods forming beige colonies on nutrient medium. **Mesophilic**, **aerobic**, catalase- and cytochrome *c* oxidase positive. **Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an**

unidentified phospholipid, and major cellular fatty acids are $C_{16:1}$ ω7c, $C_{16:0}$, cyclo $C_{17:0}$ and $C_{18:1}$ ω7c. Ubiquinone 8 is the major respiratory quinone. The type and unique species is *Caenimicrobium hargitense*; represented by the type strain CGII-59m2^T isolated from activated sludge. *Caenimicrobium hargitense* strain CGII-59m2^T shares 16S rRNA gene sequence similarity >97% with members of the genera *Bordetella*, *Candidimonas*, *Paracandidimonas* and *Parapusillimonas*.

The DNA G+C content (mol %) is 62.3 (HPLC method).

Type species: *Caenimicrobium hargitense*, Felföldi, Schumann, Mentes, Kéki, Máthé and Tóth, 2017, 631^{VP}.

Number of species with validated names: 1.

7. FAMILY CLASSIFICATION:

Alcaligenaceae (fbm00180)

8. FURTHER DESCRIPTIVE INFORMATION:

8.1. Cell, colonial and cultural characteristics:

The genus *Caenimicrobium* comprises a single species, *C. hargitense*, represented by a single strain, CGII-59m2^T (Felföldi et al., 2017). Cells are Gram-negative non-motile, non-spore forming rods, with 0.6–0.8 µm width and 1.1–2.2 µm length. On nutrient agar media, forms beige circular and raised colonies, with 2-3 mm of diameter. *C. hargitense* CGII-59m2^T presents heterotrophic aerobic metabolism in the mesophilic temperature range.

8.2. Nutrition and growth conditions:

C. hargitense strain CGII-59m2^T can grow between pH 7 and 11 and in NaCl concentrations up to 8% (w/v). Good growth is reported at 28 ºC. The growth temperature range is 4-55 ºC, and not 4-65 ºC as is incorrectly reported in the species description (Felföldi et al., 2017; Felföldi, Personal Communication). Strain CGII-59m2^T is reported as being unable to assimilate any of the API 50CH carbon sources, which might be due to some growth requirements such as vitamins or amino acids. Strain CGII-59m2^T was able to grow on complex nutritive media such as R2A or TSA.

8.3. Metabolism:

C. hargitense strain CGII-59m2^T is aerobic, capable of reducing nitrate, unable to produce acid from D-glucose either through fermentation or oxidative metabolism. Reported positive enzymatic assays include cytochrome *c* oxidase, catalase, esterase (C4), leucine arylamidase, alkaline phosphatase (weakly), esterase lipase (C8) (weakly), and naphthol-AS-BI-phosphohydrolase.

8.4. Chemotaxonomic characteristics:

As other members of the family *Alcaligenaceae*, strain CGII-59m2^T presents ubiquinone Q-8 as the major isoprenoid quinone and a polar lipids profile characterized by the presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, along with other minor unknown phospholipids and phosphatidylserine. Predominant fatty acids are the saturated $C_{16:0}$ (28.5%) and $C_{12:0}$ (5.4%), the unsaturated $C_{16:1}$ ω7c (28.7%) and $C_{18:1}$ ω7c (13.6%), the cyclo $C_{17:0}$ (13.7%) and the hydroxyl $C_{14:0}$ 3-OH (7.0%). The diagnostic diamino acid *meso*-2,6-diaminopimelic acid is present in the cell wall.

9. ENRICHMENT/ISOLATION PROCEDURES:

Strain CGII-59m2T was isolated in Odorheiu Secuiesc (Harghita County, Romania) from a bioreactor digesting a landfill leachate. Isolation was performed on a diluted formulation of R2A medium in a proportion (v/v) of 3.6/10, amended with 1.33 g L^{-1} $CaCl_2$ and 1.81 g L^{-1} NH_4Cl and a final pH 8.0, solidified with 10 g L^{-1} of gellan gum (Gelzan CM, Sigma). Serial dilutions were plated on this medium and incubated at room temperature (~22 ºC).

10. MAINTENANCE PROCEDURES:

Maintenance growth can be on R2A and nutrient agar (DSMZ medium 1; www.dsmz.de) media or TSA agar, at pH 7.0 and 28 ºC.

11. DIFFERENTIATION OF THE GENUS *CAENIMICROBIUM* FROM OTHER GENERA:

The genus *Caenimicrobium* comprises a single species, *C. hargitense*, represented by a single strain, CGII-59m2^T. This strain has the closest 16S rRNA gene sequence based phylogenetic neighbors in the genera *Parapusillimonas (Parapusillimonas granuli*, 97.7%*)*, *Bordetella (Bordetella flabilis*, 97.4%), *Paracandidimonas* (*Paracandidimonas caeni*, 97.2%) and *Candidimonas (Candidimonas nitroreducens*, 97.2%). Whereas according to the 16S rRNA gene-based phylogenetic definition of species *C. hargitense* represents a new species, a limited number of phenotypic differentiating traits were listed in the description publication (Felföldi et al., 2017). The new species phenotypic differentiation was supported on the absence of motility, which is observed in the genus *Parapusillimonas*, the higher salt tolerance than that observed in members of the genus *Bordetella*, and on the absence of acid

phosphatase enzyme activity, and the distinct fatty acids profile when compared with members of the genera *Bordetella*, *Paracandidimonas* and *Candidimonas*. However, with the exception of *Parapusillimonas granuli*, this comparative analysis was based on non-simultaneous testing. Different culture conditions may influence the results obtained for the fatty acid methyl esters (FAMES) composition. This is illustrated by the comparison of the FAMEs data reported by Kim et al. (2010) and by Felföldi et al. (2017) for the supposedly same strain LMG 24012^T (*Parapusillimonas granuli* Ch07^T), in which 2 or 3 days of incubation at 28 ºC were used, respectively. In both studies the components are respectively: $C_{16:1}$ ω7c, 12.3% and 28.5% (included in summed feature 3, SF3); $C_{16:0,}$ 30.8% and 33.2%; cyclo $C_{17:0,}$ 29.3% and 18.2%; $C_{18:1}$ ω7c, 7.7% and not detected; and $C_{14:0}$ 3-OH, 7.7% and possibly included in summed feature 2 (SF2), which represents 7.4%.

<Figure 1 near here>

12. TAXONOMIC COMMENTS:

Caenimicrobium hargitense strain CGII-59m2^T presents 16S rRNA gene sequence similarity >97% with members of genera *Bordetella* (see gbm00928), *Candidimonas*, *Paracandidimonas* and *Parapusillimonas*. Lower 16S rRNA gene sequence similarity values are observed with members of the genera *Pusillimonas* (*Pusillimonas soli*, 96.8%), *Achromobacter* (*Achromobacter aloeverae*, 96.8%) *Paralcaligenes* (*Paralcaligenes ginsengisoli*, 96.4%), *Eoetvoesia* (*Eoetvoesia caeni*, 96.4%), *Saccharedens* (*Saccharedens versatilis*, 96.3%) and *Advenella* (*Advenella mimigardefordensis*, 96.2%). Although the 16S rRNA gene sequence based phylogenetic analyses allows the definition of a new taxon represented by strain CGII-59m2^T (Figure 1), in the description publication (Felföldi et al., 2017) phenotypic

differentiation is mainly supported by chemotaxonomic traits, in particular the fatty acid methyl esters composition, which were, nevertheless, not tested in parallel for all the closest neighbours.

13. LIST OF SPECIES OF THE GENUS *CAENIMICROBIUM*:

Caenimicrobium hargitense [har.git.en'se. N.L. neut. adj. *hargitense* of, or belonging to Hargita, referring to the Harghita Mountains (Latin, Hargita), from where the landfill leachate originated which was treated by the bioreactor and gave rise to the isolation of the type strain].

Rod-shaped (0.6–0.8 x 1.1–2.2 µm) and non-motile cells, able to grow on nutrient agar medium, where can form beige-coloured, circular and raised colonies with 2–3 mm diameter. Growth occurs in a pH range of 7 to 11 and in concentrations of NaCl up to 8%. It reacts positively to oxidase, catalase, and nitrate reduction tests and for the activity of the enzymes esterase (C4), leucine arylamidase, alkaline phosphatase, esterase lipase (C8), and naphthol-AS-BI-phosphohydrolase. None of the API 50 CH carbon sources are assimilated, acid is not produced from D-glucose under either fermentation or oxidation conditions, and enzyme activity tests are negative for urease, starch hydrolysis, for acid phosphatase, lipase (C14), valine arylamidase, trypsin, cystine arylamidase, alpha-chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase.

The DNA G+C content (mol %) is 62.3 mol% (HPLC).

Type strain: CGII-59m2 (=DSM 29806 =NCAIM B.02615)

GenBank accession number (16S rRNA): NR_156161; KM083134.

RELATED ARTICLES:

gbm00928

BIBLIOGRAPHY:

Felföldi, T., Schumann, P., Mentes, A., Kéki, Z., Máthé, I. & Tóth, E. M. (2017) *Caenimicrobium hargitense* gen. nov., sp. nov., a new member of the family *Alcaligenaceae* (*Betaproteobacteria*) isolated from activated sludge. *Int J Syst Evol Microbiol* 67: 627-632.

Kim, Y. J., Kim, M. K., Im, W. T., Srinivasan, S. & Yang, D. C. (2010) *Parapusillimonas granuli* gen. nov., sp. nov., isolated from granules from a wastewater-treatment bioreactor. *Int J Syst Evol Microbiol* 60: 1401-6.

Figures:

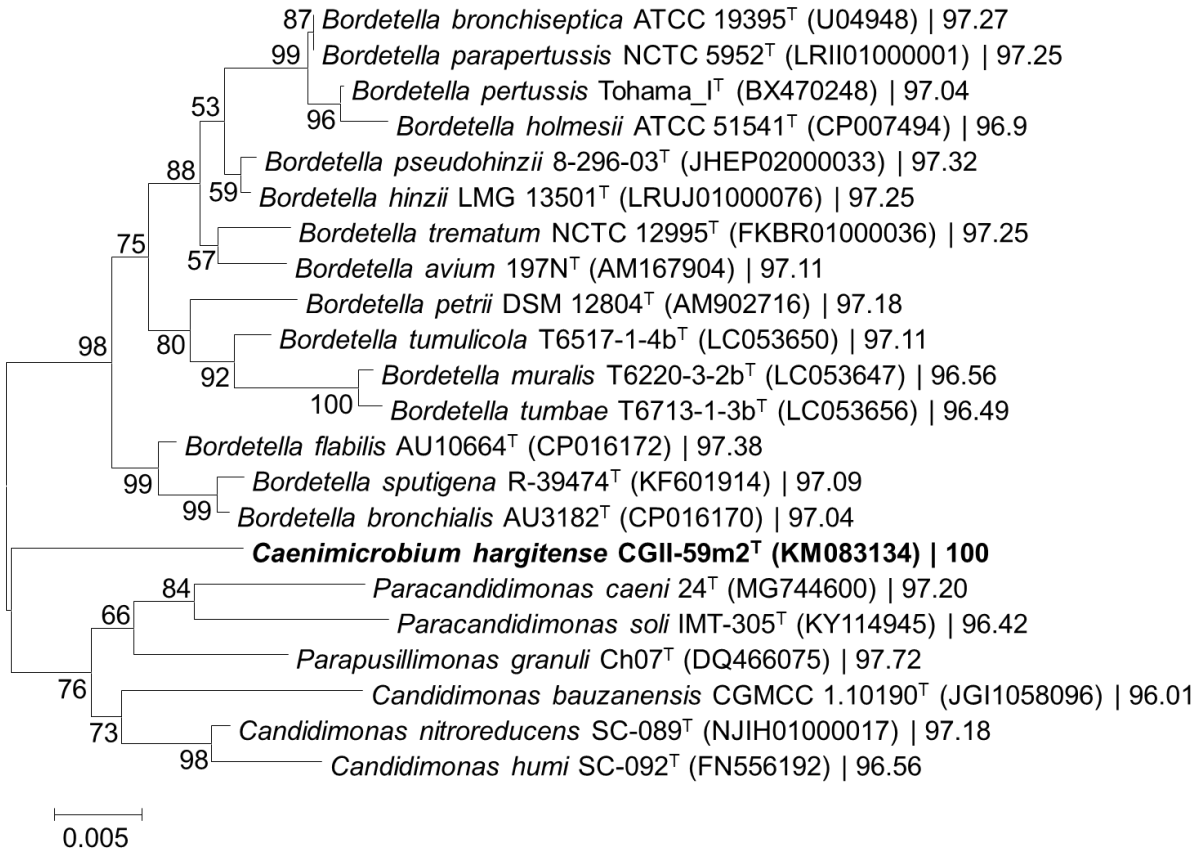

Figure 1. Dendrogram representing the relationship of *Caenimicrobium hargitense* CGII-59m2^T with the type strains of the species of genera with which it shares >97% 16S rRNA gene sequence identity. The dendrogram was reconstructed based on 1366 16S rRNA gene sequence positions using the neighbour-joining method. Bootstrap values >50 are indicated in the respective branches, as well as the species name, strain designation, GenBank accession numbers, and 16S rRNA sequence identity with strain CGII-59m2^T. Bar, 0.005 substitutions per nucleotide.