Parapusillimonas

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2. KEYWORDS: *Parapusillimonas*; *Alcaligenaceae*; granules; UASB reactor;

3. ABSTRACT

Gram-staining-negative motile short **rods**, with **three flagella**. Facultative anaerobic. Catalase- and cytochrome *c* oxidase positive. **Chemo-organotrophic**. Sugar, alcohols, organic acids, and amino acids can be used as single carbon sources. **Ubiquinone 8** is the major respiratory quinone and the predominant fatty acids are **$C_{16:0}$**, **summed feature 3** ($C_{16:1}$ ω7*c*/$C_{15:0}$ iso 2-OH), **$C_{17:0}$ cyclo**, and **summed feature 5** ($C_{18:1}$ ω7*c*/ω9*t*/ω12*t*). Major polar lipids are **phosphatidylethanolamine**, **phosphatidylglycerol**, and **diphosphatidylglycerol**. **Putrescine** is the major polyamine.

4. DEFINING PUBLICATION

Parapusillimonas, Kim, Kim, Im, Srinivasan and Yang 2010, 1405^{VP}.

5. ETYMOLOGY

Parapusillimonas (Pa.ra.pu.sil.li.mo´nas. Gr. prep. *para* like; N.L. fem. n. *Pusillimonas* bacterial genus name; N.L. fem. n. *Parapusillimonas* a bacterium like *Pusillimonas*).

6. GENERIC DEFINITION

Gram-staining-negative motile short **rods**, with **three flagella**. Facultative anaerobic. Catalase- and cytochrome *c* oxidase positive. **Chemo-organotrophic**. Sugar, alcohols, organic acids, and amino acids can be used as single carbon sources. **Ubiquinone 8** is the major respiratory quinone and the predominant fatty acids are **$C_{16:0}$**, **summed feature 3** ($C_{16:1}$ ω7*c*/$C_{15:0}$ iso 2-OH), **$C_{17:0}$ cyclo**, and **summed feature 5** ($C_{18:1}$ ω7*c*/ω9*t*/ω12*t*). Major polar lipids are **phosphatidylethanolamine**, **phosphatidylglycerol**, and **diphosphatidylglycerol**. **Putrescine** is the major polyamine.

The DNA G+C content (mol %) is 67.9 (HPLC) and 64.6 (genome).

Type species: ***Parapusillimonas granuli***, Kim, Kim, Im, Srinivasan and Yang 2010, 1405^{VP}.

Number of species with validated names: 1.

7. FAMILY CLASSIFICATION

Alcaligenaceae (fmb00180)

8. FURTHER DESCRIPTIVE INFORMATION

8.1. Cell morphology

The genus *Parapusillimonas* currently contains a single species, *Parapusillimonas granuli*, described upon the characterization of a single isolate, named Ch07^{T} (Kim *et al.*,

2010). Cells are Gram-negative short rods, 0.9 µm wide and 1.5 µm long, motile by means of three lophotrichous flagella. Fimbriae of 7-8 nm width are also produced by cells.

8.2. Colonial and cultural characteristics

On R2A medium, the type strain of *Parapusillimonas granuli* (Ch07^T) forms beige-coloured, smooth and circular colonies, with 2 mm of diameter, after 5 days of incubation at 30 ºC.

8.3. Nutrition and growth conditions

The type strain of *Parapusillimonas granuli* (Ch07^T) is able to use a wide range of sugars, alcohols, organic acids, and amino acids as sole carbon sources, such as D-glucose, D-sorbitol, adipate, phenylacetate, sodium acetate, lactate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, potassium 2-ketogluconate, malate, citrate, propionate, valerate, L-proline, L-alanine, L-serine, L-histidine, itaconate, and gluconate. Able to grow on R2A medium in the temperature and pH ranges of 25-37 ºC and 6.5-7.5, respectively, with optimal at 30 ºC and pH 7.0. It is capable of growth on R2A agar supplemented with 0.1% (w/v) thioglycolate under anaerobic conditions.

8.4. Chemotaxonomic characteristics

The major respiratory quinone is ubiquinone 8 and the major polyamine is putrescine. The polar lipids are dominated by phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and diphosphatidylglycerol (DPG). The predominant fatty acids (> 10%) are $C_{16:0}$ (33.2%), summed feature 3 ($C_{16:1}$ *ω7c*/$C_{15:0}$ iso 2-OH) (28.5%), $C_{17:0}$ cyclo (18.2%), and summed feature 5 ($C_{18:1}$ *ω7c*/*ω9t*/*ω12t*) (11.3%).

8.5. Genome features

The genome sequence of the type strain *Parapusillimonas granuli* LMG 24012^T obtained based on Illumina MiSeq is available in the GenBank under the Bioproject reference PRJNA626537, Biosample SAMN15438394. The draft genome assembly resulted in 42 contigs and scaffolds, with an N50 contig size of 443,577 kb and L50 of 5, a coverage of 269.5 x, total length of 4,65 Mb, and a G+C content (mol %) of 64.6 (and 67.9 based on HPLC method, Kim *et al.*, 2010). The draft genome sequence had 4,274 candidate protein-coding genes (CDSs), 5 rRNAs, and 47 tRNAs. Similar data was obtained for the draft genome of *Parapusillimonas granuli* DSM 18079^T (Bioproject reference PRJNA632213).

8.6. Ecology and Habitat

The type strain of the single species of the genus *Parapusillimonas* was isolated from granules collected from a wastewater-treatment bioreactor of an alcohol fermentation plant located in Daejeon, Republic of Korea (Kim *et al.*, 2010).

Other isolates identified as members of the genus *Parapusillimonas* (strain ES-QY-3 and strains Beta-28 and Beta-66) were isolated from an activated sludge sample collected from a wastewater treatment plant from Shangai (Huang *et al.*, 2009, 2010), and a soil sample from a forest inside the campus of Kyonggi University, in South Korea (Chaudhary *et al.*, 2019), respectively. Strains ES-QY-3 (16S rRNA gene accession number FJ529031), Beta-28 (16S rRNA gene accession number MH698878), and Beta-66 (16S rRNA gene accession number MH698916) showed 16S rRNA gene sequence identity values of 98.2, 98.0, and 97.9%, respectively, with *P. granuli* Ch07^T (DQ466075).

Other representatives currently related to the genus *Parapusillimonas* were identified by culture-independent techniques in bioaugmented zeolite-biological aerated filters (Z-BAFs) from China (Bai *et al.*, 2011), in the washing water of an exhaust air treatment system of a large-scale pig housing facility in Germany (Haneke *et al.*, 2010), in chromium-contaminated soils from Hunan Province (China) (He *et al.*, 2016), in a liquid asphalt from Pitch Lake, in Trinidad and Tobago (Schulze-Makuch *et al.*, 2011), and from a mesophilic biogas fermentor of beet silage (Krakat *et al.*, 2011).

9. ENRICHMENT AND ISOLATION PROCEDURES

Strain Ch07^T, the type strain of the species *Parapusillimonas granuli*, was isolated from a uniform mixture prepared from the brownish-black granules, about 2 mm in diameter, collected from an upflow anaerobic sludge blanket (UASB) reactor, by utilizing an Ace Homogenizer (Nihonseiki Co.). For bacterial isolation, the sample was serially diluted in 50 mM phosphate buffer at pH 7.0, plated on R2A (Difco), and incubated under aerobiosis. Strain Ch07^T was purified among the resulting colonies.

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

The genus *Parapusillimonas* comprises a single species, *P. granuli*, represented by a single strain, Ch07^T. Based on the EzBiocloud comparison (Yoon *et al.*, 2017), the closest 16S rRNA gene sequence-based phylogenetic neighbours of this strain are members of the genera *Paracandidimonas* (*Paracandidimonas caeni* 24^T, 98.1%), *Candidimonas*

(*Candidimonas nitroreducens* SC-089^T, 98.0%), *Pusillimonas* (*Pusillimonas thiosulfatoxidans* YE3^T, 97.9%; *Pusillimonas soli* MJ07^T, 97.8%; *Pusillimonas caeni* KCTC 42353^T, 97.7%) and *Caenimicrobium* (*Caenimicrobium hargitense* CGII-59m2^T, 97.7%). Other closely related neighbours include members of the genera *Candidimonas*, *Eoetsevia* or *Orrella*, with percentages of similarity ≤ 97.5%. Figure 1 exhibits the phylogenetic position of *Parapusillimonas granuli* in relation to other close neighbours.

The phylogenetic trees reconstructed by the neighbour-joining and maximum-likelihood algorithms showed that strain Ch07^T fell into a distinct clade with *Caenimicrobium hargitense* CGII-59m2^T, although it shared a higher similarity with the genus *Paracandidimonas*.

Parapusillimonas granuli Ch07^T could be distinguished from members of the genera *Caenimicrobium* and *Paracandidimonas* based on its narrowest temperature and pH growth ranges (25-37 ºC and pH 6.5-7.5 for *Parapusillimonas*; while 4-65 ºC and pH 7-11, and 15-42 ºC and pH 6-8 for *Caenimicrobium* and *Paracandidimonas*, respectively), its ability to hydrolize urea and assimilate gluconate, and by the presence of summed feature 5 ($C_{18:1}\omega7c/\omega9t/\omega12t$) (Kim *et al.*, 2010; Felföldi *et al.*, 2017; Kämpfer *et al.*, 2017; Yao *et al.*, 2019). In addition, some other differences in biochemical properties and substrate assimilation patterns are observed, such as its capacity to grow anaerobically, to produce acids from D-glucose or its mobility by contrast to *Caenimicrobium*, as well as, the positive enzymatic activity valine arylamidase, the negative enzymatic activities alkaline phosphatase and napthol-AS-BI-phosphohydrolase or the ability to use some substrates as sole carbon sources (e.g. D-glucose, potassium 2-ketogluconate or sorbitol) (Kim *et al.*, 2010; Felföldi *et al.*, 2017).

<Figure 1 near here>

11. TAXONOMIC COMMENTS

Parapusillimonas granuli presents high pairwise 16S rRNA gene sequence similarity with members of the genera *Paracandidimonas*, *Candidimonas*, *Pusillimonas*, *Caenimicrobium*, *Eoetsevia* or *Orrella*, above the threshold (95%) proposed for genus definition (Tindall *et al.*, 2010; Rosselló-Móra & Amann, 2015), although higher 16S rRNA gene sequence similarities (even around 97-98%) have been assumed for the differentiation of genera within the family *Alcaligenaceae* (Blümel *et al.*, 2001; Stolz *et al.*, 2005; Vaz-Moreira *et al.*, 2011; Felföldi *et al.*, 2014, 2017; Kämpfer *et al.*, 2017). Besides the phylogenetic differentiation of *Parapusillimonas granuli* from its closest neighbours, distinctive phenotypic and chemotaxonomic features were limited in number (e.g. *Parapusillimonas granuli* Ch07^T presented narrow temperature and pH growth ranges [25-37 ºC and pH 6.5-7.5] in comparison to *Caenimicrobium* [4-65 ºC and pH 7-11] and *Paracandidimonas* [15-42 ºC and pH 6-8], could hydrolize urea and assimilate gluconate, and presented summed feature 5 [$C_{18:1}\omega7c/\omega9t/\omega12t$] [Kim *et al.*, 2010; Felföldi *et al.*, 2017; Kämpfer *et al.*, 2017; Yao *et al.*, 2019]).

In addition, the Average Amino-acid Identity (AAI) values between *Parapusillimonas granuli* and the type strains of *Pusillimonas harenae*, *Pusillimonas thiosulfatoxidans*, *Pusillimonas ginsengisoli*, *Candidimonas bauzanensis* and *Pusillimonas soli* are 74, 73, 72, 71, and 70%, respectively. Except for *Paracandidimonas caeni*, *Candidimonas humi* or *Caenimicrobium hargitense*, for which genome sequences are not available, AAI values < 69% are obtained between *Parapusillimonas granuli* and the type strains of the other species represented in Figure 1. All these AAI values are above the threshold (65%) proposed for genus definition (Konstantinidis *et al.*, 2017), suggesting that different criteria should be used to define genera within the family *Alcaligenacae* or that the definition of the genus *Parapusillimonas* may need a revision.

12. LIST OF SPECIES OF THE GENUS *PARAPUSILLIMONAS*

1. Parapusillimonas granuli

Kim, Kim, Im, Srinivasan and Yang 2010, 1405^{VP}.

granuli (gra.nu´li. L. gen. n. *granuli* of a small grain, pertaining to a granule, from which the type strain was isolated).

In addition to the genus description, the type strain tests positive for glucose acidification, and assimilation of D-glucose, D-sorbitol, adipate, phenylacetate, sodium acetate, lactate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, potassium 2-ketogluconate, malate, citrate, propionate, valerate, L-proline, L-alanine, L-serine, L-histidine, itaconate, and gluconate. Tests negative for the assimilation of L-arabinose, mannose, caprate, L-fucose, melibiose, maltose, D-mannitol, sucrose, inositol, D-ribose, L-rhamnose, salicin, glycogen, potassium 5-ketogluconate, *N*-acetyl-D-glucosamine, *p*-nitrophenyl-*β*-D-galactopyranoside, gelatin, sodium malonate, or suberate. H_2S and indole are not produced. Nitrate is not reduced. Reacts positively for arginine dihydrolase, urease, esterase (C4), esterase (C8), leucine arylamidase, and valine arylamidase activity. Reacts negatively for *β*-glucosidase, *α*-glucosidase, *β*-galactosidase, *α*-galactosidase, *β*-glucuronidase, alkaline phosphatase, acid phosphatase, protease, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, *α*-fucosidase, *α*-mannosidase, *N*-acetyl-*β*-glucosaminidase, *α*-chymotrypsin, trypsin, and lipase (C14) activity.

The DNA G+C content (mol %) is 67.9 (HPLC) and 64.6 (genome).

Type strain: Ch07 (= DSM 18079 = KCTC 12668 = LMG 24012).

GenBank accession number (16S rRNA): DQ466075.

GenBank accession number (genome): JACCEM000000000.

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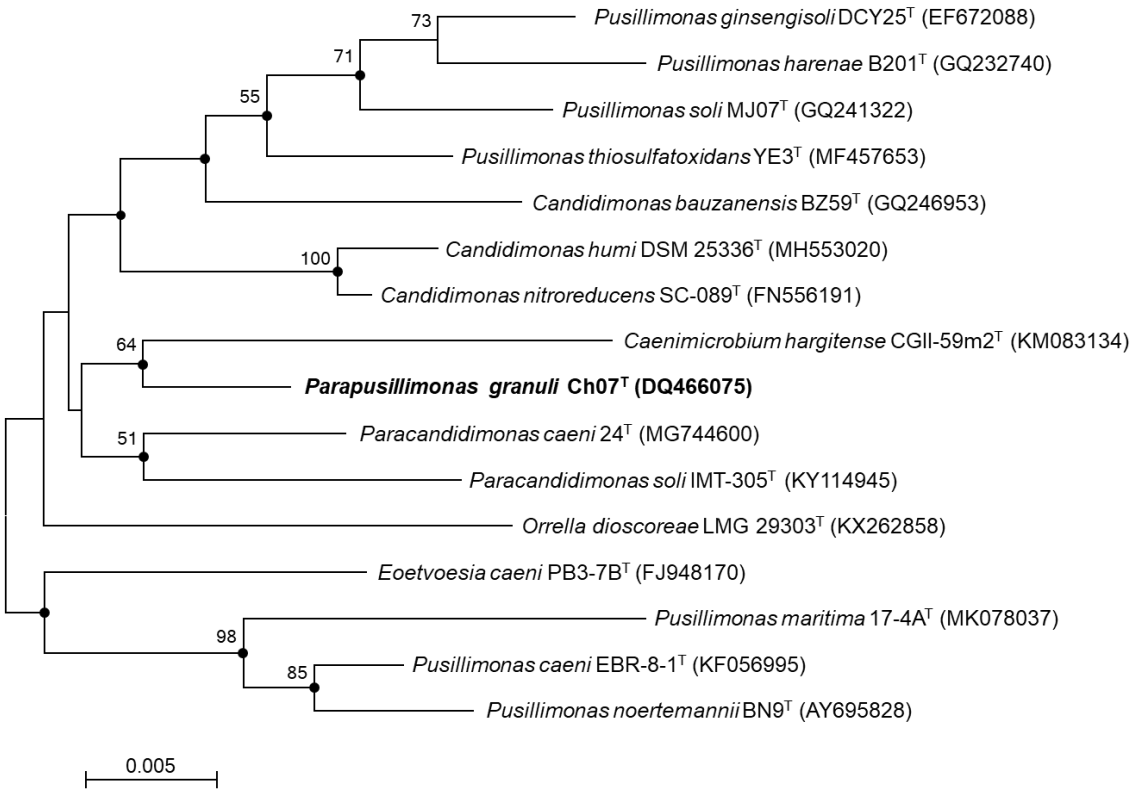

Figure 1. Neighbour-joining phylogenetic tree reconstruction based on the 16S rRNA gene sequences comparison showing the phylogenetic position of *Parapusillimonas* species in relation to other representatives of the family *Alcaligenaceae*. Bootstrap values ≥ 50% are indicated at branch points. Filled circles indicate branches on the tree that were also recovered in the tree generated using the maximum-likelihood algorithm. Bar, 0.005 substitutions per nucleotide position.