Candidimonas - (gbm01821)

1. CONTRIBUTORS DETAILS

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2. KEYWORDS

KEYWORDS: *Candidimonas*; sewage sludge; soil; *Alcaligenaceae*;

3. ABSTRACT

Rods, non-spore forming, Gram-stain-negative. Non-motile or motile by polar flagella. **Aerobic** or facultative aerobic chemo-organotrophic respiratory metabolism. **Mesophilic** with optimal growth at around 30 ºC. Growth can occur in the psychrophilic range of 1-5 ºC. Reacts positively for the catalase- and cytochrome *c* oxidase tests. Sole carbon sources

include amino acids and organic acids. Glucose can be oxidized or assimilated, but not fermented. Anaerobic growth can be observed in the presence of nitrate. Depending on the species, nitrate is reduced to nitrite or to nitrogen. The major respiratory quinone is **ubiquinone 8** and the DNA G+C content ranges 62–65 mol%. Fatty acid composition is variable among species, with $C_{16:0}$ predominating in all species, and $C_{17:0}$ cyclo, summed feature 3 ($C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH), $C_{18:1}$ *ω7c*, and summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I) represented among the major components in the validly named species. The polar lipids comprise phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and some minor unidentified aminophospholipids and aminolipids. Phylogenetically, the genus *Candidimonas* belongs to the family *Alcaligenaceae*. The type species is *Candidimonas nitroreducens* and the genus includes also *Candidimonas humi* and *Candidimonas bauzanensis.*

4. DEFINING PUBLICATION

Candidimonas, Vaz-Moreira, Figueira, Lopes, De Brandt, Vandamme, Nunes and Manaia 2011, 2243^{VP} *emend*. Zhang, Busse, Wieser, Liu, Zhou, Schinner and Margesin 2012, 2087.

5. ETYMOLOGY

Candidimonas [Can.di.di.mo'nas. L. adj. *candidus -a –um* white; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Candidimonas* a unit (rod) that produces white colonies]

6. GENERIC DEFINITION

Rods, non-spore forming, Gram-stain-negative. Non-motile or motile by polar flagella. **Aerobic** or facultative aerobic chemo-organotrophic respiratory metabolism. **Mesophilic** with optimal growth at around 30 ºC. Growth can occur in the psychrophilic range of 1-5 ºC. Reacts positively for the catalase- and cytochrome *c* oxidase tests. Sole carbon sources include amino acids and organic acids. Glucose can be oxidized or assimilated, but not fermented. Anaerobic growth can be observed in the presence of nitrate. Depending on the species, nitrate is reduced to nitrite or to nitrogen. The major respiratory quinone is **ubiquinone 8** and the DNA G+C content ranges 62–65 mol%. Fatty acid composition is variable among species, with $C_{16:0}$ predominating in all species, and $C_{17:0}$ cyclo, summed feature 3 ($C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH), $C_{18:1}$ *ω7c*, and summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I) represented among the major components in the validly named species. The polar lipids comprise phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and some minor unidentified aminophospholipids and aminolipids. Phylogenetically, the genus *Candidimonas* belongs to the family *Alcaligenaceae*. The type species is *Candidimonas nitroreducens* and the genus includes also *Candidimonas humi* and *Candidimonas bauzanensis.*

The DNA G+C content (mol %) is 61.6 – 65.2.

Type species: *Candidimonas nitroreducens*, Vaz-Moreira, Figueira, Lopes, De Brandt, Vandamme, Nunes and Manaia 2011, 2243^{VP}

Number of species with validated names: 3.

7. FAMILY CLASSIFICATION

Alcaligenaceae (fbm00180)

8. FURTHER DESCRIPTIVE INFORMATION

8.1. Cell and colony morphology and culture conditions

Three species are validly named within the genus *Candidimonas*: *C. nitroreducens*, *C. humi*, and *C. bauzanensis* (Vaz-Moreira et al., 2011, Zhang et al., 2012). *Candidimonas* spp. form white, convex, circular (~1.0-1.2 mm diameter) colonies after 48 h incubation on Plate Count Agar (PCA) (*C. nitroreducens* and *C. humi*) or after 72 h on R2A (*C. bauzanensis*). Cells stain Gram-negative, are non-spore forming coccobacilli of about 0.5 - 0.6 µm (*C. nitroreducens* and *C. humi*) or short rods of about 0.7-0.9 µm x 1.2-1.9 µm (*C. bauzanensis*). The latter is the only species described as being motile by means of polar flagella. *Candidimonas* spp. can be routinely cultured on PCA or R2A for 2-3 days at 25 ºC-30 ºC, at pH 6–8 and up to 3% (w/v) NaCl (Vaz-Moreira et al., 2011, Zhang et al., 2012).

8.2. Nutrition and metabolism

Candidimonas spp. are chemoorganotrophic heterotrophic bacteria with respiratory metabolism that occurs mainly in the presence of oxygen as final electron acceptor. The *C. nitroreducens* and *C. bauzanensis* type strains grow anaerobically in the presence of nitrate, which is reduced to N_2 or to NO_2^-, respectively (Vaz-Moreira et al., 2011, Zhang et al., 2012). Amino acids, organic acids or sugars can be used as sole carbon sources, although these are variable traits within the genus. For example, glucose and arabinose but not phenylacetic acid can be assimilated by the type strain of *C. bauzanensis*, while opposite reactions are observed for the type strains of *C. nitroreducens* and *C. humi* (Zhang et al., 2012). As common traits to

the three type strains are the production of catalase, cytochrome *c* oxidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase or the assimilation of adipic acid.

8.3. Chemotaxonomic characteristics

The major respiratory quinone is ubiquinone 8. Polar lipids, described based on the type strains of the three species *C. nitroreducens*, *C. humi* and *C. bauzanensis*, comprise phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol among other uncharacterized aminolipids or aminophospholipids. The predominant fatty acid is $C_{16:0}$, together with $C_{17:0}$ cyclo, summed feature 3 ($C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH), $C_{18:1}$ *ω7c*, and summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I) (Vaz-Moreira et al., 2011, Zhang et al., 2012).

8.4. Genome features

The whole genome sequences of strains *C. nitroreducens* SC-089^T and *C. bauzanensis* BZ59^T are available in the GenBank under the Bioproject references PRJNA391542 and PRJEB18240, respectively. *C. nitroreducens* SC-089^T genome sequence obtained based on Illumina HiSeq, with a coverage of 145x presents a total length of 5.61 Mb, with 34 contigs, an N50 value of 370,610 and L50 value of 6. The identified encoding protein regions sum 4873 at the moment of writing and the DNA G+C content (mol %) is determined to be 63.6 (and 63.9% based on HPLC method, Vaz-Moreira et al., 2011). *C. bauzanensis* BZ59^T genome sequence obtained with a coverage of 174x presents a total length of 5.64 Mb, with 48 contigs, a contig N50 value of 224,312 and L50 value of 7. The identified encoding protein regions sum 5093 at the moment of writing and the DNA G+C content (mol %) is determined to be 62.2 (and 61.6% based on HPLC method, Zhang et al., 2012).

8.5. Ecology and Habitat

Candidimonas spp. were isolated so far from human-impacted environments in Southern European countries (Portugal and Italy). The type strains of the species *C. nitroreducens* and *C. humi* were isolated from compost produced by thermophilic digestion of sewage sludge and co-isolated with other Gram-negative bacteria, yeasts and Gram-positive bacteria that represented more than 50% of the isolates (Vaz-Moreira et al., 2008). The thermophilic compost had a pH of 4.2, > 80% of organic matter and metals at the following concentrations (mg kg^{-1} ms): Zn (637), Cu (299), Ni (38), Cd (1.9), Cr (62.3), Pb (79). The digestion process can reach temperatures of 60 ºC, explaining the predominance of Gram-positive endospore forming bacteria in this environment and also the thermotolerant character of these *Candidimonas* isolates (Vaz-Moreira et al., 2008). In contrast, the psychrotolerant strain *C. bauzanensis* $BZ59^T$ is able to grow at temperatures of 1-5 ºC. This strain was isolated from a heavily contaminated soil of an industrial site with large amounts of oil and metals (Zhang et al., 2010).

9. ENRICHMENT/ISOLATION PROCEDURES

Strains $SC\text{-}089^T$ and $SC\text{-}092^T$ were the first *Candidimonas* spp. representatives whose isolation was reported in the literature (Vaz-Moreira et al., 2008). The type strains of the species *C. nitroreducens* ($SC\text{-}089^T$) and *C. humi* ($SC\text{-}092^T$) were isolated from thermophilic compost derived from activated sludge. Briefly, 10 g of compost were suspended in 90 mL of sterile saline solution (0.85% (w/v) NaCl) and shaken for 30 min. A volume of 1 mL of serial dilutions was filtered through cellulose nitrate membranes with 0.45 μm pore size that were placed onto PCA. After 24 h incubation at 30 ºC, 1-5 colonies with distinct morphologies

were inspected for inferring the bacterial diversity of culturable bacteria in the respective composts. Strains SC-089^T and SC-092^T were not affiliated to any previously named species or genus and were further characterized (Vaz-Moreira et al., 2011). Strain BZ59^T, the type strain of the species *C. bauzanensis* was isolated from industrial soil contaminated with heavy (high density) crude oil and heavy metals in the South Tyrol region, Italy (Zhang et al., 2010). For bacterial isolation, soil samples were suspended in sodium pyrophosphate solution (1%), homogenized (20 min, 150 rpm) serially diluted, and plated onto R2A. After 10 days incubation at 20 ºC, strain BZ59^T formed white colonies and was further characterized as representing a putative new taxon.

10. MAINTENANCE PROCEDURES

Candidimonas spp. can be routinely grown on R2A or PCA agar at 25-30 ºC. For long term preservation, cultures can be stored suspended in a nutritive broth supplemented with 15% (v/v) glycerol at -80 ºC. These strains are supplied as freeze dried cultures by different culture collections (e.g. DSMZ).

11. DIFFERENTIATION OF THE GENUS *CANDIDIMONAS* FROM OTHER GENERA

The closest related species to *Candidimonas* are members of the genera *Bordetella* (*B. flabilis, B. petrii, B. sputigena, B. bronchialis*), *Parapusillimonas* (*Parap. granuli*), *Paracandidimonas* (*Parac. caeni*),"*Paralcaligenes"* ("*P. ginsengisoli"*), and *Pusillimonas* (*Pus. thiosulfatoxidans*). Other closely related neigbhours include *Eoetvoesia caeni* and

Achromobacter aloeverae. At the time of the genus name description, *Candidimonas* spp. could be distinguished from members of *Achromobacter*, *Pusillimonas* and *Parapusillimonas*, due to the absence of motility, from members of the genera *Achromobacter*, *Bordetella*, and *Parapusillimonas* due to the inability to assimilate L-alanine, and from members of the genus *Bortedella* based on the nutritional profile, specifically the ability to assimilate 3-hydroxybenzoic acid observed in *Candidimonas* and absent in members of the genus *Bordetella*, which can use sugars and organic acids not assimilated by *Candidimonas* (Vaz-Moreira et al., 2011). These distinctive features were supported by the 16S rRNA gene sequence based phylogenetic analysis, however loosed relevance with the description of additional species in those genera and also with the description of the species *C. bauzanensis*.

12. TAXONOMIC COMMENTS

Based on 16S rRNA gene sequence phylogenetic analysis, the type strains of the species *C. nitroreducens* and *C. humi* share 99.3% sequence identity and <98% with the type strain of *C. bauzanensis*, whose closest neighbor is the type strain of the species *Pusillimonas thiosulfatoxidans* with which shares 98.2% sequence identity. The first two species that were described, *C. nitroreducens* and *C. humi*, based on the strains SC-089^T and SC-092^T (Vaz-Moreira et al., 2011), presented high pairwise 16S rRNA gene sequence similarity, and a low level of DNA–DNA relatedness between them (41 %), much lower than the 70 % threshold value proposed for separation of strains at the species level (Wayne et al., 1987). These two species were differentiated based on phenotypic traits such as ability to grow anaerobically, reduce nitrate, oxidize glucose and assimilate potassium 2-ketogluconate, 4-hydroxybenzoic acid, trisodium citrate, malate and potassium gluconate. Additionally, *C. nitroreducens* SC-089^T and *C. humi* SC-092^T differed in the cellular fatty acid profile and DNA G+C content

(Vaz-Moreira et al., 2011). The third species that was described, *C. bauzanensis* (Zhang et al., 2012) did not form a stable 16S rRNA gene-based phylogenetic relationship with other *Candidimonas* species, as was demonstrated by a low bootstrap value (Zhang et al., 2012). Other genomic data also suggest the inadequate inclusion of the *C. bauzanensis* BZ59^T in the genus *Candidimonas*. On one hand, *C. bauzanensis* BZ59^T shares low average nucleotide identity (ANI) value (79.54%) with the type species of the genus *C. nitroreducens* SC-089^T, below the defined species boundary (95%) (Richter et al., 2016), and an amino acid identity (AAI) value of 66.45%, close to the genus threshold of 65% (Konstantinidis et al., 2017). On the other hand, *C. bauzanensis* BZ59^T (=CGMCC 1.10190^T) shares with *Pus. thiosulfatoxidans* YE3^T ANI and AAI values of 78.94% and 73.14%, respectively, in addition to the higher 16S rRNA gene sequence identity. While the phenotypic differentiation of *C. bauzanensis* BZ59^T from *C. nitroreducens* SC-089^T and *C. humi* SC-092^T, specifically the presence of flagella and motility or the psychrophilic character alone, would not exclude this species of the genus *Candidimonas*, combined with phylogenomics data may suggest that a reclassification should be considered. Indeed, in spite of the relatively close taxonomic relatedness between distinct genera within the family *Comamonadaceae* (Willems, 2014), the proximity between *C. bauzanensis* BZ59^T and *Pus. thiosulfatoxidans* YE3^T suggests that the definition of this *Candidimonas* species may need a revision.

<Figure 1 near here>

13. LIST OF SPECIES OF THE GENUS *CANDIDIMONAS*

1. *Candidimonas nitroreducens* Vaz-Moreira, Figueira, Lopes, De Brandt,Vandamme, Nunes, Manaia 2011, 2243^{VP}

nitroreducens (ni.tro.re.du'cens. N.L. n. *nitras -atis* nitrate; N.L. pref. *nitro-* pertaining to nitrate; L. part. adj. *reducens* leading back, bringing back and, in chemistry, converting to a different oxidation state; N.L. part. adj. *nitroreducens* reducing nitrate).

In addition to the genus description, the species is described as comprising Gram-negative, coccobacilli that form white colonies on PCA, growth occurs at 15–40 ºC, pH 5–8 and in the presence of up to 3% (w/v) NaCl. Anaerobic growth is slow and weak, occuring with nitrate reduction to nitrite. Able to oxidze glucose and to produce acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase. Able to assimilate the following sole carbon sources: adipate, phenyl acetate, itaconic acid, sodium acetate, lactic acid, 3-hydroxybenzoic acid, propionic acid, valeric acid, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, malate and potassium gluconate.

The DNA G+C content (mol %) is 63.9 (HPLC method).

Type strain: SC-089; CCUG 55806; LMG 24812.

GenBank accession number (16S rRNA): FN556191.

GenBank accession number (genome): NJIH00000000.1

2. *Candidimonas humi* Vaz-Moreira, Figueira, Lopes, De Brandt,Vandamme, Nunes, Manaia 2011, 2244[VP]

humi (hu'mi. L. n. *humus* earth, soil and, in earth sciences or agriculture, humus; L. gen. n. *humi* of the soil, of the humus).

In addition to the genus description, the species is described as comprising Gram-negative coccobacilli that form white colonies. Growth occurs at 15–40 ºC, pH 5–8 and in the presence of up to 3% (w/v) NaCl. Unable to grow under anaerobic conditions or to reduce nitrate.

Produces acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase and α-chymotrypsin. Able to assimilate the following sole carbon sources: adipate, citrate, phenyl acetate, itaconic acid, sodium acetate, lactic acid, 3-hydroxybenzoic acid, propionic acid, valeric acid, trisodium citrate, potassium 2-ketogluconate and 3-hydroxybutyric acid.

The DNA G+C content (mol %) is 65.2 (HPLC method).

Type strain: SC-092; CCUG 55807; LMG 24813.

GenBank accession number (16S rRNA): FN556192.

3. ***Candidimonas bauzanensis*** Zhang, Busse, Wieser, Liu, Zhou, Schinner, Margesin 2012, 2087^{VP}

bauzanensis (bau.zan.en´sis. N.L. fem. adj. *bauzanensis* referring to *Bauzanum* medieval Latin name of Bozen/Bolzano, a city in South Tyrol, Italy, where the type strain was isolated).

In addition to the genus description, the species is described as comprising Gram-negative, facultative anaerobic rods, motile by polar flagella, forming creamy white, smooth colonies on R2A agar. Tests positive for catalase- and cytochrome *c* oxidase and is able to reduce nitrate to nitrogen gas. Psychrotolerant, with good growth between 1 and 30 ºC, and unable to grow at 42 ºC. Able to grow at pH 6–8 and 0–3 % (w/v) NaCl. Is positive for urease, leucine arylamidase, esterase (C4) and naphthol-AS-BI-phosphohydrolase, and reacts weakly for esterase lipase (C8) and acid phosphatase activity. Able to assimilate D-glucose, adipic acid, malic acid, potassium gluconate, trisodium citrate and L-arabinose. Putrescine and spermidine and minor amounts of 2-hydroxyputrescine, spermine and cadaverine are part of

the polyamine pattern.

The DNA G+C content (mol %) is 61.6 (HPLC method).

Type strain: BZ59; DSM 22805; LMG 26046; CGMCC 1.10190.

GenBank accession number (16S rRNA): GQ246953.

GenBank accession number (genome): FQXE00000000.1.

RELATED ARTICLES

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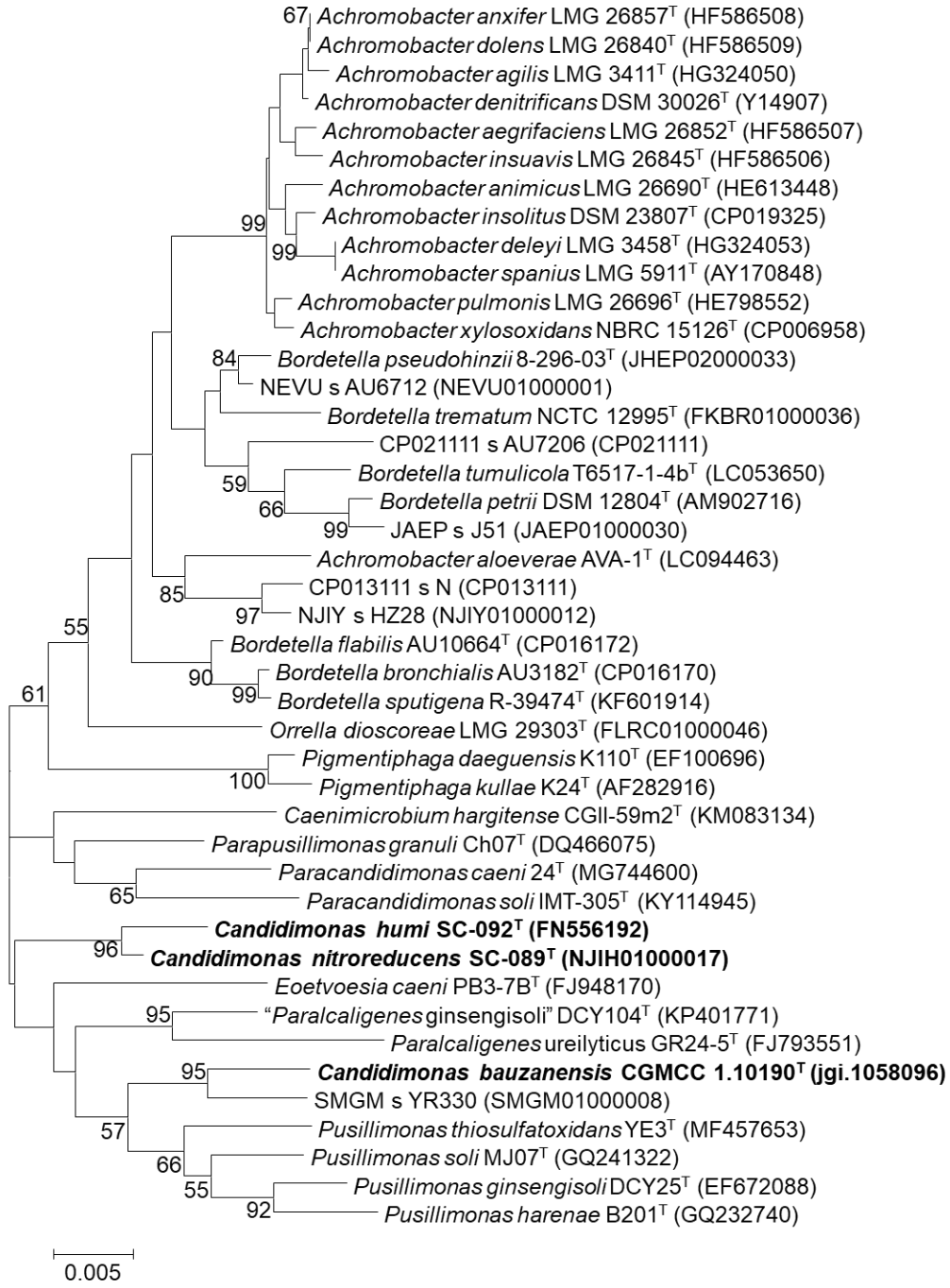

Figure 1. Dendrogram based on 16S rRNA gene sequences, showing the position of the *Candidimonas* species in relation to the type strains of species with which any of the three *Candidimonas* types strains shared ≥97% sequence identity. The dendrogram was generated by the Neighbor-Joining method. Bootstrap values, generated from 1000 re-samplings, are indicated at branch points. Bar, 1 substitution per 200 nucleotide positions.