Paracandidimonas - (gbm01822)

1. CONTRIBUTORS DETAILS

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2. KEYWORDS

KEYWORDS: *Paracandidimonas*; soil; industrial activated sludge; *Alcaligenaceae*;

3. ABSTRACT

Rods, non-spore forming, Gram-staining-negative. **Aerobic** or facultative aerobic chemo-organotrophic metabolism. Motility and the presence of cytochrome *c* oxidase test are variable traits. **Mesophilic** with optimal growth at 20-25°C or 30 ºC. The major respiratory quinone is **ubiquinone 8** and the DNA G+C content is 56.8-63.9 mol%. Putrescine and 2-hydroxyputrescine are the major polyamines. Polar lipids and fatty acids composition is variable among species. Phylogenetically, belongs to the family *Alcaligenaceae*. The genus includes two species, the type species *Paracandidimonas soli* and *Paracandidimonas caeni*.

4. DEFINING PUBLICATION

Paracandidimonas, Kampfer, Busse, Mclnroy, and Glaeser, 2017, 1743^{VP}

5. ETYMOLOGY

Paracandidimonas [Pa.ra.can.di.di.mo'nas. Gr. prep. *para* like; N.L. fem. n. *Candidimonas* bacterial genus name; N.L. fem. n. *Paracandidimonas* a bacterium like *Candidimonas*]

6. GENERIC DEFINITION

Rods, non-spore forming, Gram-staining-negative. **Aerobic** or facultative aerobic chemo-organotrophic metabolism. Motility and the presence of cytochrome *c* oxidase test are variable traits. **Mesophilic** with optimal growth at 20-25°C or 30 ºC. The major respiratory quinone is **ubiquinone 8** and the DNA G+C content is 56.8-63.9 mol%. Putrescine and 2-hydroxyputrescine are the major polyamines. Polar lipids and fatty acids composition is

variable among species. Phylogenetically, belongs to the family *Alcaligenaceae*. The genus includes two species, the type species *Paracandidimonas soli* and *Paracandidimonas caeni.*

The DNA G+C content (mol %) is 63.9 (fluorimetric thermal denaturation); 56.8-62.5 (whole genome sequencing).

Type species: *Paracandidimonas soli*, Kampfer, Busse, Mclnroy, and Glaeser, 2017, 1744^{VP}

Number of species with validated names: 2.

7. FAMILY CLASSIFICATION

Alcaligenaceae (fbm00180)

8. FURTHER DESCRIPTIVE INFORMATION

8.1. Cell and colony morphology and culture conditions

Currently the genus *Paracandidimonas* comprises two validly named species, described based on a single strain, *P. soli* IMT-305^{T} and *P. caeni* 24^{T} (Kampfer et al., 2017, Yao et al., 2019). The type strain of *P. soli* forms slightly glistening yellowish-beige, convex, circular (~2 mm diameter) colonies after 48 h incubation on Tryptic Soy Agar (TSA) at 30°C (Kampfer et al., 2017). After the same period and temperature of incubation on Reasoner's 2A agar (R2A), *P. caeni* 24^{T} forms beige, convex, circular colonies (Yao et al., 2019). *Paracandidimonas* spp. are non-spore forming cells that stain Gram-negative with the

morphology of 0.5 µm x 1.5 µm in *P. soli* MT-305^T and of 0.5-0.7 µm x 1.8-2.0 in *P. caeni* 24^T. Both type strains differ in motility, only observed in *P. soli* IMT-305^T.

P. soli IMT-305^T grows well on Nutrient Agar, Brain Heart Infusion Agar, R2A and TSA for 2 days at 30 ºC. The cardinal temperature values allow the distinction of the type strains of the two *Paracandidimonas* species; *P. soli* IMT-305^T does not grow below 10 ºC or above 45 ºC, showing optima at 20-25 ºC, whereas *P. caeni* 24^T grows in the range 15-42 °C, with optimum at 30 °C (Kampfer et al., 2017, Yao et al., 2019). The capacity of *P. soli* IMT-305^T to grow at pH 5.0 also distinguishes these two strains, as *P. caeni* 24^T grows at pH 6.0–8.0 with optimum pH 7.0. Also salt (NaCl) tolerance distinguishes both type strains; *P. caeni* 24^T is capable of growing up to 2% (w/v), (optimum 0.5%) and *P. soli* IMT-305^T can grow up to 6% (optimum 1-2%) (Kampfer et al., 2017, Yao et al., 2019).

8.2. Nutrition and metabolism

Paracandidimonas spp. are chemoorgano-heterotrophic bacteria with a aerobic respiratory metabolism. Anaerobic growth is reported in the type strain of the species *P. soli* IMT-305^T but not in *P. caeni* 24^T. In addition, the latter strain is unable to reduce nitrate (Yao et al., 2019). The nutritional pattern also differs between these two type strains, with *P. caeni* 24^T showing the abitily to assimilate several sugars and alcohols, and *P. soli* IMT-305^T with weak growth in some organic acids and amino acids (Kampfer et al., 2017, Yao et al., 2019). Accordingly, also the enzymatic activity profile differs in these strains, with *P. soli* IMT-305^T testing positive for cytochrome *c* oxidase, esterase (C4) and esterase liase (C8) and negative for alkaline and acid phosphatase and *P. caeni* 24^T showing the opposite traits (Kampfer et al., 2017, Yao et al., 2019). Examples of traits that are shared by both type strains are the

absence of activity of beta-galactosidase and the inhability to hydrolise gelatin and casein and to produce indol (Kampfer et al., 2017, Yao et al., 2019).

Comparative genomics revealed the presence of the *hmf*ABCDE gene cluster, associated with 5-hydroxymethylfurfural (HMF) and furfural degradation, in *P. soli* IMT-305^T, suggesting the ability of this organism to transform 2-furoic acid, an heterocyclic carboxylic acid widely used as a food preservative as well as a microbial metabolite of furfural oxidation, into 2-oxoglutarate (Donoso et al., 2021). Such trait seems to be widely destributed among *Proteobacteria* (Donoso et al., 2021).

8.3. Chemotaxonomic characteristics

As determined for *P. soli* IMT-305^T, the peptidogolycan diagnostic diamino acid is *meso*-diaminopimelic acid (Kampfer et al., 2017). The respiratory quinone system of the two *Paracandidimonas* type strains is similar, with ubiquinone 8 as the major respiratory quinone, and ubiquinone 7 and ubiquinone 9 constituting minor components (Kampfer et al., 2017, Yao et al., 2019). Accordingly, these type strains share the same polyamine pattern with putrescine constituting the major component [82.4-96.3 μmol (g dry weight)$^{-1}$] followed by 2-hydroxyputrescine [7.0-7.5 μmol (g dry weight)$^{-1}$], 1,3-diaminopropane [2.1-3.3 μmol (g dry weight)$^{-1}$] and trace amounts of spermine and cadaverine (Kampfer et al., 2017, Yao et al., 2019).

In contrast, the polar lipids and fatty acids profiles allow the distinction of the two *Paracandidimonas* species. In both type strains phosphatidylethanolamine and phosphatidylglycerol are major components, whereas an unidentified aminophospholipid is present at trace amounts. Other predominant polar lipids include phosphatidylmethylethanolamine and one unidentified phospholipid in *P. caeni* 24^T, and diphosphatidylglycerol in *P. soli* IMT-305^T. In addition, phosphatidylserine, an unidentified

aminolipid and an unidentified lipid lacking any functional group (amino-, glyco- or phospo-) are present at moderate amounts in the latter strain (Kampfer et al., 2017, Yao et al., 2019). According to Yao et al. (2019), in *P. soli* IMT-305^T the major cellular fatty acids (>5 %) are $C_{16:0}$ and $C_{17:0}$ cyclo, followed by summed feature 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c) and summed feature 2 (iso-$C_{16:1}$ I/$C_{14:0}$ 3-OH/$C_{12:0}$ aldehyde), whereas in *P. caeni* 24^T summed feature 3 is the major fatty acid, followed by summed feature 8 ($C_{18:1}$ ω7c/$C_{18:1}$ ω6c) and summed feature 2.

8.4. Genome features

The whole genome sequence of strain *P. soli* IMT-305^T is available in the GenBank under the accession number NZ_SMBX00000000 (Bioproject PRJNA520328). *P. soli* IMT-305^T genome sequence, obtained based on Illumina HiSeq with a coverage of 385x, presents a total length of 3.89 Mb, with 24 contigs, 23 scaffolds, an N50 value of 469,828 and L50 value of 3. At the moment of writing, 3,623, 46 and 3 regions respectively encoding proteins, tRNAs and rRNAs were identifyed and the DNA G+C content (mol%) is determined to be 62.5 (and 63.9 based on the fluorimetric thermal denaturation temperature method, Kampfer et al., 2017).

According to Yao et al. (2019) the draft genome sequence of *P. caeni* 24^T, also obtained based on Illumina HiSeq with a coverage of 212x, is available (raw data) under the accession number SRP154956 (Bioproject PRJNA482497). The size of the genome is 5.46 Mb and 2880 genes are predicted; the N50 value is 293 770 bp and the DNA G+C content is estimated to be 56.83 mol% (Yao et al. 2019).

8.5. Ecology and Habitat

Up to the moment of writing, the type strains of the *Paracandidimonas* species were isolated from human-impacted environments. *P. soli* IMT-305^T was isolated from soil that was used for 50-70 years for cultivating earthworms to be further used as fish bait, in a field located in Malvern, Alabama (USA) (Kampfer et al., 2017). *P. caeni* 24^T was isolated from activated sludge of the wastewater treatment plant of a pesticide manufacturing factory in Nantong, Jiangsu Province (China) (Yao et al., 2019). Other strains of the genus *Paracandidimonas* were isolated from an enrichment culture of surface soil collected at the coastal fen of Keri lake (Zakynthos island, Greece), characterized by increased plant biomass decay and natural oil seeps (Georgiadou et al., 2021). These isolates were enriched on Minimal Salt Medium supplemented with 1% (w/v) birchwood xylan, aiming at isolating aerobic lignocellulolytic bacteria, where represented 9% of the bacteria growing in this culture (Georgiadou et al., 2021). The respective 16S rRNA gene sequences are available under the accession numbers MT683187.1 and MT683189.1.

9. ENRICHMENT/ISOLATION PROCEDURES

P. caeni 24^T was isolated from an enrichment culture established to recover bacteria able to degrade the herbicide methoprolone. Briefly, an inoculum of 10 g of activated sludge from the pesticide manufacturing factory was incubated in mineral medium (g/L, 1.3 K_2HPO_4, 0.86 KH_2PO_4, 0.66 $(NH_4)_2SO_4$, 0.097 $MgSO_4$, 0.025 $MnSO_4 \cdot H_2O$, 0.005 $FeSO_4 \cdot 7H_2O$, 0.001 $CaSO_4 \cdot 6H_2O$) with 50 mg methoprolone as the single carbon source at 30 °C, 180 rpm for 5 days. After this period, a volume of 10 mL of the culture was transferred into 100 mL of fresh medium, and incubated under the same conditions. After four successive transfers, the final enrichment culture was diluted and spread onto R2A supplemented with 50 mg/L methoprolone. The colonies observed after 2 days of incubation at 30 °C were purified on R2A medium, leading to the isolation of strain *P. caeni* 24^T (Yao et al., 2019). *P. soli* IMT-

164 305^T was isolated from soil of a field with long history of earthworms cultivation using NA
165 medium at 30 °C for 2 days (Kampfer et al., 2017).
166

167 **10. MAINTENANCE PROCEDURES**

168 *Paracandidimonas* spp. can be routinely cultured on R2A agar at 30 ºC. For long term
169 preservation, *P. caeni* 24^T cultures can be stored suspended in a nutritive broth supplemented
170 with 30% (v/v) glycerol at -80 ºC. *P. soli* IMT-305^T is supplied as freeze dried culture by
171 different culture collections (e.g. DSMZ, BCCM/LMG; are also available in CCTCC and
172 KACC, although the respective websites are not available).
173

174 **11. DIFFERENTIATION OF THE GENUS *PARACANDIDIMONAS* FROM OTHER**
175 **GENERA**

176 The closest related species to *Paracandidimonas* are members of the genera *Candidimonas*
177 (*C. nitroreducens*) and *Parapusillimonas* (*Pp. granuli*). *Paracandidimonas* spp. can be
178 distinguished from members of these genera based on the polar lipid profile, namely the
179 absence of the unidentified aminophospholipids present in *C. nitroreducens* and in *Pp.*
180 *granuli* (Kampfer et al., 2017, Kim et al., 2010, Vaz-Moreira et al., 2011).
181

182 **12. TAXONOMIC COMMENTS**

183 Based on the 16S rRNA gene sequence phylogenetic analysis, the closest neigbour of the
184 type strain of the *Paracandidimonas* type species is *P. caeni* 24^T, with 97.8% sequence
185 identity. The DNA-DNA hybridization values of less than 45%, well below the 70%
186 threshold for the delineation of genomic species (Wayne et al., 1987), confirmed the
187 separation of these organisms into two distinct species (Yao et al., 2019). Other organisms

188 related with *Paracandidimonas soli* are *C. nitroreducens* and *Pp. granuli*, with which strain
189 IMT-305^T shares 16S rRNA pairwise sequence identity above 97% (97.4 and 97.2%,
190 respectively). In contrast, *Paracandidimonas caeni* shares highest 16S rRNA pairwise
191 sequence identity with *Pp. granuli* (98.1%) and *C. nitroreducens* (98.0%), followed by a
192 wide range of species of the family *Alcaligenaceae* (*Pusillimonas ginsengisoli*, *Pusillimonas*
193 *thiosulfatoxidans*, *Pusillimonas soli*, *Paralcaligenes ginsengisoli*, *Bordetella flabilis*,
194 *Pusillimonas caeni*, *Candidimonas humi*, *Caenimicrobium hargitense*, *Pusillimonas*
195 *noertemannii*, *Candidimonas bauzanensis*, *Paralcaligenes ureilyticus*, and *Pusillimonas*
196 *harenae*) with which strain 24^T shares between 97.7 and 97.0% sequence identity. In
197 summary, the closest neighbour of *P. soli* IMT-305^T is *P. caeni* 24^T (97.8%), but the closest
198 neighbours of the latter strain include also members of other genera. Despite this observation
199 and the numerous phenotypic and chemotaxomic distinctive traits described above, according
200 to the 16S rRNA gene sequence based phylogenetic tree (Figure 1), the *Paracandidimonas*
201 species validly named at the time of writing form a distinct clade.
202
203 <Figure 1 near here>
204
205 **13. LIST OF SPECIES OF THE GENUS *PARACANDIDIMONAS***
206 **1. *Paracandidimonas caeni*** Yao, Lai, Xue, Sun, and Wang, 2019, 3336^{VP}
207 *caeni* (cae'ni. L. gen. n. *caeni* of sludge).
208 In addition to the genus description, the species is described as comprising Gram-negative,
209 non-spore-forming, nonmotile rods that form beige colonies on R2A, growth occurs at 15–42
210 ºC, pH 6–8 and in the presence of up to 2% (w/v) NaCl. Able to assimilate glucose, ribose,
211 fructose, rhamnose, D-arabinose, L-arabinose, D-mannose, D-tagatose, D-fucose, maltose,

sucrose, trehalose, melezitose, raffinose, mannitol, glycerol, erythritol, dulcitol, D-sorbitol, D-arabitol and L-arabitol, methyl alpha-D-glucopyranoside, capric acid, phenylacetic acid, amygdalin, arbutin, salicin, inulin, and glycogen. Unable to assimilate mannose, galactose, sorbose, sorbitose, turanose, L-xylose, adonite, xyloside, lactose, melibiose, cellobiose, gentiobiose, inositol, xylitol, methyl alpha-D-mannopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, adipic acid, malic acid, trisodium citrate, and starch. Tests positive for acid phosphatase, alkaline phosphatase, leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase. Tests negative for esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, alpha-chymotrypsin, alpha-galactosidase, beta-galactosidase,beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase alpha-fucosidase, catalase, oxidase, urease, hydrolysis of casein and gelatin, indole production, anaerobic growth and nitrate reduction.

The DNA G+C content (mol %) is 56.83 (whole genome sequencing).

Type strain: 24 (=CCTCC AB 2018057 =KACC 19692 =Query 24).

GenBank accession number (16S rRNA): MG744600.

GenBank accession number (genome): SRP154956

2. *Paracandidimonas soli* Kampfer, Busse, Mclnroy, and Glaeser, 2017, 1744^{VP}

soli (so'li. L. neut. gen. n. *soli* of soil, the isolation source of the type strain).

In addition to the genus description, the species is described as comprising rods unable to grow below 10 °C or above 45 °C, with optima at 20–25 °C and 1–2% (w/v) NaCl. Facultatively anerobic and cytochrome *c* oxidase-positive. Weak reaction for the utilization of the following compounds as sole carbon source: acetate, cisaconitate, trans-aconitate,

citrate, fumarate, glutarate, DL-lactate, malate, 2-oxoglutarate, pyruvate, L-alanine, L-serine, and L-proline. N-acetylgalactosamine, N-acetylglucosamine, L-arabinose, cellobiose, D-galactose, D-glucose, D-mannose, D-fructose, maltose, melibiose, L-rhamnose, D-ribose, salicin, sucrose, D-xylose, adonitol, i-inositol, D-sorbitol, glycerol, D-mannitol, maltitol, putrescine,4-aminobutyrate, adipate, azelate, DL-3-hydroxybutyrate, itaconate, gluconate, propionate, suberate, mesaconate, beta-alanine, L-ornithine, L-phenylalanine, L-aspartate, L-histidine, L-leucine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate, and phenylacetate are not utilised as sole carbon sources.

The chromogenic substrates L-alanine-p-nitroanilide and 2-deoxythymidine-2'-p-nitrophenyl-phosphate are hydrolysed, but not p-nitrophenyl-alpha-D-glucopyranoside, bis-p-nitrophenyl-phosphate, bis-p-nitrophenyl-phosphoryl-choline, gamma-L-glutamate-p-nitroanilide, L-proline-p-nitroanilide, p-nitrophenyl-beta-D-glucopyranoside p-nitrophenyl-beta-D-galactopyranoside, p-nitrophenyl-beta-D-xylopyranoside, and p-nitrophenyl-beta-D-glucuronide. Acid production from D-glucose, maltose, trehalose, L-arabinose, adonitol, Darabitol, dulcitol, erythritol, *i*-inositol, lactose, D-mannitol, melibiose, methyl a-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, and D-xylose test negative. Casein, gelatin, starch, DNA, and tyrosine are not hydrolised. Indol and hydrogen sulphide are not produced. Does not show activity of urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and beta-galactosidase.

The DNA G+C content (mol %) is 63.9 (fluorimetric thermal denaturation) - 62.5 (whole genome sequencing).

Type strain: MT-305 (=DSM 100048 =CIP 110902 =LMG 28740 =CCM 8599).

GenBank accession number (16S rRNA): KY114945.

GenBank accession number (genome): NZ_SMBX00000000

260

261

262 **RELATED ARTICLES:**

263 gbm01821

264 gbm01824

265 gbm01823

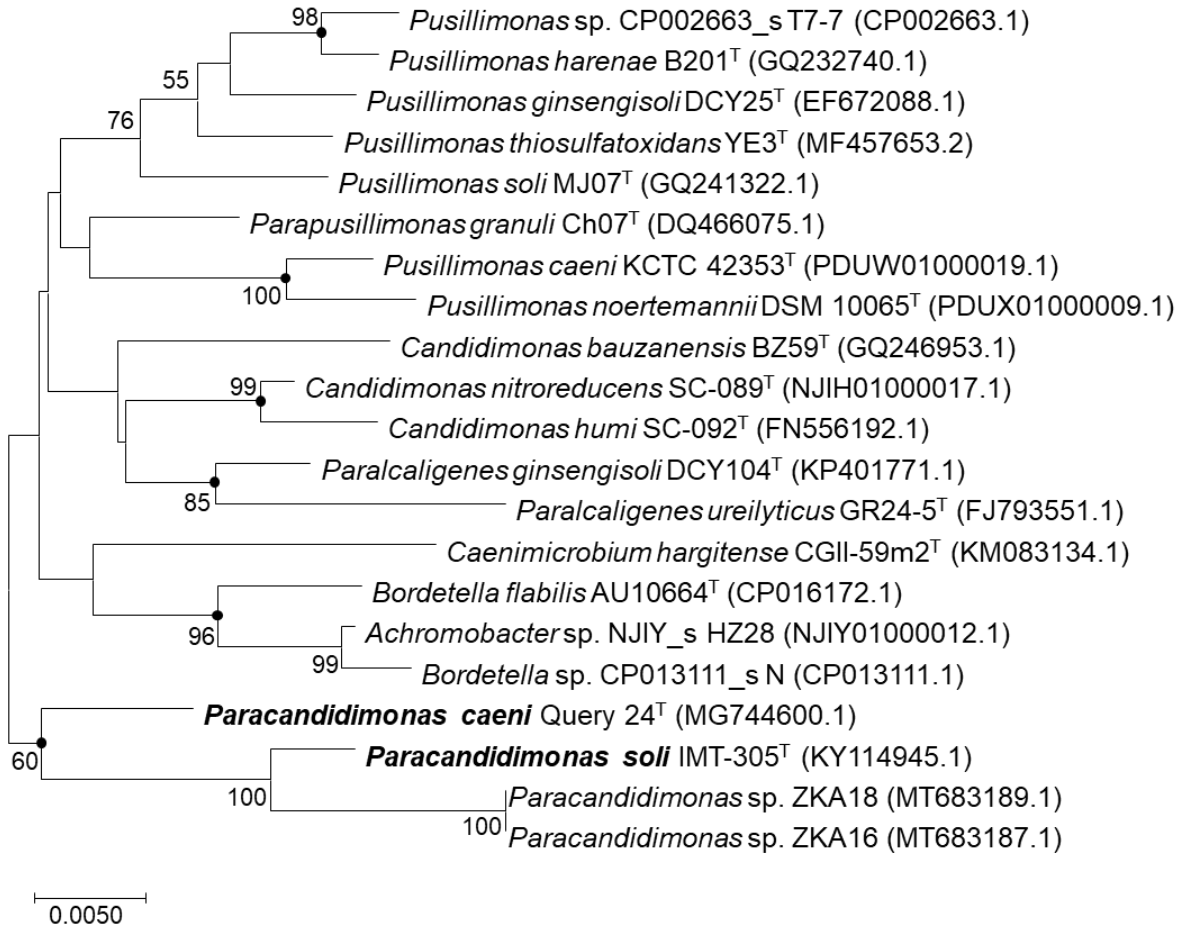
266

267 **BIBLIOGRAPHY:**

268 Donoso, R. A., Gonzalez-Toro, F. & Perez-Pantoja, D. (2021) Widespread distribution of
269 hmf genes in *Proteobacteria* reveals key enzymes for 5-hydroxymethylfurfural
270 conversion. *Comput Struct Biotechnol J* 19: 2160-2169.
271 Georgiadou, D. N., Avramidis, P., Ioannou, E. & Hatzinikolaou, D. G. (2021) Microbial
272 bioprospecting for lignocellulose degradation at a unique Greek environment. *Heliyon*
273 7: e07122.
274 Kampfer, P., Busse, H. J., Mcinroy, J. A. & Glaeser, S. P. (2017) *Paracandidimonas soli* gen.
275 nov., sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 67: 1740-1745.
276 Kim, Y. J., Kim, M. K., Im, W. T., Srinivasan, S. & Yang, D. C. (2010) *Parapusillimonas*
277 *granuli* gen. nov., sp. nov., isolated from granules from a wastewater-treatment
278 bioreactor. *Int J Syst Evol Microbiol* 60: 1401-6.
279 Vaz-Moreira, I., Figueira, V., Lopes, A. R., De Brandt, E., Vandamme, P., Nunes, O. C. &
280 Manaia, C. M. (2011) *Candidimonas nitroreducens* gen. nov., sp. nov. and
281 *Candidimonas humi* sp. nov., isolated from sewage sludge compost. *Int J Syst Evol*
282 *Microbiol* 61: 2238-46.

283 Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. a. D., Kandler, O., Krichevsky, M.
284 I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & Authors, O. (1987)
285 International Committee on Systematic Bacteriology. Report of the *ad hoc* committee
286 on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 37:
287 463–464.
288 Yao, L., Lai, Y., Xue, F., Sun, L. & Wang, J. (2019) *Paracandidimonas caeni* sp. nov.,
289 isolated from sludge. *Int J Syst Evol Microbiol* 69: 3332-3337.

290



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293 Figure 1. Dendrogram based on 16S rRNA gene sequences, showing the position of the
294 *Paracandidimonas* species in relation to the type strains of species with which any of the two
295 *Paracandidimonas* types strains shared ≥97% sequence identity. The dendrogram was
296 generated by the Neighbor-Joining method. Bootstrap values ≥50%, generated from 1000 re-
297 samplings, are indicated at branch points. Filled circles indicate branches on the tree that
298 were also recovered in the trees generated using the maximum-likelihood and maximum
299 parsimony algorithms. Bar, 1 substitution per 200 nucleotide positions.

300