Pusillimonas

1. CONTRIBUTORS DETAILS

Ana Durán-Viseras

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169-005 Porto, Portugal

Célia M. Manaia

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169-005 Porto, Portugal

Ivone Vaz-Moreira

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho 1327, 4169-005 Porto, Portugal

Olga C. Nunes

LEPABE, Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

2. KEYWORDS:

Pusillimonas; *Alcaligenaceae*; soil; sludge; enrichment culture;

3. ABSTRACT

Rods, Gram-stain-negative, non-motile or motile by polar or lateral flagella. Strictly **aerobic**. Optimal growth temperature ranges 30 ºC - 37 ºC. Catalase and cytochrome *c* oxidase tests are positive in most species. The major respiratory quinone is **ubiquinone Q-8**. Cellular fatty acid composition is characterized by the predominance of $C_{16:0.}$ The other most abundant fatty acids ($C_{17:0}$ cyclo, $C_{19:0}$ cyclo *ω8c* and $C_{18:0}$) vary among species. The polar lipids composition comprises phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine, as well as some unidentified amino- and phospholipids. Phylogenetically, belongs to the family *Alcaligenaceae*.

4. DEFINING PUBLICATION

Pusillimonas, Stolz, Bürger, Kuhm, Kämpfer and Busse 2005, 1080^{VP} *emend*. Park, Park, Jung, Lee, Park, Lee and Jeon 2011, 2904^{VP}.

5. ETYMOLOGY

Pusillimonas (Pu.sil.li.mo´nas. L. adj. *pusillus* very small/minute; Gr. fem n. *monas* unit/monad; N.L. fem. n. *Pusillimonas* very small monad/unicell, referring to the small size of cells and colonies of the type species).

6. GENERIC DEFINITION

Rods, Gram-stain-negative, non-motile or motile by polar or lateral flagella. Strictly **aerobic**. Optimal growth temperature ranges 30 ºC - 37 ºC. Catalase and cytochrome *c* oxidase tests are positive in most species. The major respiratory quinone is **ubiquinone Q-8**. Cellular fatty acid composition is characterized by the predominance of $C_{16:0.}$ The other most abundant fatty acids ($C_{17:0}$ cyclo, $C_{19:0}$ cyclo *ω8c* and $C_{18:0}$) vary among species. The polar lipids composition comprises phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine, as well as some unidentified amino- and phospholipids. Phylogenetically, belongs to the family *Alcaligenaceae*.

The DNA G+C content (mol%) is 53.1-63.3 (Tm/HPLC) and 53.4-63.1 (WGS).

Type species: ***Pusillimonas noertemannii***, Stolz, Bürger, Kuhm, Kämpfer and Busse 2005, 1080^{VP} *emend*. Park, Park, Jung, Lee, Park, Lee and Jeon 2011, 2904^{VP}.

Number of species with validated names: 7.

7. FAMILY CLASSIFICATION

Alcaligenaceae (fmb00180)

8. FURTHER DESCRIPTIVE INFORMATION

8.1. Cell morphology

The genus *Pusillimonas* currently includes seven validly named species, *P. noertemannii*, the type species, and *P. ginsengisoli*, *P. soli*, *P. harenae*, *P. caeni*, *P. thiosulfatoxidans* and *P. maritima* (Stolz *et al.*, 2005; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Jin *et al.*, 2017; Koh *et al.*, 2019; Li *et al.*, 2020). *Pusillimonas* spp. cells are rod-shaped and stain Gram-negative. The dimensions of the cells of the type strains of the seven species are described as (μm, diameter x length): 0.5-0.8 x 1.0-1.5 for *P. noertemannii*, 0.3-0.6 x 0.5-0.8 for *P. ginsengisoli*, 0.3-0.5 x 0.7-1.0 for *P. soli*, 0.5-0.7 x 0.6-0.9 for *P. harenae*, 0.6-0.7 x 0.8-1.6 for *P. caeni*, 0.7-1.2 x 2.0-2.8 for *P. thiosulfatoxidans* and 0.6-0.7 x 0.7-1.7 for *P. maritima* (Table 1) (Stolz *et al.*, 2005; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Koh *et al.*, 2019; Li *et al.*, 2020). *P. noertemannii*, *P. soli*, *P. harenae* and *P. thiosulfatoxidans* are described as motile (Stolz *et al.*, 2005; Lee *et al.*, 2010; Park *et al.*, 2011; Koh *et al.*, 2019). Polar flagella and two flagella on the rods side are described in *P. noertemannii* and *P. harenae*, respectively (Stolz *et al.*, 2005; Park *et al.*, 2011). *P. ginsengisoli*, whose type strain DCY25^{T} was originally described as being motile (Srinivasan *et al.*, 2010), is later reported as non-motile by Park *et al.*, 2011. *P. caeni* and the strains *P. maritima* 174A^{T} and L52-1-41 are described non-motile or non-flagellated, respectively (Jin *et al.*, 2017; Li *et al.*, 2020).

<Table 1 near here>

8.2. Colonial and cultural characteristics

Pusillimonas spp. form visible colonies after 2-6 days of incubation on different nutritive media. *P. noertemannii* BN9^T forms brownish circular colonies with 1-2 mm of diameter and entire margin after incubation 5-6 days at 30 ºC on Nutrient Broth (NB) agar medium or peptone yeast extract (PYE; 0.3% yeast extract, 0.3% peptone, 1.5% agar and pH 7.2); whereas on chocolate blood agar (Columbia agar base; Oxoid CM331) supplemented with 10% defibrinated sheep blood, colonies reach 2-3 mm of diameter after 3-4 days at 37 ºC (Stolz *et al.*, 2005). Colonies of *P. ginsengisoli* strains DCY25^T and DCY28 are pale yellow and circular with an entire margin after 5 days of growth on Reasoner's 2A agar (R2A) agar at 30 ºC (Srinivasan *et al.*, 2010). On Luria-Bertani (LB) agar *P. soli* MJ07^T forms yellow, circular, low-convex colonies with irregular margins and 1-3 mm in diameter, after 5 days of incubation at 25 or 30 ºC, and smaller colonies (0.3-0.5 mm) on R2A, Trypticase Soy Agar (TSA), MacConkey or NB media when incubated at the same temperature (Lee *et al.*, 2010). *P. harenae* B201^T forms ivory, convex, circular colonies with entire margins after 3 days incubation on R2A agar (Park *et al.*, 2011), whereas in the same medium, *P. caeni* forms creamy white, smooth, convex and entire-margin colonies after 2 days of incubation at 30 ºC (Jin *et al.*, 2017). *P. thiosulfatoxidans* forms pale yellow, circular and smooth colonies of 1 mm of diameter on R2A after 3 days of incubation at 30 ºC (Koh *et al.*, 2019). *P. maritima* colonies are creamy white, circular and convex with smooth surfaces and 1.0-2.0 mm in diameter after 2 days incubation on Marine Agar 2216 (MA) at 37 ºC (Li *et al.*, 2020).

8.3. Nutrition and growth conditions

Pusillimonas spp. grow in a wide variety of complex nutritive media, such as LB, NB, chocolate blood agar, PYE, R2A, TSA, MA and MacConkey agar under aerobic conditions (Stolz *et al.*, 2005; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011;

Jin *et al.*, 2017; Koh *et al.*, 2019; Li *et al.*, 2020). Chocolate blood agar with 10% defibrinated sheep blood favours the growth of *P. noertemannii* BN9^T, whereas LB agar and R2A favours *P. soli* MJ07^T and *P. harenae* B201^T, respectively (Stolz *et al.*, 2005; Lee *et al.*, 2010; Park *et al.*, 2011). Increased CO_2 concentrations (5%) do not favour the growth of *P. noertemannii* BN9^T (Stolz *et al.*, 2005).

The cardinal temperature values range 10-15 ºC and 37-45 ºC, with optimum at 30 ºC or 37 ºC, the later value reported for *P. noertemannii* BN9^T (Stolz *et al.*, 2005) and *P. maritima* 17-4A^T (Li *et al.*, 2020). Growth is observed within the pH range 5.0/5.5-9.0, with optima around neutrality, in all species (Koh *et al.* 2019; Lee *et al.*, 2010; Park *et al.*, 2011; Li *et al.*, 2020). The majority of the *Pusillimonas* strains are halotolerant, growing between 0 and 6-10% (w/v) NaCl, with optima ranging 0-3% (w/v) NaCl (Park *et al.*, 2011; Li *et al.*, 2020). However, *P. maritima* 17-4A^T, *P. caeni* EBR-8-1^T and *P. thiosulfatoxidans* YE3^T are described as requiring 0.5-1% (w/v) NaCl to grow (Jin *et al.*, 2017; Koh *et al.*, 2019; Li *et al.*, 2020) (Table 1). The detection of genes encoding for the synthesis of the compatible solute ectoine in the genomes of all the *Pusillimonas* type strains corroborates their capacity to cope with NaCl (Li *et al.*, 2020). In addition, also the genes encoding for choline dehydrogenase (BetA) and betaine aldehyde dehydrogenase (BetB), involved in the synthesis of the compatible solute betaine from choline, were detected in the genomes of *P. harenae* B201^T, *P. thiosulfatoxidans* YE3^T and *P. maritima* 174A^T and L52-1-41 (Li *et al.*, 2020).

8.4. Metabolism

Pusillimonas spp. are organo-heterotrophic aerobic bacteria. *P. thiosulfatoxidans* YE3^T is the only strain in the genus described at the moment of writing as being capable of oxidizing thiosulfate (Koh *et al.*, 2019). Oxidation of thiosulfate occurred in the presence

144 of organic nutrients (R2A medium) but not in artificial freshwater media, suggesting their
145 inability of litho-autotrophic growth (Koh *et al.*, 2019). Growth under anaerobic
146 conditions was not reported for any *Pusillimonas* spp. Nitrate reduction is a variable
147 characteristic among *Pusillimonas* strains, with some being able to reduce nitrate into
148 nitrite (*P. ginsengisoli* strains DCY25^T and DCY28) or to N_2 (*P. soli* strain MJ07^T).
149 Catalase and cytochrome-*c*-oxidase activities were described as being positive for
150 *Pusillimonas* spp. (Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Jin *et al.*,
151 2017; Koh *et al.*, 2019; Li *et al.*, 2020), except in *P. maritima* strains 17-4A^T and L52-1-
152 41 for which Li *et al.* (2020) reported negative results. Nevertheless, genes encoding
153 catalase–peroxidase (KatG) and peroxidase were reported to be present in the genomes
154 of these strains (Li *et al.*, 2020).
155 The nutritional and enzymatic activities profiles have been used to distinguish the
156 different species of this genus (Table 1). Despite some contradictory observations
157 reported in different publications, till the moment of writing all *Pusillimonas* spp. are
158 described as testing positive for the activity of esterase (C4) and negative for α-
159 fucosidase, β-galactosidase, β-glucuronidase and α-mannosidase, for the assimilation of
160 N-acetylglucosamine, D-maltose, D-mannose and D-mannitol as single carbon sources,
161 as well as for indole production and aesculin hydrolysis (Stolz *et al.*, 2005; Lee *et al.*,
162 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Koh *et al.*, 2019; Li *et al.*, 2020).
163 The potential of *P. noertemannii* BN9^T to degrade environmental pollutants has been
164 described. This strain mineralizes 3-aminobenzoate (3AB), commonly used as an
165 intermediate in the synthesis of xenobiotic organic compounds, such as dyes and
166 pesticides. Through the action of a 3AB-6-hydroxylase, *P. noertemannii* BN9^T
167 transformed 3AB into 5-aminosalicylate (5AS), which in turn, through the action of a
168 5AS-1,2-dioxygenase was transformed into a non-aromatic aminated ring-fission product

(cis-4-amino-6-carboxy-2-oxo-hexa-3,5-dienoate), which was further degraded into fumarate and pyruvate (Russ et al., 1994). Interestingly, 5AS, which is the active agent of the azo compound sulphasalazine used in the treatment of human ulcerative colitis, was also the product of the degradation of 6-aminonaphthalene-2-sulphonic acid (6A2NS) by strain BN6 (identified as *Sphingobium xenophagum*; Pal et al., 2006). Consequently, the mixed culture of these two strains (BN6 and BN9^T) cooperatively mineralized 6A2NS, used in the synthesis of azo dyes (Nörtemann *et al.*, 1986; Stolz et al., 1992; Stolz & Knackmuss, 1993a; Russ *et al.*, 1994). In addition, *P. noertemannii* BN9^T was shown to mineralize 2,4-dihydroxybenzoate (2,4-DHB) and gentisate (2,5-dihydroxybenzoate, 2,5-DHB) (Stolz & Knackmuss, 1993b). According to Stolz et al. (1992), a gentisate 1,2-dioxygenase was involved in the transformation of 2,5-DHB into maleylpyruvate, which was further channelled into the 5AS degradative pathway. The 2,4-DHB was transformed by a 2,4-dihydroxybenzoate 1-monooxygenase into 1,2,4-trihydroxybenzene (1,2,4-THB), which, by the successive action of a 1,2,4-THB 1,2-dioxygenase and a maleylacetate reductase, was converted into 3-oxoadipate (Stolz & Knackmuss, 1993b).

Other strains of *Pusillimonas* spp. were described as able to degrade N-heterocyclic compounds, such as 5-hydroxypicolinic acid, 3-hydroxypyridine, and nicotinic acid. *Pusillimonas* sp. strain 5HP, presenting 99.32% 16S rRNA gene sequence similarity with the *P. noertemannii* BN9^T, was reported to harbour three different inducible metabolic pathways for degradation of 5-hidroxypicolinic acid, 3-hydroxypyridine, and nicotinic acid, all resulting in the formation of 2,5-dihydroxypyridine. The initial degradation step of 5-hydroxypicolinic acid was described as being catalyzed by a 5-hydroxypicolinate 2-monooxygenase (Karvelis et al., 2014). *Pusillimonas* sp. strain T2, presenting 98.16% 16S rRNA gene sequence similarity with *P. thiosulfatoxidans* YE3^T, was described as

194 being able to degrade nicotine with the intermediary accumulation of 6-hydroxy-nicotine,
195 6-hydroxy-N-methylmyosmine, 6-hydroxypseudooxynicotine, 2,6-dihydroxypyridine, 6-
196 hydroxy-3-succinoyl-pyridine and 2,5-dihydroxypyridine (Ma et al., 2015). In addition,
197 strain T2 was reported as capable of nicotinic acid degradation through the activity of a
198 three-component nicotinic acid hydroxylase ($NahAB_1B_2$), which transformed nicotinic
199 acid into 6-hydroxynicotinic acid, transiently accumulated by cells (Yuan et al., 2018).
200

201 **8.5 Chemotaxonomic characteristics**

202 The major respiratory quinone reported in the *Pusillimonas* species is ubiquinone 8, with
203 minor amounts of menaquinone-6 (MK-6) being also reported in *P. harenae* (Stolz *et al.*,
204 2005; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Jin *et al.*, 2017; Koh *et*
205 *al.*, 2019; Li *et al.*, 2020). The fatty acid methyl esters profile distinguishes the
206 *Pusillimonas* species, which is, nevertheless, characterized by the predominance of $C_{16:0}$
207 (Table 2). The relative abundance of other fatty acids varies among species. According to
208 Li *et al.* (2020), who analysed simultaneously the fatty acid methyl esters profile of all
209 *Pusillimonas* type strains, *P. thiosulfatoxidans* $YE3^T$ showed the most distinct profile.
210 According to that study, $C_{19:0}$ cyclo *ω8c* and $C_{17:0}$ cyclo were also among the most
211 abundant components in *P. noertemannii*, $C_{17:0}$ cyclo and $C_{18:0}$ in *P. ginsengisoli*, $C_{18:0}$ in
212 *P. caeni* and *P. maritima*, and $C_{17:0}$ cyclo in *P. soli*, *P. harenae* and *P. thiosulfatoxidans*
213 (Li *et al.*, 2020; Koh *et al.*, 2019) (Table 2).
214 *Pusillimonas* species are characterized by the presence of the polar lipids
215 phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine,
216 complemented by unidentified phospholipids and/or aminolipids (Stolz *et al.*, 2005; Lee
217 *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Jin *et al.*, 2017; Koh *et al.*, 2019;

Li *et al.*, 2020). The number, type and relative proportion of the unknown polar lipids may vary according to the species or strain (Table 1).

The polyamine pattern in *P. noertemannii* BN9^T comprise putrescine, spermidine and 2-hydroxyputrescine. Putrescine is also the major polyamine in *P. maritima* strains 17-4A^T and L52-1-41 and *P. soli* strain MJ07^T. In addition, this last strain contains 2-hydroxyputrescine, spermidine and a minor amount of cadaverine.

<Table 2 near here>

8.6 Genome features

The whole genome sequences of the type strains of the seven species validly named are available in the GenBank: *Pusillimonas noertemannii* BN9^T (accession no. PDUX00000000), *Pusillimonas ginsengisoli* DCY25^T (accession no. SDQE00000000), *Pusillimonas soli* MJ07^T (accession no. SDQC00000000), *Pusillimonas harenae* B201^T (accession no. SDQD00000000), *Pusillimonas caeni* EBR-8-1^T (accession no. PDUW00000000), *Pusillimonas thiosulfatoxidans* YE3^T (accession no. CP022987) and *Pusillimonas maritima* 17-4A^T (accession no. NQOU00000000). *P. noertemannii* BN9^T, *P. ginsengisoli* DCY25^T, *P. soli* MJ07^T, *P. harenae* B201^T, *P. caeni* EBR-8-1^T and *P. maritima* 17-4A^T genome sequences were obtained based on Illumina HiSeq 2000, with a sequencing depth coverage higher than 60x (Li *et al.*, 2020). *Pusillimonas thiosulfatoxidans* YE3^T genome sequencing was performed using a PacBio RSII platform and Illumina HiSeq 4000 (Koh *et al.*, 2019).

The genome length, number of contigs, N50 value and G+C content are described in Table 3.

<Table 3 near here>

8.7 Ecology and Habitat

Members of the genus *Pusillimonas* are widely distributed in aquatic and terrestrial environments, frequently man-made or impacted environments (Elliott *et al.*, 2010; Cao *et al.*, 2011; Jin *et al.*, 2014; Chikere *et al.*, 2017; Grouzdev *et al.*, 2018; Koh *et al.*, 2019). *Pusillimonas* genus members have been reported as microorganisms with a key role in the consumption of non-easily biodegradable pollutants (such as high-molecular-weight fraction polycyclic aromatic hydrocarbon [HMW-PAHs]; endosulfan or nicotine) and thus as potential microorganisms with *in-situ* bioremediation applications (Lladó *et al.*, 2013; Ma *et al.*, 2015; Obi *et al.*, 2016; Chikere *et al.*, 2017; Remmas *et al.*, 2017; Kong *et al.*, 2018; Sun *et al.*, 2019). The seven validly named species of the genus were isolated from samples collected from the River Elbe (Germany) (*P. noertemannii*) (Nörtemann *et al.*, 1986), soil of a ginseng field (*P. ginsengisoli*) and soil of a farm (*P. soli*), both near the Daejeon city (South Korea) (Srinivasan *et al.*, 2010; Lee *et al.*, 2010), sand from a beach in the Taean coast (South Korea) (*P. harenae*) (Park *et al.* 2011), sludge from a biofilm reactor and from a municipal wastewater treatment plant (*P. caeni* and *P. thiosulfatoxidans*, respectively) both in Daejeon, South Korea (Jin *et al.*, 2014; Koh *et al.*, 2019) and from the surface of seawater of the Indian Ocean and South China Sea (*P. maritima*) (Li *et al.*, 2020).

Accordingly, representatives of the genus *Pusillimonas* have been identified in different samples from soils (Negus & Taylor, 2014; Karvelis *et al.*, 2014), creosote-contaminated aged soils (Lladó *et al.*, 2013), crude oil-polluted soils (Chikere *et al.*, 2017), activated sludge systems (Zhang *et al.*, 2015; Kong *et al.*, 2018), sludge from crude oil (Obi *et al.*, 2016) or from different wastewater treatment systems in China (Ma *et al.*, 2015; Sun *et*

al., 2019) and Brazil (Sant' Anna *et al.*, 2020), leachate from landfills in Peloponnese (Greece) (Remmas *et al.*, 2017) and Shangai (China) (Song *et al.*, 2015), sediments from the Eastern Arabian Sea (India) (Mandal *et al.*, 2020) and from the River Elizabeth (USA) (Hilyard *et al.*, 2008), petroleum-polluted seabed in the Bohai Sea (China) (Cao *et al.*, 2011) or contaminated groundwater from UK (Elliott *et al.*, 2010) and Russia (Grouzdev *et al.*, 2018) by using different culture-dependent and culture-independent methodologies.

9. ENRICHMENT AND ISOLATION PROCEDURES

Pusillimonas noertemannii BN9^T was the first strain of this genus reported in the literature (Nörtemann *et al.*, 1986). It was isolated from a 6-aminonaphthalene-2-sulphonate (6A2NS)-degrading mixed bacterial culture directly enriched from water collected in the River Elbe (Germany) in an Erlenmeyer flask with baffles containing 200 mL of mineral salts medium supplemented with 8 mM 6A2NS and 2 mM naphthalene-2-sulfonic acid, incubated at 30 ºC, 120 rpm for 6 days. Subculturing was performed in mineral salts medium with 10 mM 6A2NS as the sole carbon source and 10 mg/L cycloheximide to supress growth of eucaryotic cells for 1 year. Different selective and nonselective culture media were used to isolate 10 individual strains from the mixed culture, including the strains BN6 and BN9^T (Nörtemann *et al.*, 1986).

P. ginsengisoli strains DCY25^T and DCY28 were isolated by Srinivasan *et al.* (2010) from the surface soil of a ginseng crop field. A suspension containing 1 g of soil in 50 mL of saline solution was serially diluted and spread on plates of 1/10 diluted R2A agar (Difco) and incubated at 30 ºC. After 3 days, single colonies were purified by sub-cultivation in the same medium. A similar procedure was used to isolate the type strain of the species *P. caeni* (EBR-8^T) from sludge samples from an aerobic ECOVISION

biofilm reactor (Jin *et al.*, 2014; Jin *et al.*, 2017). Samples were placed in saline solution (0.85%), serially diluted and 100 µL sub-sample volumes were streaked on modified 1/10 R2A agar. After incubation for 8 days at 25 ºC in the dark, the colonies formed were selected for further studies (Jin *et al.*, 2017).

Strain MJ07^T, the type strain of the species *P. soli* was isolated from a soil sample collected in a farm close to Daejeon city (South Korea) (Lee *et al.*, 2010). For bacterial isolation, the sample was diluted in 50 mM phosphate buffer at pH 7.0 and plated on LB agar (Difco). Plates were incubated for 2 weeks at 30 ºC. Colonies were purified by re-streaking on fresh plates of the same medium and incubation conditions.

P. harenae B201^T was isolated from a sand sample from a beach located in Taean coast (Yellow Sea, South Korea) (Park *et al.*, 2011). The sand sample was suspended in saline solution (0.9%, w/v), serially diluted and plated on MA (Difco). After incubation at 25 ºC in aerobiosis for 5 days, colonies were arbitrarily picked.

P. thiosulfatoxidans YE3^T was isolated from an activated sludge sample collected from a municipal wastewater treatment plant in Daejeon city (South Korea) (Koh *et al.*, 2019). After removing the debris, the sludge sample was diluted (1:10) in phosphate saline buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4, pH 7.4), and cultured for 2 weeks at 30 ºC under aerobic conditions in R2A broth (Difco) containing 3 mM thiosulfate (Sigma-Aldrich). Dilutions of the culture (up to 10^{-5}) were plated on R2A supplemented with 3 mM thiosulfate and incubated for one week at 30 ºC. Colonies were inoculated onto new R2A plates with 3 mM thiosulfate for pure culture and subsequently cultivated in the same broth medium for 5 days.

P. maritima strains 17-4A^T and L52-1-41 were originally isolated from seawater samples from the Indian Ocean and from the South China Sea, respectively (Li *et al.*, 2020). Seawater (~ 400 mL) was added to 1% (v/v) sterile petroleum and incubated for one

month at room temperature. The resulting enrichment culture was spread on MA (BD) after four dilution series with sterile seawater, and incubated at 28 ºC for 5 days. Colonies were purified by streaking onto new MA plates.

10. MAINTENANCE PROCEDURES

Pusillimonas spp. can be routinely grown on R2A, Luria-Bertani (LB) agar and Marine Agar or Broth 2216 (Difco) at 30 ºC (Nörtemann *et al*., 1986; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Jin *et al.*, 2017; Koh *et al.*, 2019; Li *et al.*, 2020). For long-term preservation, cultures can be stored as a suspension in broth supplemented with 10% (*P. harenae*), 15% (*P. caeni*), 20% (*P. soli*; *P. maritima*) or 30% (*P. thiosulfatoxidans*) (v/v) glycerol at -80 ºC (Lee *et al.*, 2010; Park *et al.*, 2011; Jin *et al.*, 2017; Koh *et al.*, 2019; Li *et al.*, 2020).

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

Based on the 16S rRNA gene sequence analyses the closest relative genera of *Pusillimonas* species are members of the genera *Parapusillimonas, Eoetvoesia*, *Paracandidimonas*, and *Candidimonas* (Table 1, Figure 1).

Pusillimonas species could be distinguished from *Pp. granuli* Ch07^T, the type strain of the single *Parapusillimonas* validly named species described up to the moment of writing, by their positive enzymatic activity of acid phosphatase (with the exception of *P. noertemannii* and *P. caeni*) and naphtol-AS-BI-phosphohydrolase (with the exception of *P. noertemannii*), by the presence of some unidentified aminolipids and phospholipids,

and $C_{19:0}$ cyclo *ω*8*c* (in *P. noertemannii*), and $C_{18:0}$ (in *P. ginsengisoli, P. caeni* and *P. maritima*) and the absence of summed feature 3 ($C_{16:1}$ *ω*7*c*, $C_{15:0}$ iso 2-OH) and summed feature 5 ($C_{18:1}$ *ω*7*c*, *ω*9*t*, *ω*12*t*) as major fatty acids (Stolz *et al.*, 2005; Kim *et al.*, 2010; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Jin *et al.*, 2017; Koh *et al.*, 2019; Li *et al.*, 2020).

The inability of *Pusillimonas* spp. to grow at 4 °C allows their differentiation from *E. caeni* PB3-7B^T, the type strain of the single species of the *Eoetvoesia* genus described up to the moment of writing (Stolz *et al.*, 2005; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Felföldi *et al.*, 2014; Jin *et al.*, 2017; Koh *et al.*, 2019; Li *et al.*, 2020). Also, the absence of esterase (C4) activity, the ability to assimilate D-mannose and the presence of phosphatidylmethylethanolamine in *Pc. caeni* 24^T; and of phosphatidylserine in *Pc. soli* IMT-305^T differentiate the type strains of the two *Paracandidimonas* species validly named at the moment of writing from *Pusillimonas* spp. (Stolz *et al.*, 2005; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Jin *et al.*, 2017; Kämpfer *et al.*, 2017; Koh *et al.*, 2019; Yao *et al.*, 2019; Li *et al.*, 2020).

Pusillimonas spp. could be distinguished from *C. bauzanensis* CGMCC 1.10190^T, the type strain of one of the three validly named species of *Candidimonas* at the moment of writing, by their inability to grow at 1-5 °C and the absence of phosphatidylmonomethylethanolamine and summed feature 3 ($C_{16:1}$ *ω*7*c* and/or iso-$C_{15:0}$ 2-OH) as one of the major polar lipids and fatty acids, respectively (Stolz *et al.*, 2005; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011; Zhang *et al.*, 2012; Jin *et al.*, 2017; Koh *et al.*, 2019; Li *et al.*, 2020). The presence of summed feature 3 ($C_{16:1}$ *ω*7*c* and/or iso-$C_{15:0}$ 2-OH) as one of the major fatty acids in strains SC-089^T and SC-092^T allows the distinction between *Pusillimonas* spp. and *C. nitroreducens* and *C. humi*, respectively (Stolz *et al.*, 2005; Lee *et al.*, 2010; Srinivasan *et al.*, 2010; Park *et al.*, 2011;

Vaz-Moreira *et al.*, 2011; Jin *et al.*, 2017; Kämpfer *et al.*, 2017; Koh *et al.*, 2019; Yao *et al.*, 2019; Li *et al.*, 2020).

12. TAXONOMIC COMMENTS

The genus *Pusillimonas* belongs to the family *Alcaligenaceae*, order *Burkholderiales* and class *Betaproteobacteria*. The genus comprises seven species, five of which, *P. noertemannii*, *P. soli*, *P. harenae*, *P. caeni* and *P. thiosulfatoxidans*, were described based on a single strain and *P. ginsengisoli* and *P. maritima* on two. The 16S rRNA gene sequence identity and DNA-DNA hybridization values confirmed the affiliation of strains DCY25^T and DCY28 and 174A^T and L52-1-41 to *P. ginsengisoli* (99.6% and 87% DNA-DNA homology) and *P. maritima* (99.45% and 70.2% DNA-DNA homology), respectively (Srinivasan *et al.*, 2010; Li et al., 2020). The genus was first proposed in 2005 to accommodate strain BN9^T, initially identified as belonging to genus *Pseudomonas*, as representative of the species *Pusillimonas noertemannii* (Nörtemann *et al.*, 1986; Stolz *et al.*, 2005).

Based on EzBiocloud comparison (Yoon *et al.*, 2017), the 16S rRNA gene sequence identity values among the type strains of the species of the genus *Pusillimonas* ranges 95.9% to 99.1%, being *P. noertemannii* and *P. caeni* the closest and *P. thiosulfatoxidans* and *P. maritima* the most distant species. For the species that share >97% 16S rRNA gene sequence identity, digital DNA-DNA hybridization (dDDH) values, or DNA-DNA hybridization values obtained experimentally, below the 70% threshold for the delineation of genomic species (Wayne *et al.* 1987; Meier-Kolthoff *et al.,* 2013) reported at the time of the description of the different species support the distinction of the seven species (Table 4).

Also, the average nucleotide identity (ANI) values between the type strains of the seven species of the genus *Pusillimonas* (69.9-86.1%) are lower than 95%, the cut-off value for species boundary definition (Richter & Roselló-Móra, 2009), confirming their condition of different species. However, according to the 16S rRNA gene sequence based phylogenetic tree (Figure 1), *Pusillimonas* species form two distinct clusters, herein named group I and group II. One cluster (group I) is comprised by the species *P. noertemannii*, *P. caeni* and *P. maritima* and a second (group II) is represented by *P. ginsengisoli*, *P. harenae*, *P. soli* and *P. thiosulfatoxidans* (Figure 1). In addition, *Candidimonas bauzanensis* is also included within the group II (Figure 1). In accordance with data previously reported (Manaia *et al.,* 2021), *P. thiosulfatoxidans* YE3^T shares the highest 16S rRNA gene sequence similarity (98.2%) and ANI (76.1%) values with *C. bauzanensis* BZ59^T, followed by the other *Pusillimonas* species clustering in group II (98.1-97.8% and 75.2-73.9%, respectively). In contrast, *P. thiosulfatoxidans* YE3^T shares pairwise 16S rRNA gene sequence similarity and ANI values ≤ 96.8% and ≤ 75.1% with the type strains of *Pusillimonas* species of group I.

The average amino acid identity (AAI) is the most well-recognized standard for classifying prokaryotic genera and whose cut-off is 65% for genus delineation (Konstantinidis *et al.,* 2017). *Pusillimonas* species shared AAI values ranging 71-76% among members of the group II, and 69-76% including *C. bauzanensis*. Whereas in group I, AAI values ranged 68-88%. Those results indicate that within each group, all members belong to the same genus. However, the AAI values between *Pusillimonas* members of the two groups ranged 63-68%, even when *C. bauzanensis* is included in the comparison. These observations suggest that groups I and II may constitute two different genera. Thus, the results from the phylogenetic analysis and the AAI estimation suggest that the *Pusillimonas* genus definition may need a revision.

<Figure 1 near here>

<Table 4 near here>

13. LIST OF SPECIES OF THE GENUS *PUSILLIMONAS*

1. Pusillimonas caeni

Jin, Ko, Cui, Lee, Oh, Ahn and Lee 2017, 130^{VP} *emend*. Li, Qi, Lai, Dong, Liu, Wang and Shao 2020, 3487^{VP}.

caeni (cae´ni. L. gen. n. *caeni* of sludge).

Comprises non-motile short rods (0.6-0.7 x 0.8-1.6 µm) forming creamy-white, smooth, circular, convex colonies on R2A agar. Growth occurs at 15-37 ºC, pH 6.0-9.0, 1.0-8.0% (w/v) NaCl and optimally at 30 ºC, pH 7.0 and 1-3% (w/v) NaCl. Other characteristics are as given for the genus, with the following additional information. Nitrate is not reduced and indole is not produced. Tests negative for urea and aesculin hydrolysis. The enzymatic activities leucine arylamidase and esterase (C4) are positive. Naphtol-AS-BI-phosphohydrolase and trypsin are weakly positive. Negative for *N*-acetyl-*β*-glucosaminidase, acid phosphatase, alkaline phosphatase, *α*-chymotrypsin, cystine arylamidase, *α*-galactosidase, *α*-glucosidase, *α*-fucosidase, *α*-mannosidase, *β*-galactosidase, *β*-glucuronidase, lipase (C14) and valine arylamidase activities. Able to assimilate 3-hydroxybenzoate and 4-hydroxybenzoate as sole carbon sources, but not able to assimilate adipate, caprate, citrate, acetate, propionate, DL-lactate, L-alanine, itaconate, L-malate, phenylacetate, L-proline, L-serine, valerate, DL-3-hydroxybutyrate, L-fucose, *N*-acetylglucosamine, L-arabinose, gluconate, D-glucose, D-maltose, D-

mannose, *α*-D-melibiose, L-rhamnose, D-ribose, D-sucrose, salicin, inositol, D-mannitol, D-sorbitol, suberate, L-histidine, glycogen, 2-ketogluconate, 5-ketogluconate and malonate. Acid is not produced from glucose. The major fatty acids (> 5%) includes $C_{16:0}$, $C_{18:0}$, $C_{17:0}$ cyclo, $C_{19:0}$ cyclo *ω8c*, $C_{18:1}$ *ω9c* and summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*).

The DNA G+C content (mol%) is 63.3 (HPLC) and 63.1 (genome).

Type strain: EBR-8-1 (= KCTC 42353 = JCM 30463).

GenBank accession number (16S rRNA): KF056995.

GenBank accession number (genome): PDUW00000000.

2. Pusillimonas ginsengisoli

Srinivasan, Kim, Sathiyaraj, Kim and Yang 2010, 1785^{VP} *emend*. Li, Qi, Lai, Dong, Liu, Wang and Shao 2020, 3489^{VP}.

ginsengisoli (gin.sen.gi.so´li. N.L. n. *ginsengum* ginseng; L. n. *solum -i* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field, the source of the type strain).

Comprises short-rods (0.3-0.6 x 0.5-0.8 µm), forming pale yellow, circular and with entire margin colonies on R2A agar after 5 days at 30 ºC. Growth in the range 15-40 ºC, pH 5.5-9.0 and optimally at 30 ºC and pH 7.0. Other characteristics are as given for the genus, with the following additional information. Nitrate is reduced into nitrite. Negative for aesculin and urea hydrolysis and indole is not produced. The enzymatic activities acid phosphatase, alkaline phosphatase, *α*-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphtol-AS-BI-phosphohydrolase, trypsin and valine arylamidase are positive. Negative for *N*-acetyl-*β*-glucosaminidase, arginine dihydrolase, *α*-fucosidase, *β*-galactosidase, *α*-galactosidase, *α*-glucosidase,

465 *β*-glucosidase, *β*-glucuronidase, lipase (C14), *α*-mannosidase or protease activities. Able
466 to assimilate the following sole carbon sources: acetate, adipate, L-alanine, citrate, 3-
467 hydroxybutyrate, itaconate, DL-lactate, L-malate, phenylacetate, L-proline, propionate,
468 L-serine and valerate, but not able to assimilate *N*-acetyl-D-glucosamine, L-arabinose,
469 caprate, L-fucose, gluconate, D-glucose, glycogen, L-histidine, N-acetylgalactosamine,
470 3-hydroxybenzoate, 4-hydroxybenzoate, 2-ketogluconate, 5-ketogluconate, maltose, D-
471 mannitol, D-mannose, melibiose, *myo*-inositol, L-rhamnose, D-ribose, salicin, D-sorbitol,
472 suberate or sucrose. Acid is not produced from glucose. The major fatty acids (> 5%)
473 includes $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:0}$, summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*), $C_{12:0}$, $C_{18:1}$
474 *ω9c*, $C_{19:0}$ cyclo *ω8c*, $C_{14:0}$ and summed feature 2 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH). The
475 species also includes strain DCY28.

476 The DNA G+C content (mol%) of the type strain is 57.3 (HPLC) and 57.9 (genome).

477 Type strain: DCY25 (= KCTC 22046 = JCM 14767 = DSM 25164).

478 GenBank accession number (16S rRNA): EF672088.

479 GenBank accession number (genome): SDQE00000000.

480

481 ***3. Pusillimonas harenae***

482 Park, Park, Jung, Lee, Park, Lee and Jeon 2011, 2904^{VP} *emend*. Li, Qi, Lai, Dong, Liu,
483 Wang and Shao 2020, 3489^{VP}.

484 *harenae* (ha.re´nae. L. gen. n. *harenae* of sand, from where the organism was isolated).

485 Comprises rods (0.5-0.7 x 0.6-0.9 µm), motile by two lateral flagella, forming ivory
486 coloured, convex and round with entire margin colonies on R2A agar. Growth occurs at
487 15-45 ºC, pH 5.0-9.0, 0-6% (w/v) NaCl and optimally at 30 ºC, pH 6.0-7.5 and 0-3%

(w/v) NaCl. Other characteristics are as given for the genus, with the following additional information. Not able to reduce nitrate or produce indole. Tests negative for aesculin and gelatin hydrolysis. The enzymatic activities esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase and acid phosphatase are positive. Alkaline phosphatase, trypsin, *α*-chymotrypsin and napthol-AS-BI-phosphohydrolase are weakly positive. Negative for lipase (C14), *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase, arginine dihydrolase and *α*-fucosidase activities. Able to assimilate the following sole carbon sources: malate, acetate, propionate, lactate, adipate and L-alanine. Not able to assimilate *N*-acetylgalactosamine, L-serine, D-glucose, L-arabinose, D-mannose, maltose, L-histidine, inositol, D-mannitol, D-sorbitol, gluconate, L-rhamnose, D-sucrose, salicin, *N*-acetylglucosamine, L-fucose, caprate, *α*-D-melibiose and phenylacetate. Acid is not produced from glucose. The major fatty acids (> 5%) includes $C_{16:0,}$ $C_{17:0}$ cyclo, summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) and $C_{18:0}$.

The DNA G+C content (mol%) is 53.1 (HPLC) and 56.3 (genome).

Type strain: B201 (= KACC 14927 = JCM 16917 = DSM 25667).

GenBank accession number (16S rRNA): GQ232740.

GenBank accession number (genome): SDQD00000000.

4. Pusillimonas maritima

Li, Qi, Lai, Dong, Liu, Wang and Shao 2020, 3489^{VP}.

maritima (ma.ri´ti.ma. L. fem. adj. *maritima* of or belonging to the seawater).

Comprises non-flagellated short-rods (0.6-0.7 x 0.7-1.7 µm), forming white, circular and smooth convex colonies in MA agar. Growth occurs at 10-45 ºC, pH 5.0-9.0, 0-10% (w/v)

NaCl and optimally at 37 ºC, pH 7.0-8.0 and 1-3% (w/v) NaCl. Other characteristics are as given for the genus, with the following additional information. Nitrate is not reduced and H_2S and indol are not produced. Tests negative for aesculin, gelatin, Tween 20, Tween 40, Tween 60 and Tween 80 hydrolysis. The enzymatic activity of napthol-AS-BI-phosphohydrolase, esterase (C4), acid phosphatase and arginine dihydrolase are positive or weakly positive, and negative for alkaline phosphatase, valine arylamidase, cysteine arylamidase, *α*-glucosidase, *β*-glucosidase, lipase (C14), trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *β*-fucosidase activities. The activity of leucine arylamidase and of esterase lipase (C8) is variable. Assimilation of adipic acid as sole carbon source is variable. Not able to assimilate D-mannitol, malic acid, D-glucose, L-arabinose, D-mannose, N-acetylglucosamine, maltose, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. Acids are not produced from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. The major fatty acids (> 5%) includes $C_{16:0}$, $C_{18:0}$ and summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*).

The DNA G+C content (mol%) of the type strain is 53.4 (genome).

Type strain: 17-4A (= MCCC 1A12670 = KCTC 62121).

GenBank accession number (16S rRNA): MK078037.

GenBank accession number (genome): NQOU00000000.

5. Pusillimonas noertemannii

Stolz, Bürger, Kuhm, Kämpfer and Busse 2005, 1080^{VP} *emend*. Li, Qi, Lai, Dong, Liu, Wang and Shao 2020, 3487^{VP}.

535 *noertemannii* (noer.te.mann´i.i. N.L. gen. n. *noertemannii* of Nörtemann, in honour of
536 Bernd Nörtemann, who isolated this and various other bacterial strains that had
537 extraordinary degradative abilities).
538 Comprises rods (0.5-0.8 x 1.0-1.5 µm), motile by polar flagella, forming brownish,
539 circular and with entire margin colonies on chocolate blood agar. Growth occurs at 15-42
540 ºC, pH 5.0-9.0, 0-5% NaCl and optimally at 37 ºC, pH 7.0-8.0 and 2-3% (w/v) NaCl.
541 Other characteristics are as given for the genus, with the following additional information.
542 Positive for L-alanine-*p*-nitroanilide hydrolysis, but not for aesculin, gelatin, *p*-
543 nitrophenyl *β*-D-galactopyranoside, *p*-nitrophenyl *β*-D-glucuronide, *p*-nitrophenyl *α*-D-
544 glucopyranoside, *p*-nitrophenyl *β*-D-glucopyranoside, *p*-nitrophenyl *β*-D-
545 xylopyranoside, bis-*p*-nitrophenyl phosphate, bis-*p*-nitrophenyl-phenyl phosphonate, bis-
546 *p*-nitrophenyl-phosphorylcholine, L-aniline-*p*-nitroanilide, *ɣ*-L-glutamate-*p*-nitroanilide
547 and L-proline-*p*-nitroanilide hydrolysis. Nitrate is not reduced and indol is not produced.
548 The enzymatic activities esterase (C4) and esterase lipase (C8) are positive, but the
549 activities of *α*-fucosidase, *β*-galactosidase, *β*-glucosidase, *β*-glucuronidase and *α*-
550 mannosidase are negative. Able to assimilate the following sole carbon sources:
551 4-hydroxybenzoate, caprate, 2-oxoglutarate and pyruvate, but not able to assimilate
552 citrate, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin,
553 D-cellobiose, D-fructose, D-galactose, gluconate, D-maltose, D-mannose, *α*-D-
554 melibiose, L-rhamnose, D-ribose, D-sucrose, salicin, D-trehalose, D-xylose, adonitol,
555 maltitol, D-mannitol, D-sorbitol, putrescine, cis-aconitate, trans-aconitate, adipate,
556 4-aminobutyrate, azelate, fumarate, glutarate, itaconate, L-malate, mesaconate, L-
557 alanine, L-fucose, L-aspartate, L-histidine, L-leucine, L-ornithine, L-phenylalanine,
558 L-serine, L-tryptophan, L-proline, glycogen, 2-ketogluconate, 5-ketogluconate, malonate
559 and phenylacetate. Acid is not produced from glucose. The major fatty acids profile

(>5%) includes $C_{16:0}$, $C_{19:0}$ cyclo *ω8c*, $C_{17:0}$ cyclo, $C_{12:0}$, summed feature 2 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH), summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*), $C_{18:0}$ and $C_{18:1}$ *ω9c*.

The DNA G+C content (mol%) is 61.8 (Tm) and 62.6 (genome).

Type strain: BN9 (= DSM 10065 = NCIMB 14020).

GenBank accession number (16S rRNA): AY695828.

GenBank accession number (genome): PDUX00000000.

6. Pusillimonas soli

Lee, Woo, Chae and Ten 2010, 2329^{VP} *emend*. Li, Qi, Lai, Dong, Liu, Wang and Shao 2020, 3489^{VP}.

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

Comprises motile non-spore-forming rods (0.3-0.5 x 0.7-1.0 µm). Growth occurs at pH 5.0-9.0 and optimally at pH 6.5-7.0 and 30 ºC. Other characteristics are as given for the genus, with the following additional information. Able to reduce nitrate to nitrogen gas. Negative for DNA, casein, gelatin, aesculin, chitin, HE-cellulose, starch and xylan hydrolysis. Indol is not produced. The enzymatic activities of esterase (C4), acid phosphatase, alkaline phosphatase, leucine arylamidase, naphtol-AS-BI-phosphohydrolase, and esterase lipase (C8) are positive. Negative for *N*-acetyl-*β*-glucosaminidase, cystine arylamidase, *α*-fucosidase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, trypsin, *β*-glucosidase, *β*-glucuronidase, lipase (C14) and *α*-mannosidase activities. Able to assimilate the following sole carbon sources: adipate, propionate, L-alanine, itaconate, L-malate, phenylacetate, L-proline, L-serine, ribose, 3-hydroxybenzoate, 4-hydroxybenzoate, valerate, suberate and malonate, but not able to

583 assimilate caprate, *N*-acetyl-D-glucosamine, L-arabinose, gluconate, D-glucose,
584 glycogen, L-fucose, 2-ketogluconate, 5-ketogluconate, maltose, D-mannitol, D-mannose,
585 melibiose, salicin, *N*-acetylgalactosamine or sucrose. Acids are produced from L-
586 arabinose, gluconate, glycerol, D-ribose, *β*-D-xylopyranoside and arbutin. The major
587 fatty acids (> 5%) includes $C_{16:0}$, $C_{17:0}$, cyclo, summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$
588 *ω6c*), summed feature 2 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH), $C_{19:0}$ cyclo *ω8c*, summed feature
589 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and $C_{14:0}$.

590 The DNA G+C content (mol%) is 59.4 (HPLC) and 59.8 (genome).

591 Type strain: MJ07 (= KCTC 22455 = JCM 16386 = DSM 25264).

592 GenBank accession number (16S rRNA): GQ241322.

593 GenBank accession number (genome): SDQC00000000.

594

595 ***7. Pusillimonas thiosulfatoxidans***

596 Koh, Song, Do, Kim and Park 2019, 1045^{VP}.

597 *thiosulfatoxidans* (thi.o.sul.fat.o´xi.dans. N.L. neut. n. *thiosulfatum* thiosulfate; N.L. part.
598 adj. *oxidans*, oxidizing; N.L. part. adj. *thiosulfatoxidans*, oxidizing thiosulfate).

599 Comprises motile rods (0.7-1.2 x 2.0-2.8 µm) forming pale yellow, circular and smooth
600 colonies on R2A agar. Growth occurs at 10-40 ºC, pH 5.5-9.0, 1.0-8.0% (w/v) NaCl and
601 optimally at 30 ºC, pH 7.0 and 1.0% (w/v) NaCl. Other characteristics are as given for
602 the genus, with the following additional information. Nitrate is not reduced and H_2S is
603 not produced. Thiosulfate is oxidized. Tests negative for DNA hydrolysis. The enzymatic
604 activities of acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8),
605 leucine arylamidase, valine arylamidase and napthol-AS-BI-phosphohydrolase are

606 positive. Negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-
607 galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-
608 β-glucosaminidase, α-mannosidase and α-fucosidase activities. Assimilates the following
609 sole carbon sources: L-alanine, L-glutamic acid, L-pyroglutamic acid, methyl pyruvate,
610 L-lactic acid, αketo-glutaric acid, D-malic acid, L-malic acid, α-hydroxy-butyric acid,
611 β-hydroxy-D, L-butyric acid, α-keto-butyric acid, propionic acid and acetic acid. D-
612 fucose, glycerol, glucuronamide, mucic acid, *p*-hydroxy-phenylacetic acid and
613 acetoacetic acid are weakly assimilated. Not able to assimilate dextrin, maltose, trehalose,
614 cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, lactose, melibiose,
615 methyl β-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine,
616 *N*-acetyl-D-galactosamine, *N*-acetyl neuraminic acid, α-D-glucose, D-mannose, D-
617 fructose, D-galactose, 3-methyl glucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-
618 mannitol, D-arabitol, *myo*-inositol, D-glucose-6-PO_4, D-fructose-6-PO_4, D-aspartic acid,
619 D-serine, glycyl-L-proline, L-arginine, L-aspartic acid, L-histidine, L-serine, pectin,
620 D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid,
621 quinic acid, D-saccharid acid, D-lactic acid methyl ester, citric acid, bromo-succinic acid,
622 Tween-40, γ-amino-butyric acid and formic acid. The major fatty acids (> 5%) includes
623 $C_{16:0}$, $C_{17:0}$ cyclo, summed feature 2 ($C_{12:0}$ aldehyde/unknown 10.928) and $C_{12:0}$.

624 The DNA G+C content (mol%) is 59.3 (genome).

625 Type strain: YE3 (= KCTC 62737 = NBRC 113113).

626 GenBank accession number (16S rRNA): MF457653.

627 GenBank accession number (genome): CP022987.

628

629

RELATED ARTICLES

gbm01821

14. BIBLIOGRAPHY

Cao, B., Ma, T., Ren, Yan, Ren, Yi, Li, G., Li, P., *et al.* (2011) Complete genome sequence of *Pusillimonas* sp. T7-7, a cold-tolerant diesel oil-degrading bacterium isolated from the Bohai sea in China. *J Bacteriol* **193**: 4021–4022.

Chikere, C.B., Azubuike, C.C., & Fubara, E.M. (2017) Shift in microbial group during remediation by enhanced natural attenuation (RENA) of a crude oil-impacted soil: a case study of Ikarama Community, Bayelsa, Nigeria. *3 Biotech* **7**: 1–11.

Elliott, D.R., Scholes, J.D., Thornton, S.F., Rizoulis, A., Banwart, S.A., & Rolfe, S.A. (2010) Dynamic changes in microbial community structure and function in phenol-degrading microcosms inoculated with cells from a contaminated aquifer. *FEMS Microbiol Ecol* **71**: 247–259.

Felföldi, T., Vengring, A., Kéki, Z., Márialigeti, K., Schumann, P., & Tóth, E.M. (2014) *Eoetvoesia caeni* gen. nov., sp. nov., isolated from an activated sludge system treating coke plant effluent. *Int J Syst Evol Microbiol* **64**: 1920–1925.

Grouzdev, D.S., Tourova, T.P., Babich, T.L., Shevchenko, M.A., Sokolova, D.S., Abdullin, R.R., *et al.* (2018) Whole-genome sequence data and analysis of type strains '*Pusillimonas nitritireducens*' and '*Pusillimonas subterraneus*' isolated from nitrate- and radionuclide-contaminated groundwater in Russia. *Data Br* **21**: 882–887.

Hilyard, E.J., Jones-Meehan, J.M., Spargo, B.J., & Hill, R.T. (2008) Enrichment,

654 isolation, and phylogenetic identification of polycyclic aromatic hydrocarbon-
655 degrading bacteria from Elizabeth river sediments. *Appl Environ Microbiol* **74**:
656 1176-1182.
657 Jin, L., Ko, S.R., Cui, Y., Lee, C.S., Oh, H.M., Ahn, C.Y., & Lee, H.G. (2017)
658 *Pusillimonas caeni* sp. nov., isolated from a sludge sample of a biofilm reactor.
659 *Antonie van Leeuwenhoek* **110**: 125–132.
660 Jin, L., Ko, S.R., Lee, H.G., Kim, B.H., Kim, H.S., Ahn, C.Y., & Oh, H.M. (2014)
661 *Flaviflexus salsibiostraticola* sp. nov., an actinobacterium isolated from a biofilm
662 reactor. *Int J Syst Evol Microbiol* **64**: 3293–3296.
663 Kämpfer, P., Busse, H.J., McInroy, J.A., & Glaeser, S.P. (2017) *Paracandidimonas soli*
664 gen. nov., sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **67**: 1740–1745.
665 Karvelis, L., Gasparavičiūtė, R., Klimavičius, A., Jančienė, R., Stankevičiūtė, J., &
666 Meškys, R. (2014) *Pusillimonas* sp. 5HP degrading 5-hydroxypicolinic acid.
667 *Biodegradation* **25**: 11–19.
668 Kim, Y.J., Kim, M.K., Im, W.T., Srinivasan, S., & Yang, D.C. (2010) *Parapusillimonas*
669 *granuli* gen. nov., sp. nov., isolated from granules from a wastewater-treatment
670 bioreactor. *Int J Syst Evol Microbiol* **60**: 1401–1406.
671 Koh, H.W., Song, M.S., Do, K.T., Kim, H., & Park, S.J. (2019) *Pusillimonas*
672 *thiosulfatoxidans* sp. nov., a thiosulfate oxidizer isolated from activated sludge. *Int*
673 *J Syst Evol Microbiol* **69**: 1041–1046.
674 Kong, L., Zhang, Y., Zhu, L., Wang, Jinhua, Wang, Jun, Du, Z., & Zhang, C. (2018)
675 Influence of isolated bacterial strains on the *in situ* biodegradation of endosulfan and
676 the reduction of endosulfan- contaminated soil toxicity. *Ecotoxicol Environ Saf* **160**:
677 75–83.

678 Konstantinidis, K.T., Rossello-Mora, R., & Amann, R. (2017) Uncultivated microbes in
679 need of their own taxonomy. *ISME J* **11**: 2399-2406.

680 Lee, M., Woo, S.G., Chae, M., & Ten, L.N. (2010) *Pusillimonas soli* sp. nov., isolated
681 from farm soil. *Int J Syst Evol Microbiol* **60**: 2326–2330.

682 Li, J., Qi, M., Lai, Q., Dong, C., Liu, X., Wang, G., & Shao, Z. (2020) *Pusillimonas*
683 *maritima* sp. nov., isolated from surface seawater. *Int J Syst Evol Microbiol* **70**:
684 3483–3490.

685 Lladó, S., Gràcia, E., Solanas, A.M., & Viñas, M. (2013) Fungal and bacterial microbial
686 community assessment during bioremediation assays in an aged creosote-polluted
687 soil. *Soil Biol Biochem* **67**: 114–123.

688 Ma, Y., Wen, R., Qiu, J., Hong, J., Liu, M., & Zhang, D. (2015) Biodegradation of
689 nicotine by a novel strain *Pusillimonas*. *Res Microbiol* **166**: 67–71.

690 Manaia, C.M., Vaz-Moreira, I., & Nunes, O.C. (2021) *Candidimonas*. In Bergey's
691 Manual of Systematics of Archaea and Bacteria (eds M.E. Trujillo, S. Dedysh, P.
692 DeVos, B. Hedlund, P. Kämpfer, F.A. Rainey and W.B. Whitman), 1-6. DOI:
693 https://doi.org/10.1002/9781118960608.gbm01821

694 Mandal, S., Rameez, M.J., Chatterjee, S., Sarkar, J., Pyne, P., Bhattacharya, S., *et al.*
695 (2020) Molecular mechanism of sulfur chemolithotrophy in the betaproteobacterium
696 *Pusillimonas ginsengisoli* SBSA. *Microbiology (United Kingdom)* **166**: 386–397.

697 Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., & Göker, M. (2013) Genome sequence-
698 based species delimitation with confidence intervals and improved distance
699 functions. *BMC Bioinformatics* **14**: 60.

700 Negus, D. & Taylor, P.W. (2014) A poly-γ-d-glutamic acid depolymerase that degrades
701 the protective capsule of *Bacillus anthracis*. *Mol Microbiol* **91**: 1136–1147.

702 Nörtemann, B., Baumgarten, J., Rast, H.G., & Knackmuss, H.J. (1986) Bacterial

communities degrading amino- and hydroxynaphthalene-2-sulfonates. *Appl Environ Microbiol* **52**: 1195–1202.

Obi, L.U., Atagana, H.I., & Adeleke, R.A. (2016) Isolation and characterisation of crude oil sludge degrading bacteria. *Springerplus* **5**: 1946.

Pal, R., Bhasin, V.K., and Lal, R. (2006) Proposal to reclassify [*Sphingomonas*] *xenophaga* Stolz et al. 2000 and [*Sphingomonas*] *taejonensis* Lee et al. 2001 as *Sphingobium xenophagum* comb. nov. and *Sphingopyxis taejonensis* comb. nov., respectively. *Int J Syst Evol Microbiol* **56**: 667–670.

Park, M.S., Park, Y.J., Jung, J.Y., Lee, S.H., Park, W., Lee, K., & Jeon, C.O. (2011) *Pusillimonas harenae* sp. nov., isolated from a sandy beach, and emended description of the genus *Pusillimonas*. *Int J Syst Evol Microbiol* **61**: 2901–2906.

Remmas, N., Roukouni, C., & Ntougias, S. (2017) Bacterial community structure and prevalence of *Pusillimonas*-like bacteria in aged landfill leachate. *Environ Sci Pollut Res* **24**: 6757–6769.

Richter, M., & Roselló-Móra, R. (2009) Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* **106**: 19126-19131.

Russ, R., Müller, C., Knackmuss, H.J., & Stolz, A. (1994). Aerobic biodegradation of 3-aminobenzoate by Gram-negative bacteria involves intermediate formation of 5-aminosalicylate as ring-cleavage substrate. FEMS *Microbiol Lett* **122**: 137-144.

Sant' Anna, D., Sampaio, J.L.M., Sommaggio, L.R.D., Mazzeo, D.E.C., Marin-Morales, M.A., Marson, F.A.L., *et al.* (2020) The applicability of gene sequencing and MALDI-TOF to identify less common gram-negative rods (*Advenella*, *Castellaniella*, *Kaistia*, *Pusillimonas* and *Sphingobacterium*) from environmental isolates. *Antonie van Leeuwenhoek* **113**: 233–252.

Song, L., Wang, Y., Zhao, H., & Long, D.T. (2015) Composition of bacterial and archaeal

728 communities during landfill refuse decomposition processes. *Microbiol Res* **181**:
729 105–111.
730 Srinivasan, S., Kim, M.K., Sathiyaraj, G., Kim, Y.J., & Yang, D.C. (2010) *Pusillimonas*
731 *ginsengisoli* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol*
732 **60**: 1783–1787.
733 Stolz, A., Bürger, S., Kuhm, A., Kämpfer, P., & Busse, H.J. (2005) *Pusillimonas*
734 *noertemannii* gen. nov., sp. nov., a new member of the family *Alcaligenaceae* that
735 degrades substituted salicylates. *Int J Syst Evol Microbiol* **55**: 1077–1081.
736 Stolz, A., Nörtemann, B., & Knackmuss, H.J. (1992). Bacterial metabolism of 5-
737 aminosalicylic acid. *Biochem J* **282**: 675-680.
738 Stolz, A., & Knackmuss, H.J. (1993a). Bacterial metabolism of 5-aminosalicylic acid:
739 enzymic conversion to L-malate, pyruvate and ammonia. *J Gen Microbiol* **139**:
740 1019-1025.
741 Stolz, A., & Knackmuss, H.J. (1993b). Degradation of 2,4-dihydroxybenzoate by
742 *Pseudomonas* sp. BN9. FEMS *Microbiol Lett* **108**: 219-224.
743 Sun, H., Narihiro, T., Ma, X., Zhang, X.X., Ren, H., & Ye, L. (2019) Diverse aromatic-
744 degrading bacteria present in a highly enriched autotrophic nitrifying sludge. *Sci*
745 *Total Environ* **666**: 245–251.
746 Vaz-Moreira, I., Figueira, V., Lopes, A.R., de Brandt, E., Vandamme, P., Nunes, O.C., *et*
747 *al.* (2011) *Candidimonas nitroreducens* gen. nov., sp. nov. and *Candidimonas humi*
748 sp. nov., isolated from sewage sludge compost. *Int J Syst Evol Microbiol* **61**: 2238–
749 2246.
750 Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky,
751 L.H., *et al.* (1987). Report of the Ad Hoc Committee on reconciliation of approaches
752 to bacterial systematics. *Int J Syst Evol Microbiol* **37**: 463-464.

753 Yao, L., Lai, Y., Xue, F., Sun, L., & Wang, J. (2019) *Paracandidimonas caeni* sp. nov.,
754 isolated from sludge. *Int J Syst Evol Microbiol* **69**: 3332–3337.
755 Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017) Introducing
756 EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and
757 whole-genome assemblies. *Int J Syst Evol Microbiol* **67**: 1613–1617.
758 Yuan, M., Zhang, Y., Zhao, L., Ma, Y., He, Q., He, J., & Qiu, J. (2018). Identification
759 and characterization of a new three-component nicotinic acid hydroxylase
760 NahAB(1) B(2) from *Pusillimonas* sp. strain T2. *Lett Appl Microbiol* **66**: 321-328.
761 Zhang, D.C., Busse, H.J., Wieser, C., Liu, H.C., Zhou, Y.G., Schinner, F., *et al.* (2012)
762 *Candidimonas bauzanensis* sp. nov., isolated from soil, and emended description of
763 the genus *Candidimonas* Vaz-Moreira *et al.* 2011. *Int J Syst Evol Microbiol* **62**:
764 2084-2089.
765 Zhang, X., Qu, Y., Ma, Q., Zhang, Z., Li, D., Wang, J., *et al.* (2015) Illumina MiSeq
766 sequencing reveals diverse microbial communities of activated sludge systems
767 stimulated by different aromatics for indigo biosynthesis from Indole. *PLoS One* **10**:
768 1–14.
769

770 **Table 1.** Characteristics that differentiate the species of the genus *Pusillimonas*.
771

Characteristic	***P. noertemannii* BN9^T**	***P. ginsengisoli* DCY25^T / DCY28**	***P. soli* MJ07^T**	***P. harenae* B201^T**	***P. caeni* EBR-8-1^T**	***P. thiosulfatoxidans* YE3^T**	***P. maritima* 17-4-A^T/ L52-1-41**
Cell morphology	Rods	Short rods	Rods	Ovoid rods	Short rods	Rods	Short rods
Cell size (µm)	1-1.5 x 0.5-0.8	0.5-0.8 x 0.3-0.6	0.3-0.5 x 0.7-1.0	0.5-0.7 x 0.6-0.9	0.6-0.7 x 0.8-1.6	0.7-1.2 x 2.0-2.8	0.6-0.7 x 0.7-1.7
Motility (flagella)	+ (polar)	+[1]	+	+ (two lateral)	-	+	-
Colonies pigmentation	Brownish	Pale yellow	Yellow	Ivory	Creamy white	Pale yellow	White
Temperature range for growth (optimum) (°C)	15-42 (40)[2]	15-40 (30)	15-42[2] (30)	15–45 (30)	15-37 (30)	10–40 (30)	10–45 (37)
pH range for growth (optimum)	5-9 (7-8)[2]	5.5-9.0 (7.0)	5.0-9.0 (6.5-7.0)	5.0-9.0 (6.0-7.5)	6.0-9.0 (7.0)	5.5-9.0 (7.0)	5.0-9.0 (7.0-8.0)
NaCl growth (optimum) (%, w/v)	0-5 (2-3)[2]	0-10 (0-1)[2]	0-8 (1-2)[2]	0-6 (0-3)[3]	1-8 (1-3)[4]	1-8 (1)	0/0.5-8/10 (1-3/3)
Nitrate reduction	-	+	+	-	-	-	-
Thiosulfate oxidation	n.a.	-[5]	-	-	n.a.	+	n.a.
Hydrolysis of:							
Urea	+[6]	-	-	+	-	-	-
Enzymatic activities:							
Cystine arylamidase	-	+	-	+	-	-	-
Trypsin	-	+	-	+[w]	+[w]	-	-
Assimilation:							
Citrate	-	+	+	-	-	-	-
L-Malate	-	+	+	+	-	+	-

Characteristic	***P. noertemannii* BN9^T**	***P. ginsengisoli* DCY25^T / DCY28**	***P. soli* MJ07^T**	***P. harenae* B201^T**	***P. caeni* EBR-8-1^T**	***P. thiosulfatoxidans* YE3^T**	***P. maritima* 17-4-A^T/ L52-1-41**
Major polar lipids	PE, DPG, PG, 2 AL	PG, PE	PG, DPG, PE, 2 AL	PG, DPG, PE, 3 AL, 1 PL	PG, DPG, PE, 1 AL	DPG, PG, PE	PE, PG, DPG, 2 / 3 AL
Other polar lipids	3 AL, 2 PL, 3 APL, 1 L	AL, DPG, APL, L	1 AL, 1 APL, 1 L	ND	ND	2 APL, 4 L	3PL / 2L
DNA G+C content (mol%)	61.8 (Tm) 62.9 (WGS)	57.3 (HPLC) 57.9 (WGS)	59.4 (HPLC) 59.8 (WGS)	53.1 (HPLC) 56.3 (WGS)	63.3 (HPLC) 63.1 (WGS)	59.3 (WGS)	53.4 (WGS)
Closest related species (% 16S rRNA similarity)	*Pp. granuli* Ch07^T (97.4%) *E. caeni* PB3-7B^T (97.3%) *Pc. caeni* 24^T (97.0%)	*Pc. caeni* 24^T (97.7%) *Pp. granuli* Ch07^T (97.3%) *C. bauzanensis* CGMCC 1.10190^T (97.0%)	*Pp. granuli* Ch07^T (97.8%) *Pc. caeni* 24^T (97.7%) *C. nitroreducens* SC-089^T (97.1%)	*C. bauzanensis* CGMCC 1.10190^T (97.1%) *Pc. caeni* 24^T (97.0%)	*E. caeni* PB3-7B^T (97.8%) *Pp. granuli* Ch07^T (97.7%) *Pc. caeni* 24^T (97.4%)	*C. bauzanensis* CGMCC 1.10190^T (98.2%) *Pp. granuli* Ch07^T (97.9%) *Pc. caeni* 24^T (97.7%)	*Pp. granuli* Ch07^T (96.7%)

[1]Park *et al.* (2011) report the type strain *P. ginsengisoli* KACC 15017^T as non-motile.
[2]Data retrieved from Li *et al.* (2020) for the type strain.
[3]Li *et al.* (2020) report 1-2 % (w/v) as optimum NaCl range growth for the type strain *P. harenae* B201^T.
[4]Li *et al.* (2020) report 2-3 % (w/v) as optimum NaCl range growth for the type strain *P. caeni* EBR-8-1^T.
[5]Koh *et al*. (2019) tested only the type strain of this species (*P. ginsengisoli* KCTC 22046^T).
[6]Srinivasan *et al.* (2010) and Jin *et al.* (2017) described the strain as positive but Park *et al.* (2011) and Li *et al.* (2020) described the strain as negative.
DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; AL, unknown aminolipid; PL, unknown phospholipid; APL, unknown aminophospholipid; L, unknown polar lipid.
n.a., not available; [w], weakly positive; ND, not described; Tm, determined by the thermal melting point; HPLC, determined by high performance layer chromatography; WGS, determined by the whole genome sequencing.

784 **Table 2.** Cellular fatty acids profile of the type strains of the different species of the genus *Pusillimonas*.

	[a]*P. noertemannii* **BN9^T**	[a]*P. ginsengisoli* **DCY25^T**	[a]*P. soli* **MJ07^T**	[a]*P. harenae* **B201^T**	[a]*P. caeni* **EBR-8-1^T**	[b]*P. thiosulfatoxidans* **YE3^T**	[a]*P. maritima* **17-4-A^T**
Saturated:							
$C_{9:0}$	ND	ND	ND	ND	1.3	ND	1.7
$C_{10:0}$	1.9	ND	ND	ND	1.1	0.5	ND
$C_{10:0}$ iso	ND	ND	ND	ND	ND	ND	1.4
$C_{11:0}$ iso	1.9	ND	ND	TR	TR	ND	1.2
$C_{12:0}$	7.7	6.4	TR	3.1	3.4	7.7	3.0
$C_{13:0}$ iso	1.6	ND	ND	ND	ND	ND	ND
$C_{14:0}$	4.5	5.3	5.8	1.9	1.3	1.0	1.9
$C_{15:0}$ anteiso	ND	TR	ND	2.1	3.6	ND	TR
$C_{16:0}$	17.9	25.9	28.2	40.4	28.5	39.2	32.5
$C_{17:0}$	ND	TR	TR	TR	1.0	TR	2.0
$C_{17:0}$ anteiso	2.3	ND	ND	TR	TR	ND	TR
$C_{17:0}$ cyclo	10.0	11.7	24.0	20.2	9.9	37.5	3.3
$C_{18:0}$	6.1	10.5	2.9	6.1	14.9	1.1	29.1
$C_{19:0}$ cyclo $\omega 8c$	11.7	5.9	7.8	4.9	7.6	ND	1.8
Unsaturated:							
$C_{14:1}$ $\omega 5c$	1.9	1.7	TR	TR	ND	ND	ND
$C_{15:1}$ iso G	3.3	4.0	TR	ND	ND	ND	ND
$C_{16:1}$ $\omega 11c$	ND	ND	ND	TR	1.1	ND	ND
$C_{16:1}$ $\omega 7c$ alcohol	ND	ND	ND	TR	1.1	ND	ND

	[a]*P. noertemannii* **BN9^T**	[a]*P. ginsengisoli* **DCY25^T**	[a]*P. soli* **MJ07^T**	[a]*P. harenae* **B201^T**	[a]*P. caeni* **EBR-8-1^T**	[b]*P. thiosulfatoxidans* **YE3^T**	[a]*P. maritima* **17-4-A^T**
SF3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*)	1.0	2.1	7.0	2.0	TR	0.6	TR
SF8 ($C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*)	6.7	7.3	9.2	7.5	5.0	TR	7.8
$C_{18:1}$ ω9*c*	6.0	6.4	2.0	3.6	5.1	ND	4.8
SF5 ($C_{18:2}$ ω6,9*c* and/or $C_{18:0}$ anteiso)	ND	ND	ND	ND	1.9	ND	1.9
Hydroxyl:							
SF2 ($C_{12:0}$ aldehyde / unknown 10.928)	ND	ND	ND	ND	ND	9.0	ND
$C_{13:0}$ 2-OH	1.5	2.0	ND	ND	TR	ND	ND
$C_{16:0}$ 2-OH	ND	ND	ND	ND	2.8	ND	1.3
SF2 ($C_{14:0}$ 3-OH and/or $C_{16:1}$iso I)	7.2	5.1	9.0	3.0	3.2	ND	TR

[a]Data from Li *et al*. 2020.
[b]Data from Koh *et al*. 2019.
ND, not detected; TR, traces (<1%).
S2, Summed feature 2 comprises $C_{14:0}$ 3-OH and/or $C_{16:1}$ iso I in the description of Li et al. (2020) and $C_{12:0}$ aldehyde/unknown 10.928 in the description of Koh et al. (2019); S3, Summed feature 3 comprises $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; S5, Summed feature 5 comprises $C_{18:2}$ ω6,9*c* and/or $C_{18:0}$ anteiso; S8, Summed feature 8 comprises $C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*.

793 **Table 3.** Genome length, number of contigs, N50 value and G+C content for the available genome sequences of the *Pusillimonas* spp. type strains

794 (Koh *et al.*, 2019; Li *et al.*, 2020).

	***P. noertemannii* BN9^T**	***P. ginsengisoli* DCY25^T**	***P. soli* MJ07^T**	***P. harenae* B201^T**	***P. caeni* EBR-8-1^T**	***P. thiosulfatoxidans* YE3^T**	***P. maritima* 17-4A^T**
Genome length (Mb)	4.21	4.25	4.15	3.37	4.44	3.55	3.25
Number of contigs	10	33	18	7	23	1	28
N50 (bp)	881,120	234,212	857,311	1,256,821	422,339	3,548,703	747,381
G+C (mol %)	62.6	57.9	59.8	56.3	63.1	59.3	53.4

795

796 **Table 4.** Digital DNA-DNA hybridization (dDDH) values and DNA-DNA hybridization values experimentally obtained (between brackets with
797 indication of the reference) for the different type strains of the *Pusillimonas* spp.

	***P. ginsengisoli* DCY25^T**	***P. soli* MJ07^T**	***P. harenae* B201^T**	***P. caeni* EBR-8-1^T**	***P. thiosulfatoxidans* YE3^T**	***P. maritima* 17-4A^T**
***P. noertemannii* BN9^T**	20.3 (20)[1]	20.3 (18 ± 7%)[2]	20.2 (n.d.)	31.4 (27.4)[4]	20.0 (n.d.)	19.4 (n.d.)
***P. ginsengisoli* DCY25^T**		21.8 (50 ± 8%)[2]	19.4 (30.2 ± 5.4%)[3]	23.7 (32.3)[4]	22.6 (21.3 ± 1.2)[5]	19.8 (n.d.)
***P. soli* MJ07^T**			19.3 (4.9 ± 1.8%)[3]	20.0 (24.9)[4]	19.7 (14.0 ± 0.7)[5]	18.8 (n.d.)
***P. harenae* B201^T**				19.6 (n.d.)	19.3 (28.7 ± 2.3)[5]	19.0 (n.d.)
***P. caeni* EBR-8-1^T**					23.1 (n.d.)	19.6 (n.d.)
***P. thiosulfatoxidans* YE3^T**						18.9 (n.d.)

798 [1]Srinivasan et al., 2010; [2]Lee *et al.*, 2010; [3]Park *et al.* 2011; [4]Jin *et al.*, 2017; [5]Koh *et al.* 2019.
799 n.d., not determined.

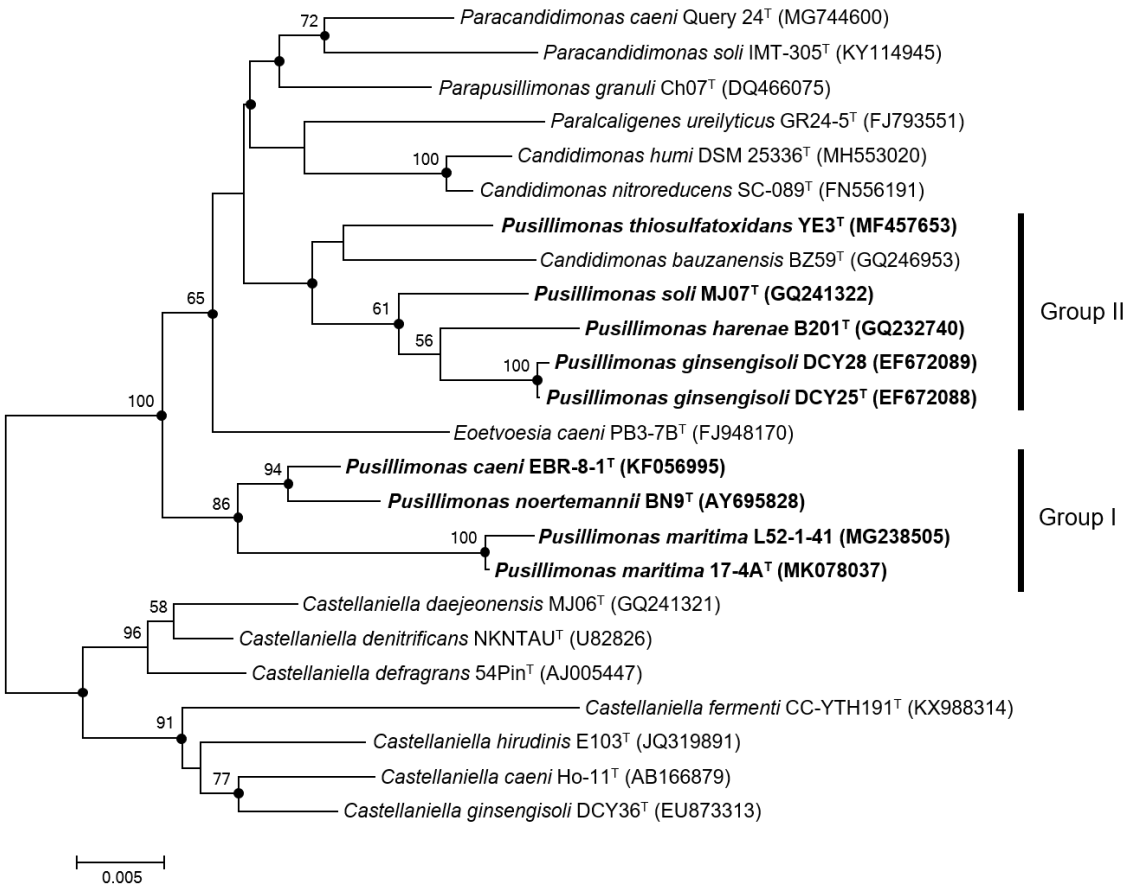

Figure 1. 16S rRNA gene-based neighbour-joining tree showing the phylogenetic position of *Pusillimonas* species in relation to other representatives of the family *Alcaligenaceae*. Bootstrap values ≥ 50% are indicated at branch points. Filled circles indicate branches on the tree that were also recovered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