Diaphorobacter

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2. KEYWORDS: *Diaphorobacter*; aerobes; denitrifying bacteria; chemo-organotroph; poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) biodegradation

3. ABSTRACT:

Rods, non-spore forming, Gram-stain-negative. Motile with a single polar flagellum or non-motile. **Aerobic or facultative aerobic**. **Mesophilic** with optimum growth conditions at a temperature around 28ºC and pH 7. No special growth requirements. Catalase positive. **Cytochrome c oxidase test varies among species**. **Chemo-organotrophic**. **Denitrifying or nitrate reduction**. **No indole production or glucose fermentation**. Some members have the capacity to degrade poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). The major respiratory quinone is **ubiquinone 8** and major fatty acids are **$C_{16:0}$, summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH), and $C_{18:1}$ ω7c**, and the predominant hydroxy fatty acid is **$C_{10:0}$ 3-OH**.

4. DEFINING PUBLICATION:

Diaphorobacter, Khan and Hiraishi 2003, 936^{VP} (Effective publication: Khan and Hiraishi 2002, 305) *emend*. Kim, Moon, Ahn, Weon, Hong, Seok and Kwon 2014, 515.

5. ETYMOLOGY:

Diaphorobacter [Di.a.pho.ro.bac'ter. Gr. adi. *diaphoros*, different, profitable; Gr. n. *bacter*, rod; M.L. masc. n. *Diaphorobacter*, distinguished and profitable rod, referring to usefulness in nitrogen removal].

6. GENERIC DEFINITION:

Rods, non-spore forming, Gram-stain-negative. Motile with a single polar flagellum or non-motile. **Aerobic or facultative aerobic**. **Mesophilic** with optimum growth conditions at a temperature around 28ºC and pH 7. No special growth requirements. Catalase positive. **Cytochrome *c* oxidase test varies among species**. **Chemo-organotrophic**. **Denitrifying or nitrate reduction**. **No indole production or glucose fermentation**. Some members have the capacity to degrade poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). The respiratory quinone is **ubiquinone 8** and major fatty acids are **$C_{16:0}$, summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH), and $C_{18:1}$ ω7c**, and the predominant hydroxy fatty acid is **$C_{10:0}$ 3-OH**.

The DNA G+C content (mol %) is 62.9 – 66.8.

Type species: *Diaphorobacter nitroreducens*, Khan and Hiraishi 2003, 936^{VP} (Effective publication: Khan and Hiraishi 2002, 305)

Number of species with validated names: 4.

7. FAMILY CLASSIFICATION:

Comamonadaceae (fbm00182)

8. FURTHER DESCRIPTIVE INFORMATION:

8.1. Cell morphology:

Four species are validly described within the genus *Diaphorobacter*: *Diaphorobacter nitroreducens*, *Diaphorobacter oryzae*, *Diaphorobacter aerolatus* and *Diaphorobacter polyhydroxybutyrativorans* (Khan and Hiraishi, 2002, Kim et al., 2014, Pham et al., 2009, Qiu et al., 2015). "*Diaphorobacter ruginosibacter*" described by Wei *et al*. (2015) is a non-validly named *Diaphorobacter* species awaiting for inclusion in the IJSEM validation list. Cells are Gram-stain-negative, non-spore forming rods (0.3-0.9 µm x 1.0-1.9 µm). *D. nitroreducens*, *D. oryzae*, *D. polyhydroxybutyrativorans*, and "*D. ruginosibacter*" cells are motile with a single polar flagellum. *D. aerolatus* cells are non-motile. Pilus-like filamentous appendages projecting from the cell surface are described in *D. nitroreducens*.

8.2. Colonial and cultural characteristics:

Diaphorobacter spp. have optimum growth temperatures around 28 ºC. *D. nitroreducens* forms rough colonies with uneven or smooth and entire margins on PBY agar medium (0.5% Bacto-peptone, 0.3% beef extract and 0.1% yeast extract). Those colonies are colorless at the earlier stages of growth but change to cream to beige at older stages. *D. oryzae* colonies are smooth and circular with regular edges, approximately 1.3-2.0 mm diameter, after 2 days of growth on R2A agar. *D. aerolatus* colonies are circular and beige colored on R2A agar. *D.*

polyhydroxybutyrativorans colonies are circular (1-2 mm diameter) and ivory on R2A or yellow on LB medium agar, after 3 days of growth. After 3 days of growth, "*D. ruginosibacter*" colonies are cream-white, sticky, with smooth-surface and slightly central-raised with near-smooth margins. The colonies become slightly orange in the center after incubation for more than a week.

8.3. Nutrition and growth conditions:

Diaphorobacter spp. grow well at the expense of simple organic compounds under aerobic conditions. *D. nitroreducens* present the same capacity with nitrate as the terminal electron acceptor. Sugars do not support or promote weak growth (Table 1). Assimilation of acetate, gluconate, succinate, DL-lactate, malate, and L-alanine is common to the type strains of the *Diaphorobacter* species, including "*D. ruginosibacter*".

With exception of *D. oryzae*, for which no information is available, all the other *Diaphorobacter* spp. are described as neutrophilic bacteria, able to grow between pH 5 and 9, with optimum pH at 7, or 7-8 in the case of *D. nitroreducens*. The optimum growth temperature is around 28 ºC for all species, and for *D. oryzae* and *D. nitroreducens* is observed until 32 ºC or 35 ºC, respectively. The species with the non-validated name "*D. ruginosibacter*" is able to grow at temperatures up to 40 ºC. *D. aerolatus* and *D. polyhydroxybutyrativorans* can grow in the presence of up to 3% (w/v) NaCl and "*D. ruginosibacter*" in the presence of up to 6% (w/v) NaCl.

<Table 1 near here>

8.4. Metabolism:

All type strains of the four validated names of *Diaphorobacter* species are aerobic chemo-organotrophic and catalase positive. *D. oryzae*, *D. nitroreducens*, *D. aerolatus*, and *D. polyhydroxybutyrativorans* are described as denitrifying bacteria (able to reduce nitrate and nitrite to nitrogen) and for "*D. ruginosibacter*" only the reduction of nitrate to nitrite is reported.

D. nitroreducens and *D. polyhydroxybutyrativorans* have the capacity to degrade poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) or PHBV, respectively. At least these species are able to degrade PHBV using oxygen or nitrate as the terminal electron acceptor. In addition, *D. nitroreducens* is able to use other carbon sources with nitrate as the terminal electron acceptor. Cytochrome *c* oxidase tests are positive for *D. nitroreducens*, *D. oryzae, D. polyhydroxybutyrativorans*, and"*D. ruginosibacter*" and negative for *D. aerolatus*. None ferment glucose or produce indole. Hydrolytic activities are variable among species. *D. nitroreducens* hydrolyzes aesculin, *D. oryzae* hydrolyzes gelatin, *D. aerolatus* hydrolyses Tween 80 and tyrosine, *D. polyhydroxybutyrativorans* hydrolyses casein, and "*D. ruginosibacter*" hydrolyses gelatin and Tween-80 (Table 1). *Diaphorobacter* spp. have been described as able to degrade compounds such as phenol, pyrene, phenanthrene, fluoranthene, 3-nitrotoluene, catechol, triazophos or chloroaniline (Ge et al., 2015, Klankeo et al., 2009, Singh and Ramanathan, 2013, Yang et al., 2011, Zhang et al., 2010). The following enzymes are produced: esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. None of the validly named *Diaphorobacter* type strains, produce alpha- and beta-galactosidase, alpha-glucosidase, lipase (C14), valine arylamidase, alpha-chymotrypsin, beta-glucuronidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase or alpha-fucosidase.

8.5. Chemotaxonomic characteristics:

The major respiratory quinone is ubiquinone 8. Polar lipids, described for the species *D. aerolatus*, *D. polyhydroxybutyrativorans*, and "*D. ruginosibacter*" comprise phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an uncharacterized aminolipid and/or phospholipid. The predominant fatty acids reported in the type strains of *Diaphorobacter*, according to Qiu et al. (2015), are $C_{16:0}$ (22.2-25.6%), summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH, 38.0-47.9 %), and $C_{18:1}$ ω7c (10.2-17.3%). The relative abundance of the fatty acid $C_{17:0}$ cyclo is higher in *D. oryzae* (3.0%) and *D. aerolatus* (3.7%), than in *D. nitroreducens* (0.7%) or *D. polyhydroxybutyrativorans* (0.6%). The predominant hydroxy fatty acid is $C_{10:0}$ 3-OH for all strains studied so far (3.3-4.4%). The fatty acid profile of "*D. ruginosibacter*" is in agreement with those described for the other *Diaphorobacter* species (Wei et al., 2015).

8.6. Genome features:

The whole genome sequences of *D. nitroreducens* and *D. polyhydroxybutyrativorans* type strains are available. The two genomes share an Average Nucleotide Identity (ANI) value of 98.2%. *D. nitroreducens* draft genome has 3.01 Mb and a GC content of 65.5%, in agreement with the 65.8 mol% determined by thermal melting point (Qiu et al., 2015). *D. polyhydroxybutyrativorans* complete genome comprises one chromosome with 4.06 Mb and a GC content of 66.7%, in agreement with the 66.8 mol% determined by the thermal denaturation method. A total of 3639 predicted protein-coding genes and nine rRNA genes were identified.

8.7. Antibiotics susceptibility:

The profiles of antibiotic susceptibility for the four *Diaphorobacter* species are described in Table 1.

8.8. Ecology and Habitat:

D. nitroreducens type strain was isolated from PHBV denitrifying acclimation cultures inoculated with activated sludge from sewage treatment plants, in Japan (Khan and Hiraishi, 2002). This type strain was the first described as a PHB-degrading denitrifying bacterium (Khan and Hiraishi, 2002, Khan et al., 2015) and members of this species have been reported among the most abundant PHB-degrading denitrifiers in activated sludge and PHBV-acclimated solid-phase denitrification processes (Khan et al., 2007, Qiu et al., 2017). The *D. nitroreducens* type strain is able to degrade PHBV under anaerobic denitrifying conditions, in the temperature range of 15–40 ºC and in the pH range of 6–9, with a yield coefficient of 0.49 (Hiraishi and Khan, 2003). The denitrification rate is of 20-47 mg NO_3^- -N g^{-1} (dry wet cells) h^{-1} and decreases with increasing concentrations of dissolved oxygen, being still more than 3 mg NO_3^- -N g^{-1} (dry wet cells) h^{-1} under fully aerobic conditions, which means the strain is capable of PHBV aerobic denitrification (Hiraishi and Khan, 2003, Khan et al., 2015).

The type strain of *D. polyhydroxybutyrativorans* was isolated from the biofilm of a PHBV denitrifying reactor inoculated with activated sludge of a sequencing batch reactor treating swine manure wastewater, in China (Qiu et al., 2015). *D. polyhydroxybutyrativorans* type strain can reduce both nitrate and nitrite and under aerobic conditions can use ammonium as electron donor while degrading PHBV as the carbon source, with average removal rates of 1.73 mg NH_4^+ -N L^{-1} h^{-1}, 6.10 mg NO_3^- -N L^{-1} h^{-1}, and 4.95 mg NO_2^--N L^{-1} h^{-1} (Zhang et al., 2017).

D. oryzae was isolated from a thiosulfate-oxidizing enrichment culture from paddy soils, in Korea (Pham et al., 2009). The *D. aerolatus* type strain was isolated from an outdoor air sample, also in Korea (Kim et al., 2014). "*D. ruginosibacter*" was isolated from soybean root nodules (Wei et al., 2015). The diversity of isolation sources suggests the ubiquitous character of this genus.

9. ENRICHMENT/ISOLATION PROCEDURES:

The isolation of *D. nitroreducens* type strain was described by Khan et al. (2002). Samples of activated sludge were centrifuged and washed with phosphate-buffered saline (PBS) (10 mM K_2HPO_4 and 130 mM NaCl adjusted to pH 7.0 with HCl). Screw-cap glass reactors (670 mL capacity) with 500 mL of mineral base RM2 medium (Hiraishi and Kitamura, 1984), 2 g of PHBV (5% hydroxyvalerate content) powder, and 2 g of KNO_3 per liter were inoculated with the pre-washed sludge (2000 mg (dry weight) L^{-1}). Incubation was carried out, at 28 ºC for 10 weeks, with gentle agitation (70 rpm min^{-1}), corresponding to dissolved oxygen tension below 0.1 mg L^{-1}. Every 3 days of incubation, half of the volume of the supernatant was replaced by fresh medium. For plate counting and isolation, 1 mL of diluted sample was plated on PHBVN agar (PHBVN medium plus 1.8% agar) supplemented with 0.05% yeast extract, and incubated anaerobically (AnaeroPak system) for 4-5 weeks. Positive colonies for PHBV degradation and denitrification (colonies showing a zone of clearance) were then purified by repeated streaking on PHBN or PHBVN agar.

The isolation of *D. polyhydroxybutyrativorans* type strain was described by Qiu et al. (2015). A sample of activated sludge (20 mL), from a swine manure wastewater treatment plant, was used as the inoculum of a cylindrical plexiglass reactor (6 cm inner diameter and 50 cm

height) packed with PHBV granules operating with an up-flow mode using tap water with approximately 50 mg nitrate-nitrogen L^{-1} as the inlet. After 48 days of operation, 10 g of the PHBV granules were collected from the reactor and aseptically transferred to a flask with 100 mL sterile water and small glass balls to help in the suspension of the biofilms attached to the granules by shaking (on a rotary shaker at 150 r.p.m. for 30 min). Suspended biofilms were plated on denitrifying medium (DM: 2 g L^{-1} KNO_3, 0.2 g L^{-1} $MgSO_4.7H_2O$, 0.5 g L^{-1} K_2HPO_4, 0.3 g L^{-1} NH_4Cl, 15 g L^{-1} agar, pH 7.2) supplemented with 5 g L^{-1} PHBV powder, and incubated at 28 ºC for one week. Positive colonies for PHBV degradation (colonies showing a zone of clearance) were purified by streaking onto Luria-Bertani agar plates, which were incubated for 3 days at 28 ºC.

The isolation of *D. oryzae* type strain was described by Pham et al. (2009). A sample of 3 g of sediment of a rice-paddy field was added to 100 mL of basal freshwater medium (BFM) (Taylor et al., 1981) supplemented with 1 mM thiosulfate, and incubated aerobically at 20 ºC in a test tube. Five subcultures were prepared, at intervals of 2-3 weeks, by transferring 10% of the previous culture to new liquid fresh medium. The last enrichment culture was plated on solid BFM containing 1.5 % (w/v) agarose and incubated for 3 days at 20 ºC. Colonies were purified from these cultures, using the same conditions.

The isolation of *D. aerolatus* type strain was described by Kim et al. (2014). An air sample was collected using a MAS-100 air sampler (single-stage multiple-hole impactor; Merck) containing an R2A agar Petri dish amended with 200 µg mL^{-1} of cycloheximide. Cultures were purified by sub-culturing on R2A.

The type strain of "*D. ruginosibacter*" was isolated from soybean root nodules samples collected in the suburb of Baoji city in Shaanxi, China (Wei et al. 2015). The root nodules were washed and surface sterilized, and further squashed and squeezed to obtain endophytic bacteria. The bacteria were isolated from the nodule juice by streaking on nutrient agar (NA)

(5.0 g peptone, 1.5 g yeast extract, 1.5 g beef extract, 5.0 g NaCl, 20 g agar, 1 L distilled water; pH 7.2) (Deng et al., 2011).

10. MAINTENANCE PROCEDURES:

D. nitroreducens can be maintained aerobically on PBY medium. *D. polyhydroxybutyrativorans* can be maintained on LB agar medium. *D. oryzae* and *D. aerolatus* can be maintained on R2A agar or in a broth with R2A composition. "*D. ruginosibacter*" can be maintained on nutrient agar. All cultures can be preserved for longer periods frozen at -70 ºC in nutritive broth supplemented with 15% (v/v) glycerol.

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

The closest related genera to *Diaphorobacter* are *Alicycliphilus* (see gbm01825), *Oryzisolibacter* (see gbm01828) and *Melaminivora* (see gbm01827) (Figure 1). *Diaphorobacter* can be distinguished from *Oryzisolibacter* and *Melaminivora* by the lower G+C content of the *Diaphorobacter* spp. (62.9-66.8 mol% *versus* 69.4 and 69.5-69.6 mol% , respectively) (Table 2). In addition, *Diaphorobacter* spp. have a lower optimum growth temperature and pH than *Melaminivora* spp. (28-35 ºC and pH=7-8 *versus* 30-45 and pH=7-9.5), and also generally shorter cells (0.9-1.9 µm *versus* 2.0-3.5 µm). The genus *Melaminivora* is also the only one for which nitrite reduction is not described. *Diaphorobacter* spp. are not producers of valine arylamidase, a phenotypic characteristic that is characteristic of all the other closely related genera (Table 2).

<Figure 1 near here>

<Table 2 near here>

12. TAXONOMIC COMMENTS:

The genus *Diaphorobacter* is classified in the family *Comamonadaceae*, order *Burkholderiales*, within the class *Betaproteobacteria*. Members of the genus *Diaphorobacter* share 96-97% 16S rRNA gene sequence similarity with members of the genus *Alicycliphilus*, and less than 95.5% with members of the genera *Oryzisolibacter* and *Melaminivora*. Members of the four species with validated names of the genus *Diaphorobacter* share 16S rRNA gene sequence similarities of 96.8-99.4%, being the species *D. nitroreducens* and *D. polyhydroxybutyrativorans* the phylogenetically closest (Figure 1). Members of the species with the non-validated name "*D. ruginosibacter*" shares 96.5-98.7% 16S rRNA gene sequence similarity with the four validly described *Diaphorobacter* species.

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

1. *Diaphorobacter aerolatus* Kim, Moon, Ahn, Weon, Hong, Seok, Kwon 2014, 516^{VP}

aerolatus (ae.ro.la'tus. Gr. n. *aer* air; L. part. adj. *latus* carried; N.L. masc. part. adj. *aerolatus* airborne).

In addition to the genus description, the species members can be characterized by the following traits: aerobic, non-motile rods (0.8–0.9 µm x 1.1–1.7 µm), forming circular and beige coloured colonies in R2A agar. Grows at 10-37 ºC (optimum 28 ºC), pH 5-9 (optimum 7), and with up to 3% NaCl (w/v). Oxidase-negative. Able to hydrolyse tyrosine and tween

80, but not casein, cellulose, chitin, DNA, hypoxanthine, starch, xanthine, aesculin or gelatin. Assimilates α-ketoglutaric acid, L-asparagine, citrate, valerate, adipate, itaconate, suberate, among others. Positive activities for alkaline and acid phosphatase, among others described for the genus. The major cellular fatty acids are $C_{16:0}$, summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH), summed feature 8 ($C_{18:1}$ ω6c and/or $C_{18:1}$ ω7c) and $C_{17:0}$ cyclo, and the predominant hydroxy fatty acid is $C_{10:0}$ 3-OH. Polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, and an unknown aminolipid.

The DNA G+C content (mol %) is 65 (fluorometric method).

Type strain: 8604S-37, KACC 16536, NBRC 108926.

GenBank accession number (16S rRNA): KC352658.

2. *Diaphorobacter nitroreducens* Khan and Hiraishi 2003, 936^{VP} (Effective publication: Khan and Hiraishi 2002, 305)

nitroreducens (nitro.re.du'cens. L. n. *nitrum* nitrate; L. part. adj. *reducens* converting to a different state; N. L. adj. *nitroreducens* reducing nitrate).

In addition to the genus description, the species members can be characterized by the following traits: facultative aerobic motile rods (0.7–0.9 µm x 1.0-1.8 µm), forming rough colonies with uneven margins or smooth colonies with entire margins, transparent in earlier stages of growth or pale to beige after a longer incubation. Grows at 20-40 ºC (optimum 28-35 ºC) and pH 5-9 (optimum 7-8). Oxidase positive. Able to degrade poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) under aerobic and anaerobic denitrifying conditions. Cannot hydrolyse starch, gelatin, casein, DNA, Tween 20 or Tween 80. Assimilates ethanol, propionate, butyrate, α-ketobutyric acid, α-

304 ketoglutaric acid, 3-hydroxibutirate, pyruvate, glutamate, L-asparagine, D-alanine, among
305 others. Positive activities for protease and urease, among others described for the genus.
306 The DNA G+C content (mol %) is 64-65 (HPLC) or 65.8 (DNA melting method).
307 Type strain: NA10B, JCM 11421, CIP 107294.
308 GenBank accession number (16S rRNA): AB064317.
309 GenBank accession number (genome): BAZJ01000001.
310

311 **3. *Diaphorobacter oryzae*** Pham, Park, Roh, Roh, Rhee 2009, 220^{VP}

312 *oryzae* (o.ry'zae. L. gen. n. *oryzae* of rice, referring to the rice-paddy fields from where the
313 type strain was isolated).
314 Description as for the genus plus the following traits: motile rods (0.5-0.8 x 1.3-1.8 µm),
315 forming circular colonies (1.3-2.0 mm diameter) with regular edges. Grows at 7-35 ºC
316 (optimum 28-32 ºC). Oxidase positive. Does not oxidize thiosulfate. Hydrolyzes gelatin, but
317 not starch, casein, DNA or aesculin. Assimilates propionate, α-ketobutyric acid, 3-
318 hydroxybutirate, pyruvate, 2-ketogluconate, D-alanine, caprate, succinate, L-histidine,
319 valerate, L-proline, L-threonine, among others. Positive activities for protease and trypsin,
320 among others described for the genus. The major cellular fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and
321 $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH.
322 The DNA G+C content (mol %) is 62.9 (HPLC).
323 Type strain: RF3, KCTC 22225, LMG 24467.
324 GenBank accession number (16S rRNA): EU342381.
325

4. *Diaphorobacter polyhydroxybutyrativorans* Qiu, Zuo, Gao, Gao, Han, Sun, Zhang, Wang 2015, 2916^{VP}

polyhydroxybutyrativorans (po.ly.hy.dro.xy.bu.ty.ra.ti.vo'rans. N.L. neut. n. *polyhydroxybutyratum* polyhydroxybutyrate; L. part. adj. *vorans* devouring; N.L. part. adj. *polyhydroxybutyrativorans* polyhydroxybutyrate-devouring).

Description as for the genus plus the following traits: facultative aerobic motile rods (0.35-0.9 µm x 1.1-1.9 µm), forming circular (1-2 mm diameter) ivory (on R2A agar) or yellow (LB medium agar) colonies. Grows at 7-40 ºC (optimum 28 ºC), pH 5-9 (optimum 7), and with 0-3 % (w/v) NaCl. Oxidase positive. Degrades poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) under aerobic and anaerobic denitrifying conditions, with an average degrading rate of 0.14±0.12 g day^{-1} under anaerobic conditions. Able to hydrolyse casein, but not gelatin or aesculin. Able to assimilate propionate, butyrate, α-ketobutyric acid, α-ketoglutaric acid, 3-hydroxybutirate, pyruvate, glutamate, L-asparagine, D-alanine, fumarate, L-serine, D-serine, L-leucine, L-proline, itaconate, glycerol, L-threonine, among others. Positive for alkaline phosphatase activity, among others described for the genus. The major cellular fatty acids are summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH), $C_{16:0}$ and $C_{18:1}$ ω7c. Polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, and an unknown phospholipid.

The DNA G+C content (mol %) is 66.8 (DNA melting method).

Type strain: SL-205, ACCC 19739, DSM 29460.

GenBank accession number (16S rRNA): JX974341.

GenBank accession number (genome): NZ_CP016278.

RELATED ARTICLES:

gbm01825

gbm01828

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419 **TABLES:**

420 Table 1. Differential features of the species of the genus *Diaphorobacter* [a]

	D. nitroreducens	*D. oryzae*	*D. aerolatus*	*D. polyhydroxybutyrativorans*	*"D. ruginosibacter"*
Colonies colour	colourless (at early growth phase), cream to beige (at older growth stages)	n.a.	beige coloured	ivory (R2A agar) or yellow (LB medium agar)	cream-white, and slightly orange in the center after 7 days incubation
Cells size	0.7-0.9 µm x 1.0-1.8 µm	0.5-0.8 µm x 1.3-1.8 µm	0.8-0.9 µm x 1.1-1.7 µm	0.35-0.9 µm x 1.1-1.9 µm	0.33 µm x 0.89-1.73 µm
Motility	+	+	-	+	+
Cytochrome c oxidase	+	+	-	+	+
Growth temperature	20-40 °C[b] (optimum 28–35°C)	7-35 ºC (optimum 28-32 ºC)	10-37 ºC (optimum 28 ºC)	7-40 ºC (optimum 28 ºC)	10-40 ºC
pH	5-9 (optimum pH 7.0–8.0)	n.a.	5-9 (optimum pH 7.0)	5-9 (optimum pH 7.0)	6-8
Hydrolytic activity					

aesculin	+	-	-	-	-
casein	-	-	-	+	n.a.
gelatin	+/-*	+	-	-	+
Tween-80	-	n.a.	+	n.a.	+
tyrosine	n.a.	n.a.	+	n.a.	n.a.
PHBV	+	-	-	+	n.a.
Assimilation					
adipate	+/-*	-	+	-	+
D-alanine	+	+	-	+	n.a.
γ-aminobutyric acid	+/-*	-	-	+	+
L-asparagine	+	+/-*	+	+	+
caprate	-	+	+/-*	n.a.	-
glycerol	+	+/-*	-	+	+
D-glucose	-	+/-*	-	n.a.	-
L-histidine	-	+/-*	-	n.a.	-
3-hydroxibutyric acid	+	+	+/-*	+	+
4-hydroxybenzoate	-	+/-*	-	n.a.	n.a.

itaconate	+/-*	+/-*	+	+	+
α-ketobutyric acid	+	+	+/-*	+	+
α-ketoglutaric acid	+/-*	+/-*	+	+	+
2-ketogluconate	-	+	-	n.a.	n.a.
5-ketogluconate	-	-	-	n.a.	n.a.
D-mannose	+/-*	-	-	n.a.	-
L-proline	+	+	+/-*	+	+
propionate	+	+	+/-*	+	+
pyruvate	+	+	-	+	+
L-serine	+/-*	+/-*	+/-*	+	+
suberate	+	-	+	n.a.	n.a.
L-threonine	+	+	+/-*	+	+
trissodium citrate	-	-	+	n.a.	-
valerate	+	+	+	n.a.	+
Enzyme activities					
acid phosphatase	+/-*	-	+	-	n.a.
alkaline phosphatase	-	-	+	+	n.a.

arginine dihydrolase	+/-*	+/-*	-	-	+[w]
cystine arylamidase	+[w]	-	-	-	n.a.
β-glucosidase	+/-*	+/-*	-	-	n.a.
trypsin	-	+	-	-	n.a.
urease	+/-*	+/-*	-	-	+[w]
Antibiotics susceptibility[ϕ]					
amikacin	+	+	-	n.a.	n.a.
kanamycin	+	-	-	n.a.	n.a.
streptomycin	+	-	-	n.a.	n.a.
penicillin	-	+	-	-	n.a.
ampicillin	-	+	-	-	n.a.
cefalotin	-	+	-	-	n.a.
imipenem	n.a.	n.a.	+	n.a.	n.a.
erythromycin	-	+	-	n.a.	n.a.
troleandomycin	+	+	+	-	n.a.
nalidixic acid	+	+/-*	+/-*	-	n.a.
tetracycline	-	+	-	n.a.	n.a.

chloramphenicol	n.a.	n.a.	+	n.a.	n.a.
Major FAMES (%)[c]	SF3 (47.9%)	SF3 (44.7%)	SF3 (38.0%)	SF3 (45.4%)	$C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6 c* (20.0%)
	$C_{16:0}$ (23.2%)	$C_{16:0}$ (22.2%)	$C_{16:0}$ (25.6%)	$C_{16:0}$ (24.2%)	$C_{16:0}$ (32.5%)
	$C_{18:1}$ *ω7c* (16.1%)	$C_{18:1}$ *ω7c* (17.3%)	$C_{18:1}$ *ω7c* (10.2%)	$C_{18:1}$ *ω7c* (16.8%)	$C_{18:1}$ *ω7c*/$C_{18:1}$ *ω6 c* (15.1%)
			$C_{15:0}$ (8.7%)		$C_{17:0}$ cyclo (10.4%)
G+C (mol %)	64-65 (HPLC); 65.8 (T_m)	62.9 (HPLC)	65 (fluorometric method)	66.8 (T_m)	65.2 (T_m)
Isolation source	activated sludge	thiosulfate-oxidizing enrichment culture from rice-paddy soils	outdoor air sample	biofilms of a denitrifying reactor using PHBV as the sole carbon source	Soybean root nodules

421 [a] Data from Khan and Hiraishi, 2002; Pham *et al.*, 2009; Kim *et al.*, 2014; Qiu *et al.*, 2015; and Wei et al., 2015.

422 [b] Sham *et al.* (2009) reports as maximum growth temperature 35 ºC and Qiu *et al.* (2015) 37 ºC.

423 [c] Data from Qiu et al. (2015) for *D. nitroreducens*, *D. oryzae*, *D. aerolatus*, and D. *polyhydroxybutyrativorans*, and from Wei et al. (2015) for "*D.*

424 *ruginosibacter*". SF3, Summed feature 3 comprises $C_{16:1}$ ω7c/$C_{15:0}$ iso 2-OH.

425 n.a., not available; [w], weakly positive; *variable result depending on the testing conditions; [ϕ] positive susceptibility means bacterial growth is inhibited in the

426 presence of the antibiotic.

427 PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); HPLC, High-Performance Liquid Chromatography; T_m (determined by the thermal melting point)

428 Table 2. Differentiating characteristics between the genus *Diaphorobacter* and the closest related genera *Alicycliphilus*, *Oryzisolibacter*, and

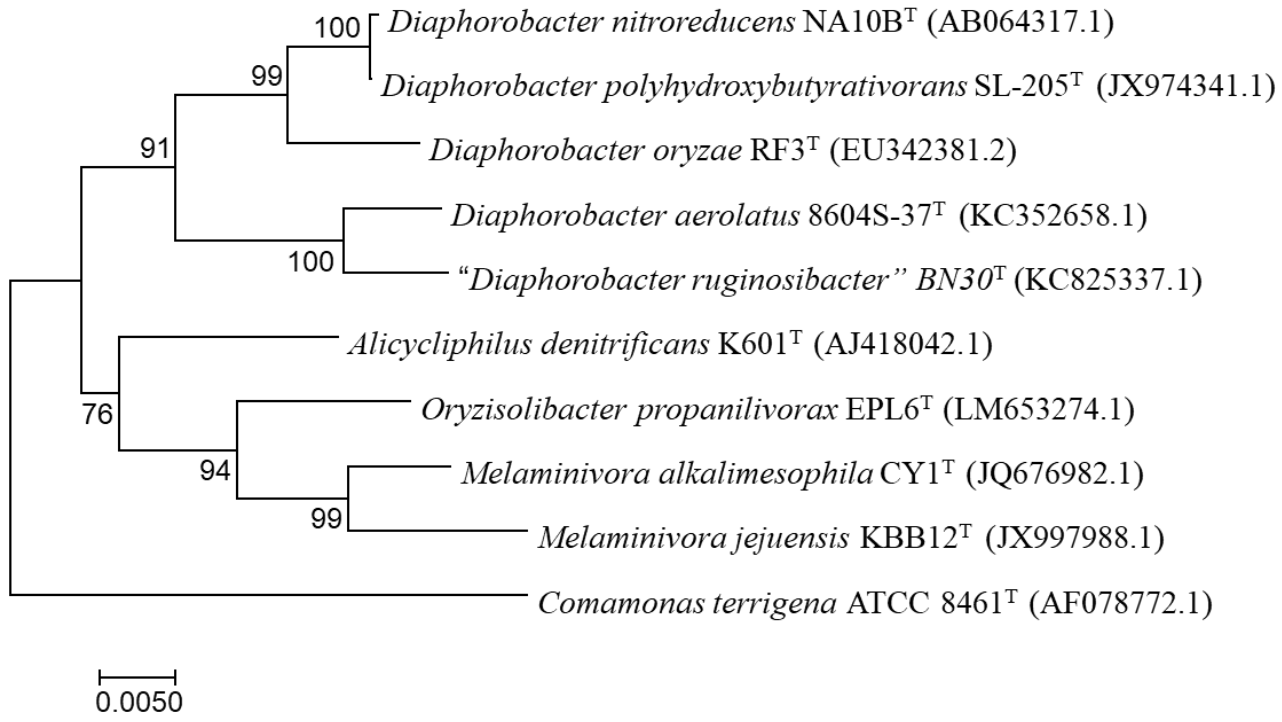
429 *Melaminivora*.

	Diaphorobacter	***Alicycliphilus***	***Oryzisolibacter***	***Melaminivora***
Cell morphology	Rods (0.3-0.9 µm x 0.9-1.9 µm)	Rods (0.6 µm x 1.0-2.0 µm)	Rods (0.6 µm x 1.9 µm)	Rods (0.5-0.9 µm x 2.0-3.5 µm)
Motility	v	+	+	+
Optimum growth temperature (ºC)	28-35	28-30	30	30-45
Optimum growth pH	7-8	7.2-7.4	n.a.	7-9.5
Nitrite reduction	+	+	+	-
Valine arylamidase	-	+	+	+
G+C (mol %)	62.9-66.8	66.0	69.4	69.5-69.6

430 +, positive; -, negative; v, variable characteristic among species of the genus.

Note: The species with the non-validated name "*Diaphorobacter ruginosibacter*" was not considered for the genera comparison.

433 **FIGURES:**



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435 Figure 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the position of the
436 *Diaphorobacter* species in relation to the closest phylogenetic genera of the family
437 *Comamonadaceae* in the *Betaproteobacteria*. The dendrogram was generated by the
438 Neighbor-Joining method. Bootstrap values, generated from 1000 re-samplings, are indicated
439 at branch points. Bar, 1 substitution per 200 nucleotide positions.

440