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Paludibacterium

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2. KEYWORDS

Paludibacterium; *Chromobacteriaceae*; wetland; marsh;

3. ABSTRACT

Curved rods, non-spore forming and Gram-negative. **Motile** by means of a single polar flagellum. **Facultative anaerobe**. Reacts positively for the catalase and cytochrome *c* oxidase tests. Nitrate reduction is variable among genus members and indole is not produced. The major respiratory quinone is ubiquinone 8 (Q-8). Fatty acid composition is variable within the genus, although summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and $C_{16:0}$ are predominant in all species. The polar lipid profile consists of phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), among other unidentified aminophospholipids, phospholipids, and polar lipids.

4. DEFINING PUBLICATION

Paludibacterium, Kwon, Kim, Kim, Yoo, Yoo, Son and Weon 2008, 192^{VP}.

5. ETYMOLOGY

Paludibacterium (Pa.lu´di.bac.te´ri.um. L. n. *palus –udis* a marsh; L. neut. n. *bacterium* a rod; N.L. neut. n. *Paludibacterium* a rod isolated from peat).

6. GENERIC DEFINITION

Curved rods, non-spore forming and Gram-negative. **Motile** by means of a single polar flagellum. **Facultative anaerobe**. Reacts positively for the catalase and cytochrome *c* oxidase tests. Nitrate reduction is variable among genus members and indole is not produced. The major respiratory quinone is ubiquinone 8 (Q-8). Fatty acid composition is variable within the genus, although summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and $C_{16:0}$ are predominant in all species. The polar lipid profile consists of phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), among other unidentified aminophospholipids, phospholipids, and polar lipids.

The DNA G+C content (mol %) is 59.2-63.0 (HPLC and Tm) and 60.7-62.2 (genome).

Type species: ***Paludibacterium yongneupense***, Kwon, Kim, Kim, Yoo, Yoo, Son and Weon 2008, 192^{VP}.

Number of species with validated names: 3.

7. FAMILY CLASSIFICATION

Chromobacteriaceae

The genus *Paludibacterium*, originally classified within the family *Neisseriaceae* (Prévot, 1933), was subsequently proposed to be transferred to the family *Chromobacteriaceae* within the order *Neisseriales*, based on 16S rRNA phylogenetic analysis and on the

presence of conserved phenotypic characteristics, such as rod-shaped morphology and flagella-based mobility (Adeolu & Gupta, 2013).

8. FURTHER DESCRIPTIVE INFORMATION

8.1. Cell morphology

Three species are validly named within the genus *Paludibacterium*: *Paludibacterium yongneupense* isolated from a wetland area in the region of Yongneup, in the Inje County in the Republic of Korea (Kwon *et al.*, 2008), *Paludibacterium paludis* isolated from a marsh in Taiwan (Sheu *et al.*, 2014), and *Paludibacterium purpuratum* isolated from a wetland soil in the Jeju island in the Republic of Korea (Kang *et al.*, 2016). Cells of all species are motile by means of a single polar flagellum, Gram-negative, non-spore forming rods, which are curved in species *P. yongneupense* and *P. purpuratum*. The dimensions (µm, diameter x length) of the cells of the type strains of the three species are described as: 0.6-0.9 x 2.0-4.0 for *P. yongneupense*, 0.6-0.8 x 2.0-5.0 for *P. paludis*, 0.5-0.7 x 1.8-2.5 for *P. purpuratum.*

8.2. Colonial and cultural characteristics

On Reasoner's 2A agar (R2A) medium, *Paludibacterium* spp. form convex, round colonies with entire margins with distinct colours. The colonies of *P. yongneupense* are typically of cream colour (Kwon *et al.*, 2008), of *P. paludis* are yellow (Sheu *et al.*, 2014) and of *P. purpuratum* produce a brown tone at an early stage, becoming purple after 3 days (Kang *et al.*, 2016).

8.3. Nutrition and growth conditions

Paludibacterium species are facultative anaerobic bacteria with a chemo-organoheterotrophic metabolism. Members of the species described up to date grow optimally at 28-30 ºC, with slight differences on the tolerated temperature range (Table 1); and have a low salt tolerance, only able to grow up to 1-2 % (w/v) NaCl (Table 1). *P. yongneupense* exhibits the broadest range of pH tolerated, with growth occurring between pH 4.0-8.0; all the *Paludibacterium* species grow optimally at 7.0-8.0 (Table 1). The three type strains of the validly named species of the genus *Paludibacterium* are catalase and oxidase positive and test negative for indole production. Features shared by *Paludibacterium* species are the positive enzymatic activities for alkaline phosphatase, esterase (C4), leucine arylamidase and acid phosphatase, and negative attributes for cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-mannosidase, and *α*-fucosidase. The range of substrates that can be used as carbon sources support the differentiation of the type strains of the known species (Table 1), being the use of D-glucose, pyruvic acid methyl ester and DL-lactic acid observed in the three. None of the three type strains are able to assimilate γ-hydroxybutyric acid, α-keto glutaric acid, α-keto valeric acid, *N*-acetyl-D-galactosamine, adonitol, arabitol, erythritol, L-fucose, gentibiose, inositol, lactose, lactulose, D-melibiose, psicose, D-raffinose, L-rhamnose, sorbitol, turanose, xylitol, cis aconitic acid, formic acid, glucosaminic acid, itaconic acid, malonic acid, quinic acid, sebacic acid, L-leucine, L-ornithine, L-phenylalanine, L-threonine, D,L-carnithine, inosine, uridine, phenylacetic acid, adipic acid, hydroxyl-L-proline, succinamic acid, L-pyroglutamic acid, D-mannose, trisodium citrate, D-mannitol, α-cyclodextrin, D-galactonic acid lactone, phenylethyl-amine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol or D,L-α-glycerol phosphate as sole carbon and energy source.

<Table 1 near here>

8.4. Chemotaxonomic characteristics

The three *Paludibacterium* type strains present a similar chemotaxonomic profile. The predominant quinone is ubiquinone 8. The most abundant fatty acids in all type strains of *Paludibacterium* species are $C_{16:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) (Kang *et al.,* 2016). The three type strains diverge in the proportion of other fatty acid components, being $C_{17:0}$ cyclo and summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) among the most abundant in *P. paludis* and summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) in *P. purpuratum* (Kang *et al.,* 2016). Hydroxyl fatty acids detected in the three species are the components $C_{12:0}$ 3-OH and $C_{10:0}$ 3-OH (Kang *et al.,* 2016). The polar lipid profile consists on phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) in all *Paludibacterium* species (Sheu *et al.,* 2014; Kang *et al.,* 2016). In addition, uncharacterized aminophospholipids and/or phospholipids or polar lipids are reported in the three type strains (Table 1) (Sheu *et al.,* 2014; Kang *et al.,* 2016).

8.5. Genome features

The whole genome sequences of strains *Paludibacterium yongneupense* DSM 18731^T and *Paludibacterium purpuratum* CECT 8976^T are available in the GenBank under the Bioproject references PRJNA185612 and PRJNA463291, respectively. The genome sequences of both strains were obtained based on Illumina HiSeq analyses. The *Paludibacterium yongneupense* DSM 18731^T genome comprises in total 4.33 Mb, and 62 contigs with an N50 of 142,622 and L50 of 8. Based on the genome data, the average

G+C content (mol%) is 62.2, whereas based on High-Performance Liquid Chromatography (HPLC) method it is 63.0 mol%, as reported in the species description (Kwon *et al.*, 2008). The genome of *Paludibacterium purpuratum* CECT 8976^{T} has a coverage of 263 x, presents a total length of 4.04 Mb, and was assembled in 40 contigs with an N50 value of 208,715 and L50 value of 7. According to the genome data, the average G+C content (mol%) is 60.7; whereas on the basis of the fluorescence melting curves method, it is 59.2 mol% (Kang *et al.*, 2016).

8.6. Ecology and Habitat

Validly named species of the genus *Paludibacterium* were isolated from samples of a wetland peat (*P. yongneupense*) and a wetland soil (*P. purpuratum*) collected in the Republic of Korea, and from a water sample from a marsh in Taiwan (*P. paludis*). *P. yongneupense* strain 5YN8-15^{T} was isolated from a 150 cm thick peat layer, described as being formed about 4000–5000 years ago, in the Yongneup (38º 12’ 53´´ N 128º 07´ 30´´ E) wetland moor located at 1200–1280 m above sea level. The original publication refers to this habitat as a “special type of ecosystem in terms of weather, soil and vegetation” due to the fact that it is a wetland located 1200–1280 m above sea level that serves as moor, indeed the only high moor in Korea (Kwon *et al.*, 2008). *P. paludis* strain KBP-21^{T} was isolated from the Banping Lake Wetland Park (22º 41´ 30´´ N 120º 18´ 32´´ E), located in the proximities of Kaohsiung City, in Taiwan. The water sample had a pH of 7.3 and a NaCl concentration of 0.5 %, its underlying geology is coral limestone (Sheu *et al.*, 2014). *P. purpuratum* strain KJ031^{T} was isolated from the top layer of a soil at 0-10 cm, belonging from a wetland in the Jeju island (33º 21´ 25´´ N 126º 27´ 48´´ E), located at 1100 m above sea level on Halla mountain and described as Ramsar site with unique freshwater marshes and pools (Kang *et al.*, 2016).

Other isolates identified as members of the genus *Paludibacterium* (strains PT71 and PT78) were isolated from water samples collected from reservoirs in South Bohemia (Czech Republic) (Salmonová *et al.*, 2018). Strains PT71 (16S rRNA gene accession number KY124210) and PT78 (16S rRNA gene accession number KY124211) were most closely related to *P. paludis* (HE981224), with 16S rRNA gene sequence identity values of 97.2% and 97.7%, respectively (Salmonová *et al.*, 2018). Also the phylotype NKB6 (16S rRNA gene accession number AB013258) identified by culture-independent techniques from sediment samples from the submarine Nankai Trough (Li *et al.*, 1999) shows 16S rRNA gene sequences similarities of 95.0 and 95.4% with *P. paludis* (HE981224) and *P. yongneupense* (AM396358) respectively.

9. ENRICHMENT AND ISOLATION PROCEDURES

No specific enrichment procedure has been recommended for members of the genus *Paludibacterium*. The three validly named species of the genus were isolated on R2A medium. For the isolation of the type strain of the genus, *P. yongneupense* 5YN8-15^{T}, the wetland sample was suspended in 0.85 % (w/v) NaCl solution and serially diluted, being the 10-fold dilutions spread onto R2A plates and incubated at 28 ºC for 4 days (Kwon *et al.*, 2008). The standard dilution plating method was also used to isolate the type strain of *P. purpuratum* on R2A agar at 30 °C (Kang *et al.*, 2016).

10. MAINTENANCE PROCEDURES

Paludibacterium spp. can be routinely grown on R2A medium at 28 ºC or 30 ºC. For long-term preservation, cultures can be stored as a suspension in R2A broth or in distilled water supplemented with 20 % (v/v) glycerol and maintained at -80 ºC (Sheu *et al.*, 2014;

Kang *et al.*, 2016). In addition, *Paludibacterium* strains can be stored lyophilized in 20 % (w/v) skimmed milk (Sheu *et al.*, 2014; Kang *et al.*, 2016).

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

Currently, the closest phylogenetic relatives of the genus *Paludibacterium* are members of the genus *Pseudogulbenkiania* (96.0-94.8% 16S rRNA identity) (see gmb01834), followed by members of the genera *Gulbenkiania* (95.8-93.6% 16S rRNA identity) (see gmb01832) and *Chromobacterium* (95.6-93.1% 16S rRNA identity) (see gbm00975), by EzBioCloud comparison (Yoon *et al.*, 2017) (Figure 1).

Paludibacterium species can be distinguished from members of the genus *Pseudogulbenkiania* based on differences on colony pigmentation, the presence of summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) in *P. paludis* and *P. purpuratum* and $C_{17:0}$ cyclo in *P. paludis*, as well as, on various biochemical properties and substrate assimilation patterns, such as a positive catalase reaction, the ability to produce arginine dihydrolase and to ferment glucose, the inability to use some substrates as sole carbon sources (e.g. succinic acid, formic acid, quinic acid or L-ornithine) and the resistance to gentamicin (10 µg) and penicillin G (10 U) (Kwon *et al.*, 2008; Lin *et al.*, 2008; Lee *et al.*, 2013; Sheu *et al.*, 2014; Kang *et al.*, 2016).

<Figure 1 near here>

12. TAXONOMIC COMMENTS

The genus *Paludibacterium* was first described in 2008 as a new member of the family *Neisseriaceae* of the order *Neisseriales* (Kwon *et al.*, 2008). Five years later, a

phylogenomic reassessment reorganized the order *Neisseriales* with the revision of the family *Neisseriaceae* and the proposal of a novel family, *Chromobacteriaceae*, which would accommodate the genus *Paludibacterium* as well as other genera (Adeolu & Gupta, 2013).

The genus *Paludibacterium* currently contains three validly named species, each described based on a single isolate and largely delineated based on 16S rRNA gene sequence analysis, supported by differentiating characteristics. According to Sheu *et al.* (2014), strain KBP-21^T, the type strain of the species *Paludibacterium paludis*, shared 96.4% 16S rRNA gene sequence similarity with the type strain of *Paludibacterium yongneupense*. This was supported by the colony pigmentation and by the ability of *P. paludis* to accumulate poly-*β*-hydroxybutyrate, to hydrolize casein, chitin, gelatin, corn oil, lecithin, Tween 20, 40 and 80. In addition, by contrast with *P. yongneupense*, *P. paludis* was not able to reduce nitrate, to hydrolize starch, or to express *α*-glucosidase and *β*-glucosidase activity. *P. paludis* was able to use Tween 40, Tween 80, *α*-hydroxybutyric acid, *β*-hydroxybutyric acid, propionic acid, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline, L-serine, caprate, glycyl-L-glutamic acid, thymidine, *γ*-amino butyric acid, glycogen and acetic acid as sole carbon and energy source, but *P. yongneupense* was not. Maltose, *α*-D-glucose-1-phosphate, D-glucose-6-phosphate and sucrose were used as sole carbon and energy sources by *P. yongneupense*, but not by *P. paludis*. The most recently described species, *Paludibacterium purpuratum*, shares a 16S rRNA gene sequence similarity of 96.2 % with *P. paludis* and 96.0 % with *P. yongneupense* (Kang *et al.*, 2016), which is supported by the differentiation based on colony pigmentation of *P. purpuratum*, its inability to hydrolyse CM-cellulose and by the assimilation of glycyl-L-aspartic acid, L-arabinose, potassium gluconate and malic acid in comparison to *P. yongneupense* and *P. paludis*.

Some other differentiating physiological and chemotaxonomic features are detailed in Table 1. Given the fact that the 16S rRNA gene sequence similarity values among the three type strains were below 97%, DNA-DNA hybridization values were not part of the species description.

13. LIST OF SPECIES OF THE GENUS *PALUDIBACTERIUM*

1. ***Paludibacterium paludis*** Sheu, Chen, Young and Chen 2014, 2500^{VP}.

paludis (pa.lu´dis. L. gen. n. *paludis* of a swamp, of a marsh, of a bog).

In addition to the genus description, the type strain of the species is described as comprising rods 0.6-0.8 µm in diameter and 2.0-5.0 µm in length that forms yellow, convex, round and smooth with entire edges colonies of 1.2-1.4 mm in diameter. Accumulation of poly-*β*-hydroxybutyrate is observed. Growth occurs at 15-40 ºC, pH 5.0-8.0 and 0-2% NaCl, and optimally at 30 ºC, pH 8.0 and 0% NaCl. Casein, DNA, corn oil, chitin, lecithin, CM-cellulose, gelatin and Tween-20, 40, 60 and 80 are hydrolysed, whereas starch, urea or alginate are not. In API 20NE tests, nitrate reduction and indole production are negative. In API ZYM strips reacts positively for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and *N*-acetyl-*β*-glucosaminidase. Negative for cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *α*-mannosidase and *α*-fucosidase activities. Cells are able to assimilate Tween 40, Tween 80, *N*-acetyl-D-glucosamine, *α*-D-glucose, pyruvic acid methyl ester, *α*-hydroxybutyric acid, *β*-hydroxybutyric acid, DL-lactic acid, propionic acid, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline, L-serine, caprate, glycyl-L-glutamic acid, thymidine, *γ*-amino butyric acid, glycogen and acetic

acid (API 20NE and GN2 microplate). Susceptible to nalidixic acid (30 µg), chloramphenicol (30 µg), rifampicin (5 and 30 µg), tetracycline (30 µg), novobiocin (30 µg), and sulfamethoxazole plus trimethoprim (23.75+1.25 µg); but resistant to penicillin G (10 U), ampicillin (10 µg), erythromycin (15 µg), gentamicin (10 µg), and vancomycin (30 µg). The polar lipid profile is phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an uncharacterized aminophospholipid and four uncharacterized phospholipids. Major fatty acids (> 10%) are summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), $C_{16:0}$, summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) and $C_{17:0}$ cyclo.

The DNA G+C content (mol%) is 62.1 (HPLC).

Type strain: KBP-21 (= BCRC 80514 = LMG 27230 = KCTC 32182 = DSM 29672).

GenBank accession number (16S rRNA): HE981224.

2. ***Paludibacterium purpuratum*** Kang, Kim, Joung, Kim and Joh 2016, 2715^{VP}.

purpuratum (pur.pu.ra´tum. L. neut. adj. *purpuratum* clad in purple-violet, referring to the colony colour).

In addition to the genus description, the type strain of the species exhibits the following properties. Cells are 1.8-2.5 in length and 0.5-0.7 µm in width. Colonies are brown to purple, circular, convex and with entire margins on R2A medium. Capable of growth at 20-37 ºC, pH 6.0-8.0 and in the presence of 0-1.5% (w/v) NaCl. Optimal growth temperature is 30 ºC, pH 7.0 and 0 % (w/v) NaCl concentration. Anaerobic growth occurs in the presence of sulfate as electron acceptor. Good growth is observed on R2A, nutrient agar (NA) and Tryptic Soy agar (TSA) media, but not on Marine 2216 (MA), blood or MacConkey agar. Casein (skimmed milk), gelatin and aesculin are hydrolysed, whereas

DNA, starch, dextrin, CM-cellulose, urea and cellulose (filter paper) are not. In the API ZYM kit, reacts positively for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *α*-glucosidase activities. Negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase activities. In API 20NE tests, positive reactions for glucose fermentation and arginine dihydrolase, and negative reactions for nitrate reduction, indole production. Cells are able to assimilate Tween 40, Tween 80, *N*-acetyl-D-glucosamine, D-fructose, *α*-D-glucose, D-trehalose, pyruvic acid methyl ester, D,L-lactic acid, bromosuccinic acid, L-alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-proline, L-arabinose, potassium gluconate, malic acid, urocanic acid, *α*-D-glucose-1-phosphate and D-glucose-6-phosphate (API 20NE and GN2 MicroPlate). Susceptible to chloramphenicol (30 µg), kanamycin (30 µg), rifampicin (30 µg), streptomycin (10 µg) and tetracycline (30 µg), but resistant to ampicillin (10 µg), erythromycin (15 µg), gentamicin (10 µg), penicillin G (10 U) and vancomycin (30 µg). The polar lipid profile is diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminophospholipid, two unidentified phospholipids and one unidentified polar lipid. Major fatty acids (> 10%) are summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), $C_{16:0}$ and summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*).

The DNA G+C content (mol%) is 59.2 (Tm) and 60.7 (genome sequence).

Type strain: KJ031 (= KCTC 42852 = CECT 8976).

GenBank accession number (16S rRNA): JN624304.

GenBank accession number (genome): SNZP00000000.

3. ***Paludibacterium yongneupense*** Kwon, Kim, Kim, Yoo, Yoo, Son and Weon 2008, 192^{VP}.

yongneupense (yong.ne.up.en´se N.L. neut. adj. *yongneupense* pertaining to Yongneup, a wetland in Korea where the organism was first isolated).

In addition to the genus description, the type strain of the species is described as cream-coloured colonies, round and convex with clear margins on R2A medium. Exponentially growing cells are 0.6-0.9 x 2.0-4.0 µm in size. Growth in the range 4-35 ºC and pH 4.0-8.0. CM-cellulose, starch, xanthine and Tween 60 are hydrolysed, whereas casein, chitin, hypoxanthine, gelatin, pectin, Tween 20, Tween 40, Tween 80, tyrosine, urea, corn oil, lecithin or alginate are not. Reacts positively for alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, *α*-glucosidase and *β*-glucosidase. Negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-mannosidase or *α*-fucosidase (API ZYM). D-glucose is fermented (API 20NE). Cells are able to assimilate D-glucose, maltose, pyruvic acid methyl ester, DL-lactic acid, *α*-D-glucose-1-phosphate, D-glucose-6-phosphate, *α*-keto butyric acid and sucrose (API 20NE and API ID 32 GN). Susceptible to nalidixic acid (30 µg), chloramphenicol (30 µg), rifampicin (5 and 30 µg), tetracycline (30 µg), novobiocin (30 µg) and sulfamethoxazole plus trimethoprim (23.75+1.25 µg), but resistant to penicillin G (10 U), erythromycin (15 µg), gentamicin (10 µg) and vancomycin (30 µg). The polar lipid profile is phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an

uncharacterized aminophospholipid and five uncharacterized phospholipids. Major fatty acids (> 10%) are $C_{16:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*).

The DNA G+C content (mol%) is 63.0 (HPLC) and 62.2 (genome sequence).

Type strain: 5YN8-15 (= KACC 11601 = DSM 18731).

GenBank accession number (16S rRNA): AM396358.

GenBank accession number (genome): AUGZ00000000.

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gmb01832

gbm00975

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Table 1. Characteristics that distinguish the species of the genus *Paludibacterium*: *P. yongneupense* KACC 11601^T, *P. paludis* KCTC 32182^T and *P. purpuratum* KJ031^T.

The three strains tested positive for oxidase, catalase, alkaline phosphatase, esterase (C4), leucine arylamidase and acid phosphatase. All assimilate D-glucose, pyruvic acid methyl ester and DL-lactic acid.

All the strains were negative for indole, hydrolysis of urea, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. No growth was observed with γ-hydroxybutyric acid, α-keto glutaric acid, α-keto valeric acid, N-acetyl-D-galactosamine, adonitol, arabitol, erythritol, L-fucose, gentibiose, inositol, lactose, lactulose, D-melibiose, psicose, D-raffinose, L-rhamnose, sorbitol, turanose, xylitol, cis aconitic acid, formic acid, glucosaminic acid, itaconic acid, malonic acid, quinic acid, sebacic acid, L-leucine, L-ornithine, L-phenylalanine, L-threonine, D,L-carnithine, inosine, uridine, phenylacetic acid, adipic acid, hydroxyl-L-proline, succinamic acid, L-pyroglutamic acid, D-mannose, trisodium citrate, D-mannitol, α-cyclodextrin, D-galactonic acid lactone, phenylethyl-amine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol or D,L-α-glycerol phosphate.

All the strains were susceptible to chloramphenicol (30 µg), rifampicin (5 and 30 µg) and tetracycline (30 µg), and resistant to penicillin G (10 U), erythromycin (15 µg), gentamicin (10 µg) and vancomycin (30 µg).

Characteristic	***P. yongneupense* KACC 11601^T**	***P. paludis* KCTC 32182^T**	***P. purpuratum* KJ031^T**
Cell morphology	Curved-rod[a]	Rod[b]	Curved-rod
Cell size (µm)	0.6-0.9 x 2.0-4.0[a]	0.6-0.8 x 2.0-5.0[b]	1.8-2.5 x 0.5-0.7
Colonies pigmentation	Cream[a]	Yellow[b]	Brown to purple
NaCl growth (optimum) (%, w/v)	0-1 (0.5)[b]	0-2 (0) [b]	0-1.5 (0)
pH growth (optimum)	4.0-8.0 (7.0) [b]	5.0-8.0 (8.0) [b]	6.0-8.0 (7.0)
Temperature growth (optimum) (ºC)	4-35 (28)[a1]	15-40 (30) [b]	20-37 (30)
Nitrate reduction	+	-	-
Hydrolysis:			
CM-cellulose	+	+	-

Characteristic	***P. yongneupense* KACC 11601^T**	***P. paludis* KCTC 32182^T**	***P. purpuratum* KJ031^T**
Starch	+	-	-
Casein	-	+	+
DNA	-[2]	+	-
Chitin	-	+	n.a.
Gelatin	-	+	+
Tween 20	-	+	n.a.
Tween 40	-	+	n.a.
Tween 80	-	+	n.a.
Corn oil	-	+	n.a.
Lecithin	-	+	n.a.
Enzymatic activities			
α-Glucosidase	+	-	+
β-Glucosidase	+	-	-
N-Acetyl-β-glucosaminidase	+[3]	+	-
Assimilation of:			
Maltose	+	-	-
Tween 40	-	+	+
Tween 80	-	+	+
α-Hydroxybutyric acid	-	+	-
β-Hydroxybutyric acid	-	+	-
Propionic acid	-	+	-
L-Alaninamide	-	+	+

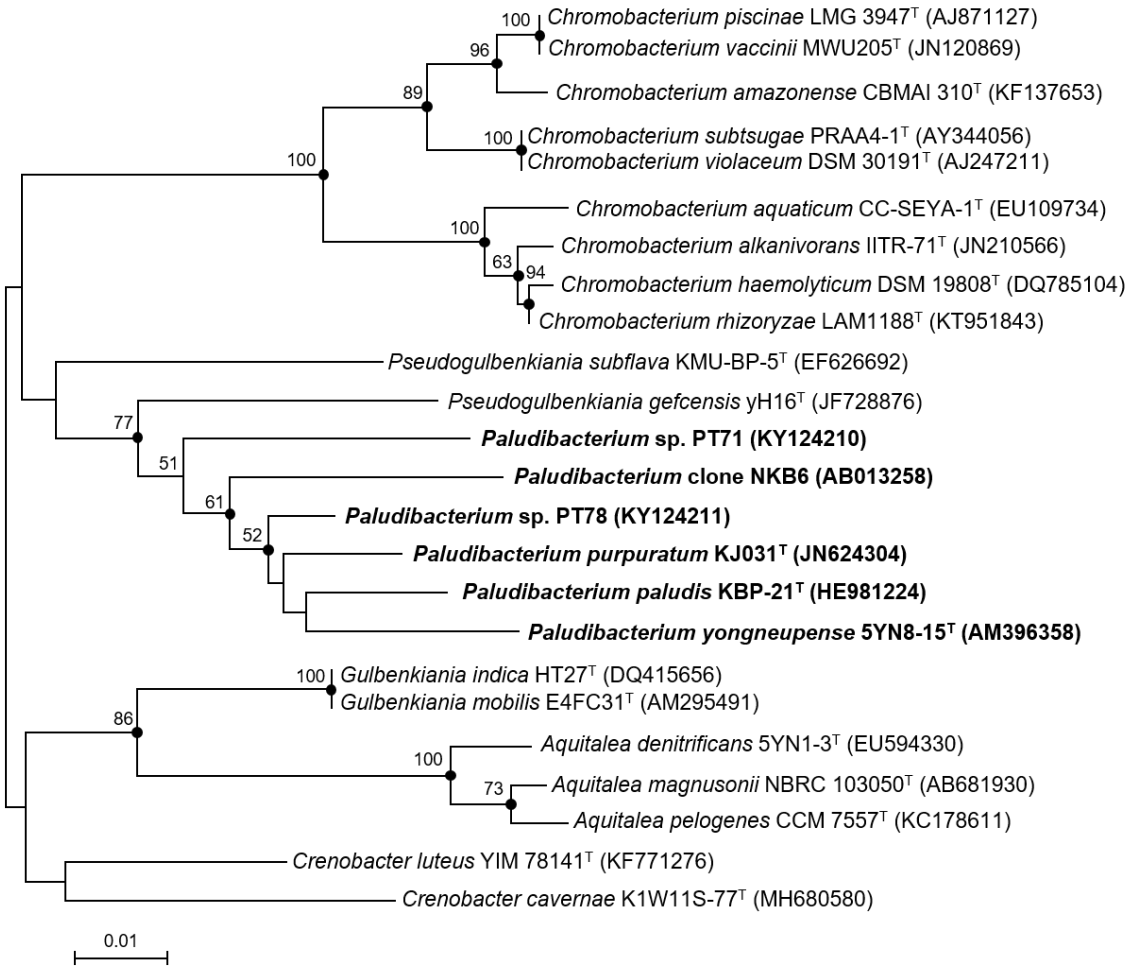
Characteristic	***P. yongneupense* KACC 11601^T**	***P. paludis* KCTC 32182^T**	***P. purpuratum* KJ031^T**
L-Alanine	-	+	+
L-Alanyl glycine	-	+	+
L-Asparagine	-	+	+
L-Aspartic acid	-	+	+
L-Glutamic acid	-	+	+
L-Proline	-	+	+
L-Serine	-	+	-
Caprate	-	+	-
D-Trehalose	-[2]	-	+
Bromosuccinic acid	-[2]	-	+
Glycyl-L-aspartic acid	-	-	+
Glycyl-L-glutamic acid	-	+	+
α-D-Glucose-1-phosphate	+	-	+
D-Glucose-6-phosphate	+	-	+
L-Arabinose	-	-	+
Potassium gluconate	-	-	+
Malic acid	-	-	+
α-Keto butyric acid	+	+[4]	-
Sucrose	+	-	-
Thymidine	-	+	-
γ-Amino butyric acid	-	+	-
Glycogen	-	+	-

Characteristic	***P. yongneupense* KACC 11601^T**	***P. paludis* KCTC 32182^T**	***P. purpuratum* KJ031^T**
Acetic acid	-	+	-
Antibiotics resistance:			
Kanamycin (30 µg)	R[5]	R[5]	S
Streptomycin (10 µg)	R[5]	R[5]	S
Ampicillin (10 µg)	S[6]	R	R
Major fatty acids (> 10%)	$C_{16:0}$ Summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*)	Summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) $C_{16:0}$ Summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*) $C_{17:0}$ cyclo	Summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) $C_{16:0}$ Summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*)
Polar lipids	PE, PG, DPG, 1 APL and 5 PL	PE, PG, DPG, 1 APL and 4 PL	PE, PG, DPG, 1 APL, 2 PL and 1 L
DNA G+C content (mol %)	63.0 (HPLC)[a7] 62.2 (genome)	62.1 (HPLC)[b8]	59.2 (Tm) 60.7 (genome)
Isolation source	Wetland	Water	Wetland

[a]Data from Kwon *et al.* (2008).
[b]Data from Sheu *et al.* (2014).
[1]Sheu *et al.* (2014) found 10-40 (15) (ºC) the temperature range growth for the type strain *P. yongneupense* 5YN8-15^T.
[2]Sheu *et al.* (2014) described the strain as positive.
[3]Kwon *et al.* (2008) and Sheu *et al.* (2014) described the strain as negative.
[4]Sheu *et al.* (2014) described the strain as negative.
[5]Sheu *et al.* (2014) described the strain as susceptible.
[6]Sheu *et al.* (2014) described the strain as resistant.

[7]Kang *et al*. (2016) found 63.6 mol %, the DNA G+C content for the type strain *P. yongneupense* KACC 11601^T, using the Fluorescent DNA melting curves.
[8]Kang *et al*. (2016) found 61.1 mol %, the DNA G+C content for the type strain *P. paludis* KCTC 32182^T, using the Fluorescent DNA melting curves.

n.a., not available; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; APL, uncharacterized aminophospholipid; PL, uncharacterized phospholipids; L, unidentified polar lipid.



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407 **Figure 1.** Neighbour-joining phylogenetic tree reconstruction based on the 16S rRNA gene
408 sequences comparison showing the phylogenetic position of *Paludibacterium* species and other
409 *Paludibacterium* representatives in relation to other genera of the order *Neisseriales*. Bootstrap
410 values ≥ 50 % are indicated at branch points. Filled circles indicate branches on the tree that were
411 also recovered in the tree generated using the maximum-likelihood algorithm. Bar, 0.01
412 substitutions per nucleotide position.

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