

Published in final edited form as:

Int J Syst Evol Microbiol. 2017 February ; 67(2): 379–385. doi:10.1099/ijsem.0.001637.

***Polynucleobacter wuianus* sp. nov., a free-living freshwater bacterium affiliated with the cryptic species complex PnecC**

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Abstract

Strain QLW-P1FAT50C-4^T, isolated from a shallow acidic freshwater pond located in the Austrian Alps at an altitude of 1300 m, was characterized by investigation of phenotypic, chemotaxonomic and genomic traits. As shown previously, phylogenetic analyses based on 16S rRNA gene sequences place the strain in the cryptic species complex PnecC within the genus *Polynucleobacter*. The major fatty acids of the strain were C_{16:1} ω7c and C_{18:1} ω7c. The strain has a genome of 2.23 Mbp with a GC content of 44.9 mol%. The strain encodes a seemingly complete gene cluster for anoxygenic photosynthesis but lacks typical genes for CO₂ assimilation. In order to resolve the phylogenetic position of the strain within the species complex PnecC, concatenated partial sequences of eight housekeeping genes were analysed. The obtained phylogenetic reconstruction did not place strain QLW-P1FAT50C-4^T close to any of the five previously described species within subcluster PnecC. Pairwise average nucleotide identity (ANI) comparisons of whole genome sequences suggested that strain QLW-P1FAT50C-4^T (DSM 24008^T =CIP 111100^T) represents a novel species for which we propose the name *Polynucleobacter wuianus* sp. nov.

The genus *Polynucleobacter* and the species *Polynucleobacter necessarius* were proposed by K. Heckmann and H. J. Schmidt (1987) for bacterial endosymbionts of benthic freshwater ciliates affiliated with the genus *Euplotes*. Later, it was discovered that free-living strains, closely related to those endosymbiotic strains, represent important planktonic freshwater bacteria (Bahr *et al.*, 1996; Hahn, 2003; Percent *et al.*, 2008; Zwart *et al.*, 2002). The genus *Polynucleobacter* can be subdivided in at least four subclusters labelled PnecA, PnecB, PnecC, and PnecD (Hahn, 2003). Subcluster PnecC, which includes the endosymbiotic *P. necessarius* and free-living species, was recognized as being a cryptic species complex, which means that this seemingly very species-rich subcluster cannot be resolved by 16S rRNA gene phylogenies (Hahn *et al.*, 2016a; Hahn *et al.*, 2016b). Taxonomic research on strains affiliated with subcluster PnecC is hampered by the lack of a type strain of the genus

DDBJ/EMBL/GenBank accession numbers

Polynucleobacter wuianus sp. nov. strain QLW-P1FAT50C-4^T: CP015922 and AJ879778

(Hahn *et al.*, 2016a). Reference material representing the species *P. necessarius* was contained in the *Euplotes aediculatus* 'stock 15' culture (=E24 =ATCC 30859) (Heckmann and Schmidt, 1987), which is unfortunately not any more available. However, recently, it was shown (Hahn *et al.*, 2016a) that the lost endosymbionts used for the species description are well represented by another endosymbiotic *P. necessarius* strain, i.e. STIR1, for which a complete genome sequence is available (Boscaro *et al.*, 2013).

Here we describe strain QLW-P1FAT50C-4^T affiliated with subcluster PnecC, which was isolated from an acidic freshwater pond, and propose to establish for this strain the species name *P. wuianus* sp. nov.

Home habitat and isolation

Strain QLW-P1FAT50C-4^T shares with the type strain of *P. asymbioticus* (Hahn *et al.*, 2016a) the same home habitat. Both strains were isolated from water from Pond-1 (geographic coordinates 47°44'23.37"N, 13°18'6.03"E) by Q. L. Wu in October 2003 (Hahn *et al.*, 2005). This pond is located in the Austrian Alps at an altitude of 1300 m (Hahn *et al.*, 2012b). It is a small, shallow (max depth < 1 m) natural freshwater pond, which was usually (years 2003-2013) characterized by a slightly acidic pH in the range of 4.5-5.5 and a quite low conductivity of usually 5-15 $\mu\text{S cm}^{-1}$.

Isolation of the strain was described in detail elsewhere (Hahn *et al.*, 2005). Briefly, the strain was isolated by using the filtration-acclimatization method and NSY medium (Hahn *et al.*, 2005; Hahn *et al.*, 2004).

Phenotypic and chemotaxonomic characterization

Cells of strain QLW-P1FAT50C-4^T are short sometimes slightly curved rods of small to medium size. Cell size depends on growth stage and medium used for cultivation. While size in NSY medium is typically 1 μm cell length (50 cells measured; SD 0.2; range 0.6-1.7 μm) and 0.5 μm cell widths (n, 50; SD, 0.1; range 0.3-0.6 μm), cell size dropped to 0.5 x 0.3 μm when cells were grown in sterile water from their home habitat (Table 1). This cell size equals to a cell volume of 0.03 μm^3 placing the strain among the smaller cells in freshwater bacterioplankton (Hahn *et al.*, 2003). Strain QLW-P1FAT50C-4^T forms small circular, convex colonies with shiny surface on NSY agar plates and lacks pigmentation. Growth at different temperatures and growth under anoxic conditions in an anaerobic chamber were examined by using NSY agar plates as described previously (Hahn *et al.*, 2009). Furthermore, growth rates of the strain in liquid NSY medium were determined after appropriate thermal acclimatization at four different temperatures. Salinity (NaCl) tolerance was determined using NSY agar supplemented with various NaCl concentrations as described previously (Hahn *et al.*, 2009). The strain showed no anaerobic growth, grew at temperatures up to 34°C and tolerated salt concentrations up to 0.5% (Table 1). Rates of growth in NSY medium determined at 8, 12, 22, and 28°C increased with temperatures and were 0.063 h⁻¹, 0.128 h⁻¹, 0.198 h⁻¹, and 0.273 h⁻¹, respectively.

Utilization of various substrates was investigated in the same way as for previously described *Polynucleobacter* species (Hahn *et al.*, 2011a; Hahn *et al.*, 2011b; Hahn *et al.*,

2009; Hahn *et al.*, 2012a; Hahn *et al.*, 2010). Briefly, growth enabled by utilization of a specific substrate was determined by comparison of OD at 575 nm established in liquid one tenth-strength NSY medium (0.3 g l^{-1}) with and without 0.5 g l^{-1} test substrate. Differences of < 10 %, 10–50% and >50% of the OD obtained in the test treatments compared to the OD obtained without test substrate (i.e. in 0.3 g l^{-1} NSY medium) were scored after 10 days of growth as no utilization (-), weak utilization (w) and good utilization (+), respectively (Table 1).

The analysis of the whole-cell fatty acid composition was carried out as described previously (Hahn *et al.*, 2012a). Biomass was harvested from R2A agar plates. Plates were inoculated with 1 ml cell suspension, incubated while keeping the agar surface moist and inspected for growth daily, starting the third day after inoculation. Once a biomass film was well visible, the cell mass was harvested. The results (Table 2) underpin the membership of strain QLW-P1FAT50C-4^T in subcluster PnecC, for example by the presence of n-dodecanoic acid, which is lacking in members of subcluster PnecD (*P. cosmopolitanus*).

Genomic characterization

DNA used for genome sequencing was extracted from biomass grown in liquid NSY medium as described previously (Meincke *et al.*, 2012). Two libraries were sequenced by an Illumina and a Roche system, respectively, and the obtained reads were *de novo* assembled. A shotgun library was paired-end sequenced on GS FLX by using Titanium chemistry (Beckman Coulter Genomics SA, Grenoble, France). Sequencing resulted in 212,176 filtered reads with a mean length of 361 nt. A 8 kb library was mate pair sequenced to receive long jumping distance pair sequences by using a Illumina MiSeq instrument (Eurofins Genomics, Munich, Germany). This sequencing resulted in 570,724 filtered reads with a mean length of 97 nt. A *de novo* hybrid assembly was conducted using an in-house pipeline that incorporates the software tool newbler 2.9 (Eurofins Genomics). The assembly resulted in 16 contigs contained in nine scaffolds. Some gaps could be closed by *in silico* analyses and all remaining gaps but one were closed by PCR amplification of gap regions and subsequent Sanger sequencing of amplicons. These efforts resulted in a closed genome sequence with a single gap. Primers constructed in order to bridge and sequence this last gap, resulted in PCR products but repeated efforts to obtain sequences from this amplicon did not result in sequences suitable for gap closure. The obtained PCR products, however, suggested a gap size of < 1000 bp. The obtained genome sequence has a coverage of about 62x and is characterized by a length of 2.23 Mbp and a G+C content of 44.9 mol% (Table 3). The genome sequence was annotated using the IMG/ER annotation pipeline (Markowitz *et al.*, 2012) and deposited in DDBJ/EMBL/GenBank (Accession Number CP015922). Gene finding resulted in 2274 protein coding and 51 RNA genes. The genome of strain QLW-P1FAT50C-4^T and the genomes of type strains of all four other previously described free-living *Polynucleobacter* species affiliated with subcluster PnecC (Hahn *et al.*, 2016a) differ in coding density from the only yet sequenced endosymbiotic *P. necessarius* strain (Boscaro *et al.*, 2013). While the five free-living strains possess coding densities in the range of 93.1–93.5%, the endosymbiotic strain is characterized by a coding density of less than 78.5%. Furthermore, the endosymbiotic and the free-living strains differ in genome size, consisting of 1.56 Mbp and 2.03–2.32 Mbp, respectively (Table 3).

While all five compared free-living strains possess similar genome sizes and G+C contents, they differ from each other pronouncedly in gene content (Table 4). Strain QLW-P1FAT50C-4^T shares, for instance, the presence of various genes putatively involved in acquisition of inorganic nitrogen with the type strains of *P. asymbioticus* and *P. yangtzensis* but shares the presence of a large gene cluster putatively encoding an anoxygenic photosynthesis system only with the type strain of *P. duraquae*. The gene content listed in Table 4 represents only an incomplete list of content differences, however, this selection shows that all five type strains possess unique combinations of genes not shared by any other so far sequenced *Polynucleobacter* strain. Currently, it is unknown if listed genes present in a type strain are also present in all strains affiliated with the species represented by the type strain.

Phylogeny

The 16S rRNA gene sequence and the 16S-23S internal transcribed spacer (ITS) sequences of strain QLW-P1FAT50C-4^T determined by previous PCR amplicon sequencing and by genome sequencing were identical, respectively. The 16S rRNA gene sequences of the strain and closely related strains (Hahn *et al.*, 2005) is characterized by the lack of a base in *E. coli* numbering position 1007. All other investigated *Polynucleobacter* strains (109 strains) possess a guanine base in this position, which is in secondary structure models of the molecule base-pairing with an adenine base in *E. coli* numbering position 1022. Interestingly, QLW-P1FAT50C-4^T and closely related strains share with all other investigated *Polynucleobacter* strains the presence of the adenine base in position 1022.

Phylogenetic reconstructions based on 16S rRNA gene sequences (Fig. 1B) place strain QLW-P1FAT50C-4^T in subcluster PnecC (Hahn, 2003) of the genus *Polynucleobacter* (Hahn *et al.*, 2016a). As reported previously, strains affiliated with this species complex share 16S rRNA genes with sequence similarities > 99% (Fig. 1B). In order to better resolve the phylogenetic position of strain QLW-P1FAT50C-4^T a tree based on concatenated multilocus sequences was calculated (Fig. 1A). Partial sequences of eight loci (*rpoB*, *trpE*, *icdA*, *glnA*, *mdh*, *fbp*, *msbA*, and *gyrA*) defined previously (Hahn *et al.*, 2016a) were extracted from genome sequences (Table 3), concatenated and aligned by using the software MEGA7 (Kumar *et al.*, 2016). This resulted in a total alignment length of 6359 bp. The same software was used for calculation of neighbour-joining (NJ), maximum likelihood (ML) and maximum parsimony (MP) trees (Fig. 1A). In previous pairwise comparisons, sequence similarity values of these concatenated sequences correlated well with average nucleotide identity (ANI) values of whole genome sequences (Hahn *et al.*, 2016a). This suggests that this locus selection is somehow representative for the entire genomes. The phylogenetic reconstruction based on this multilocus sequence set also placed strain QLW-P1FAT50C-4^T in subcluster PnecC but did not suggest that the strain is affiliated with any previously described species.

Ecology

Until recently, all free-living *Polynucleobacter* strains affiliated with subcluster PnecC including strain QLW-P1FAT50C-4^T were considered to belong to *P. necessarius* subspecies

asymbioticus (Hahn *et al.*, 2009). All these free-living strains have a planktonic lifestyle in common, that is, they dwell freely suspended in the water column of freshwater habitats (Wu & Hahn, 2006). Jezbera and colleagues, however, recognized that subcluster PnecC represents an ecologically heterogeneous taxon not resolvable by methods targeting 16S rRNA sequences (Jezbera *et al.*, 2011). They could characterize a couple of subgroups within subcluster PnecC by using signature sequences contained in the 16S-23S ITS sequences of the strains and demonstrated differences in ecological preferences among the subgroups by using a cultivation-independent detection method. A major ecological difference between some groups was the preferences for either acidic or alkaline habitats. Strain QLW-P1FAT50C-4^T belongs to subgroup F4 characterized by the signature sequence 5'-ACCATCAGCAGCAGTGATA-3' (Jezbera *et al.*, 2011). In a cultivation-independent survey covering a large area located in Central Europe (Austria and Czech Republic), this subgroup was only detected in acidic (pH 4 – 6.5) ponds located at altitudes > 1000 m in the Austrian Alps (Jezbera *et al.*, 2011), however, strains sharing the diagnostic sequence of group F4 were also isolated from habitats in Denmark and Finland (Hahn *et al.*, 2015; Jezbera *et al.*, 2011). The distribution of subgroup F4 was found to be negatively correlated with pH and conductivity of freshwater habitats, and positively with a proxy for the concentration of dissolved humic matter (Jezbera *et al.*, 2011). Currently, it is unknown if subgroup F4 consists only of a single or multiple species. A preference of strain QLW-P1FAT50C-4^T for acidic habitats is also suggested by the presence of genes putatively encoding a Fe(II) transporter (Table 4) and the absence of genes encoding Fe(III) transporters (Hahn *et al.*, 2016b).

Proposal of the new species *Polynucleobacter wuianus* sp. nov.

We tested if strain QLW-P1FAT50C-4^T has to be considered to be affiliated with one of the four previously described free-living species affiliated with subcluster PnecC by performing average nucleotide identity (ANI) analyses with whole genome sequences by using the IMG/ER system (Markowitz *et al.*, 2012). This resulted in all pairwise comparisons in ANI values of 77.3-79.7% (Fig. 1A), which suggests that the strain is not affiliated with any of these four species (Kim *et al.*, 2014; Konstantinidis & Tiedje, 2005; Konstantinidis & Tiedje, 2007; Konstantinidis *et al.*, 2006; Rosselló-Móra & Amann, 2015). A comparison of strain QLW-P1FAT50C-4^T with the type material of the species *P. necessarius* cannot be performed due to the lack of a genome sequence or pure genomic DNA of the endosymbionts, however, previous investigations suggested that the genome sequence of the endosymbiont *P. necessarius* STIR1 has to be considered to share a highly similar genome sequence with the endosymbiont described as *P. necessarius* (Hahn *et al.*, 2016a). An ANI comparison of the genomes of QLW-P1FAT50C-4^T and STIR1 resulted in an ANI value of 79.6%, which is very similar to those values obtained for the four free-living type strains (Fig. 1A). Thus, strain QLW-P1FAT50C-4^T has to be considered to represent a new species affiliated with subcluster PnecC of the genus *Polynucleobacter*.

Strain QLW-P1FAT50C-4^T can be discriminated from the type strains of *Polynucleobacter* species not affiliated with subcluster PnecC by chemotaxonomic traits. As for other species affiliated with subcluster PnecC, strain QLW-P1FAT50C-4^T can be discriminated from the type strains of *P. rarus* (Hahn *et al.*, 2011a), *P. acidiphobus* (Hahn *et al.*, 2011b) and *P.*

difficilis (Hahn *et al.*, 2012a) based on the G+C content of their DNA (Hahn *et al.*, 2016a). The discrimination of strain QLW-P1FAT50C-4^T from *P. cosmopolitanus* is possible by the absence of the fatty acid C_{12:0}3-OH (Hahn *et al.*, 2010), which was so far only found in *Polynucleobacter* strains affiliated with the species *P. cosmopolitanus*. Furthermore, strain QLW-P1FAT50C-4^T, like all other PnecC strains, contains the signature sequence 5'-GAGCCGGTGTTCCTCC-3' at *Escherichia coli* position 445–463 of the 16S rRNA, which is absent in *Polynucleobacter* strains not affiliated with this subcluster (Hahn *et al.*, 2016a). A feature distinguishing strain QLW-P1FAT50C-4^T from previously described type strains of species affiliated with subcluster PnecC is the assimilation of L-alanine (Table 1). Only this strain showed a substantial assimilation of this amino acid while the other type strains showed no or only a weak assimilation.

Description of *Polynucleobacter wuianus* sp. nov.

Polynucleobacter wuianus (wu.i.a' nus. N.L. masc. adj. wuianus, of Wu, named after Qinglong L. Wu, Chinese microbiologist and microbial ecologist, who isolated the type strain and strongly contributed to research on diversity of *Polynucleobacter* and other freshwater bacteria).

Contains free-living *Polynucleobacter* strains dwelling in the water column of acidic or circum-neutral freshwater systems. Never found in alkaline waters (Jezbera *et al.*, 2011). Cells are short sometimes slightly curved rods, 0.5–1.7 µm in length and 0.3–0.6 µm in width, depending on cultivation conditions. Chemo-organotrophic, aerobic, anaerobic growth was not observed. The type strain encodes a gene cluster for anoxygenic photosynthesis but pigmentation of cells or colonies indicating expression of a photosynthesis system was not observed so far. Does not encode genes for synthesis of flagella. Colonies grown on NSY agar are non-pigmented, circular and convex with smooth surface. Growth occurs up to 34 °C. Growth occurs in 0–0.5% (w/v) NaCl. Assimilates acetate, propionate, pyruvate, oxaloacetate, malate, fumarate, succinate, L-glutamate, L-aspartate, L-histidine, and L-alanine. Does not assimilate glycolate, oxalate, citrate, D-mannose, D-glucose, D-galactose, D-lyxose, L-fucose, D-sorbitol, L-leucine, L-serine, or betaine. Major cellular fatty acids are C_{16:1} ω7c and C_{18:1} ω7c. The type strain is QLW-P1FAT50C-4^T (=DSM 24008^T = CIP 111100^T), which was isolated from acidic Pond-1 located in the Austrian Alps. The genome of the type strain is characterized by a size of 2.23 Mbp and a G+C content of 44.9 mol%. Genome and 16S rRNA gene sequences characterizing the type strain are available under the accession numbers CP015922 and AJ879778, respectively.

Acknowledgements

We thank Gabriele Pötter for carrying out the fatty acid measurements. This study was supported by the Austrian Science Fund (FWF) project I482-B09 (Ecological diversification in *Polynucleobacter*), and the European Science Foundation (ESF) project FREDI.

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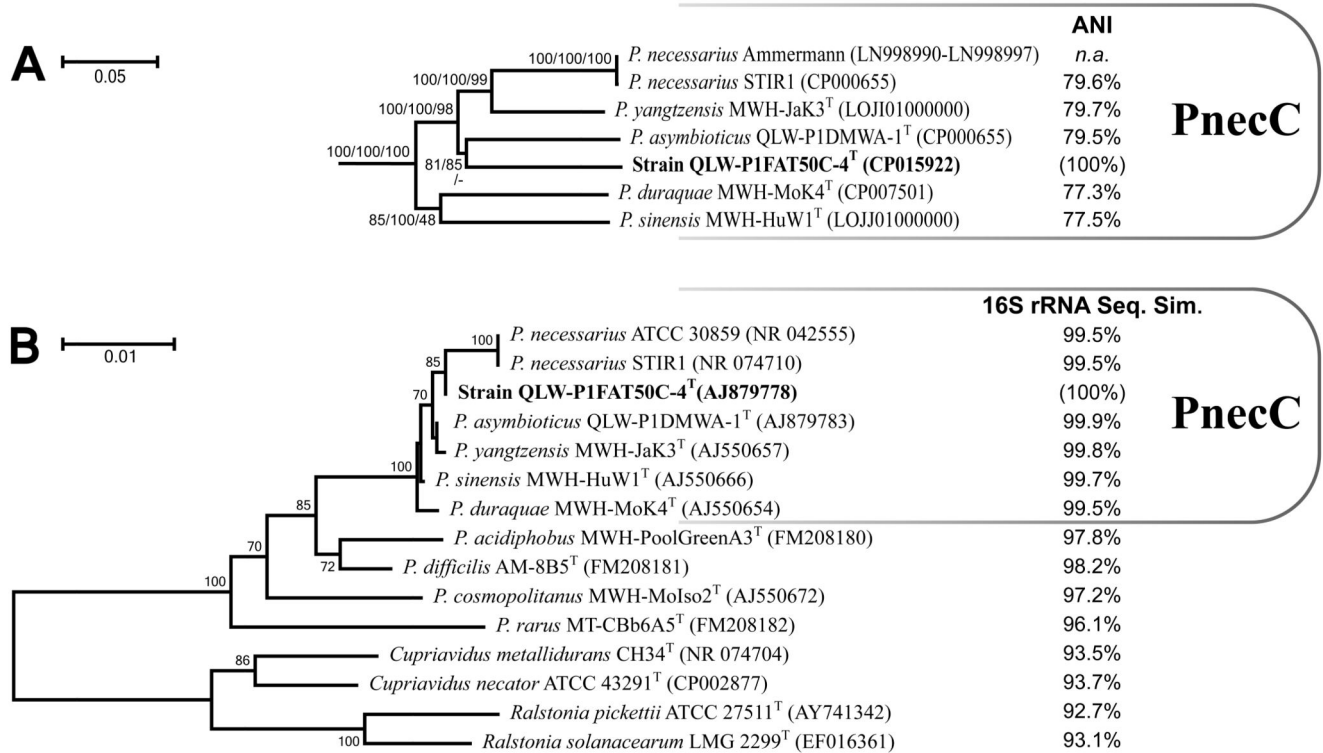


Fig. 1. Phylogenetic position of strain QLW-P1FAT50C-4^T.

A, neighbour-joining (NJ) tree calculated with concatenated multilocus sequences of eight protein-encoding genes. The tree was rooted with sequences of two *Cupriavidus* strains (not shown). Bootstrap values are shown from left to right for NJ, maximum likelihood, and maximum parsimony trees calculated with the same sequence set. Percent values behind the strain names indicate ANI values obtained in comparison with strain QLW-P1FAT50C-4^T.

B, NJ tree calculated with 16S rRNA gene sequences. Percent values behind the strain names indicate 16S rRNA sequence similarity values obtained in comparison with strain QLW-P1FAT50C-4^T.

Table 1

Traits characterizing the five investigated *Polynucleobacter* taxa affiliated with subcluster PnecC. All type strains have the following characteristics in common. In motility tests negative; catalase oxidase positive; assimilation of acetate and pyruvate; weak assimilation of D-galacturonic acid; no assimilation of glycolate, oxalate, citrate and L-serine. n.d., not determined; w, weak reaction.

	<i>P. wuianus</i> sp. nov. QLW-P1FAT50C-4 ^T (DSM 24008 ^T)	<i>P. asymbioticus</i> QLW-P1DMWA-1 ^T (DSM 18221 ^T)	<i>P. duraquae</i> MWH-MoK4 ^T (DSM 21495 ^T)	<i>P. sinensis</i> MWH-HuW1 ^T (DSM 21492 ^T)	<i>P. yangtzensis</i> MWH-JaK3 ^T (DSM 21493 ^T)
Cell morphology	short rods	short rods	curved rods	short curved rods	short rods
Cell length (µm)	0.6-1.7	0.7-1.2	0.9-2.9	0.6-1.4	0.5-1.5
Cell width (µm)	0.3-0.6	0.4-0.5	0.4-0.5	0.4-0.5	0.3-0.5
Temperature range of growth (°C)	5 - 34	5 - 34(w)	5 - 30	5 - 35	5 - 35
NaCl tolerance (%NaCl, w/v)	0 - 0.5	0 - 0.5(w)	0 - 0.3	0 - 0.5	0 - 0.3(w)
Anaerobic growth	-	+	-	-	+
Assimilation of:					
Glyoxylic acid	w	w	-	-	-
Propionic acid	▨	+	-	+	+
Malonic acid	w	w	-	+	w
Oxaloacetic acid	▨	-	+	+	+
Malic acid	▨	+	w	+	+
Fumaric acid	▨	+	w	+	+
Succinic acid	▨	+	+	+	+
Levulinic acid	w	w	-	-	-
D-Mannose	▨	w	-	-	-
D-Glucose	▨	w	w	-	-
D-Galactose	▨	w	-	-	-
D-Lyxose	▨	w	w	-	w
D-Fructose	w	w	w	-	w
L-Fucose	▨	w	-	-	w
D-Sorbitole	▨	w	-	-	-
L-Glutamate	▨	+	-	+	-
L-Histidine	▨	n.d.	n.d.	n.d.	n.d.
L-Aspartate	▨	+	-	-	-
L-Cysteine	w	+	+	w	+
L-Alanine	▨	w	-	-	-
L-Asparagine	w	w	-	-	-
L-Leucine	▨	n.d.	n.d.	n.d.	n.d.
Betaine	-	w	-	-	-

Table 2

Major fatty acid compositions of the *Polynucleobacter* strains representing subcluster PnecC. Strains were grown on R2A agar. Data for strains QLW-PIDMWA-1^T, MWH-HuW1^T, MWH-JaK3^T were taken from (Hahn *et al.*, 2009) and data for MWH-Mok4^T from Hahn *et al.* (2016b).

Fatty acid	QLW-PIEAT50C-4 ^T (DSM 24008 ^T)	QLW-PIDMWA-1 ^T (DSM 18221 ^T)	MWH-Mok4 ^T (DSM 21495 ^T)	MWH-HuW1 ^T (DSM 21492 ^T)	MWH-JaK3 ^T (DSM 21493 ^T)
C _{12:0}	3.3	3.4	3.8	5.5	3.7
C _{14:0}	0.3	0.9	0.3	0.3	1.2
C _{15:0}	0.2	0.3	-	0.3	-
C _{16:0}	18.6	22.2	15.9	29.6	15.5
C _{17:0}	-	-	-	0.5	-
C _{18:0}	0.5	1.2	0.5	2.4	0.5
C _{20:0}	-	1.1	-	-	-
C _{14:1} ω5c	-	-	-	0.2	0.6
C _{15:1} ω6c	-	-	-	0.6	-
C _{16:1} ω5c	0.4	0.9	0.4	-	0.4
C _{16:1} ω7c	40.8	41.3	38.6	45.0	35.6
C _{18:1} ω9c	-	-	0.3	0.4	-
C _{18:1} ω7c	20.4	12.9	19.8	1.1	20.4
11-methyl C _{18:1} ω7c	4.6	3.1	4.2	1.1	8.1
C _{12:0} 2-OH	0.3	2.5	1.3	1.3	2.2
C _{16:0} 2-OH	1.5	-	1.8	-	-
Feature 1	-	0.4	-	1.0	0.5
Feature 2	7.1	9.6	11.9	9.9	9.2
Feature 7	1.2	0.4	-	0.3	2.0

Summed features represent groups of two fatty acids which could not be separated by GLC and the MIDI system, such as summed feature 2 containing C_{16:1} isoI and C_{14:0}-3OH and summed feature 7 containing C_{19:1} ω6c and an unknown compound with an ECL of 18.846, or represent compounds with uncertain identity such as summed feature 1 probably containing C_{12:0} ALDE?.

Table 3

Genome characteristics of the investigated *Polynucleobacter* strains.

Strain	Life style	Genome size (Mbp)	Scaffolds	G+C content (mol%)	DDBJ/EMBL/GenBank accession number	IMG Genome ID	Reference
QLW-PIFAT50C-4 ^T (=DSM 24008 ^T)	free-living	2.23	1	44.9	CP015922	2596583503	This study
QLW-PIDMWA-1 ^T (=DSM 18221 ^T)	free-living	2.16	1	44.8	CP000655	640427129	Meinke <i>et al.</i> , 2012
MWH-MoK4 ^T (=DSM 21495 ^T)	free-living	2.03	1	45.2	CP007501	2505313000	Hahn <i>et al.</i> , 2016b
MWH-HuW1 ^T (=DSM 21492 ^T)	free-living	2.32	19	45.5	LOJ1000000000	2630969031	Hahn <i>et al.</i> , 2016a
MWH-JaK3 ^T (=DSM 21493 ^T)	free-living	2.05	42	45.4	LOJ1000000000	2608642177	Hahn <i>et al.</i> , 2016as
STIR1 (<i>Euplotes aediculatus</i>)	endosymbiont	1.56	1	45.6	CP001010	2503982034	Boscaro <i>et al.</i> , 2013

Table 4

Comparison of the presence and absence of selected genes.

Genes putatively encoding	<i>P. wuianus</i> sp. nov.	<i>P. asymbioticus</i>	<i>P. duraquae</i>	<i>P. sinensis</i>	<i>P. yangtzensis</i>
	QLW-PIEAT50C-4 ^T	QLW-PIDMWA-1 ^T	MWH-MoK4 ^T	MWH-HuW1 ^T	MWH-JaK3 ^T
Inorganic nutrients					
ABC-type Fe ³⁺ transport system	█	█	█	█	█
feoAB genes (uptake of Fe ²⁺)	█	█	█	█	█
ABC-type Nitrate/Nitrite/Cyanate transporter	█	█	█	█	█
Nitrate reductase (assimilatory)	█	█	█	█	█
Nitrite reductase (assimilatory)	█	█	█	█	█
Cyanate lyase (releases NH ₃ and CO ₂ from cyanate)	█	█	█	█	█
Urease and ABC-type urease transporter	█	█	█	█	█
Oxidative phosphorylation/Energy metabolism					
Cytochrome bd-I terminal oxidase (CydAB)	█	█	█	█	█
Fumarate reductase	█	█	█	█	█
Carbon monoxide dehydrogenase	█	█	2 clusters	█	█
Acetate permease actP	█	█	█	█	█
Anoxygenic photosynthesis					
Photosynthesis gene cluster	█	█	█	█	█
Motility					
Flagella genes	█	█	█	█	█
Oxidative stress					
Catalase	█	2 genes	█	█	1 gene
Other					
Cellulose synthase operon protein C	█	█	█	█	█
Cellulose synthase catalytic subunit [UDP-forming]	█	█	█	█	█