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Polynucleobacter acidiphobus sp. nov., a representative of an abundant group of planktonic freshwater bacteria

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The heterotrophic, aerobic, facultatively anaerobic, catalase- and oxidase-positive, non-motile strain MWH-PoolGreenA3^T, isolated from a rock pool filled with freshwater, was characterized. The strain grew on NSY medium over a NaCl range of 0.0–0.3 % (w/v). Whole-cell fatty acids were dominated by $C_{16:1}\omega7c$ (feature 3), $C_{18:1}\omega7c$ and straight-chain $C_{16:0}$; furthermore, the components $C_{12:0}$ and $C_{14:0}$ 2-OH were present. The DNA G+C content was 48.3 mol%. Phylogenetic analysis as well as strong similarities in phenotypic and chemotaxonomic traits indicated the affiliation with the genus *Polynucleobacter*. 16S rRNA gene similarity values with the three described species of the genus *Polynucleobacter* ranged from 96.7 to 97.8 %. DNA–DNA hybridization experiments did not reveal that the strain belongs to a previously described species of the genus *Polynucleobacter*. The strain can be discriminated from previously established species of the genus *Polynucleobacter* by chemotaxonomic and phenotypic traits. The bacterium possesses a free-living lifestyle and represents a group of planktonic freshwater bacteria occurring with high cell numbers in many freshwater lakes. Based on the phylogeny revealed and the chemotaxonomic and phenotypic differences from previously described species of the genus *Polynucleobacter*, we propose to establish the novel species *Polynucleobacter acidiphobus* sp. nov. with the type strain MWH-PoolGreenA3^T (=DSM 21994^T =CIP 110079^T).

K. Heckmann and H.-J. Schmidt described the genus *Polynucleobacter* to accommodate bacteria thriving as obligate endosymbionts in cells of several species of freshwater ciliates belonging to the genus *Euplotes* (*Hypotrichia*), and the species *Polynucleobacter necessarius* for obligate endosymbionts of *Euplotes aediculatus* (Heckmann & Schmidt, 1987). In 2003, strains closely related to endosymbiotic *P. necessarius* were isolated from several freshwater habitats (Hahn, 2003), and recently it was concluded that these strains represent obligately free-living organisms, which strongly contrasts with the obligately endosymbiotic *P. necessarius* investigated previously (Vannini *et al.*, 2007). Consequently, the description of the genus *Polynucleobacter* and the species *P. necessarius* was emended by adding descriptions of free-living strains (Hahn *et al.*, 2009) and separation of the two groups of organisms differing in lifestyle in the two subspecies *P. necessarius* subsp. *necessarius* (for endosymbionts of *E. aediculatus* and *Euplotes harpa*) and *P. necessarius* subsp. *asymbioticus* (for obligately free-living strains) (Hahn *et al.*, 2009). Other cultivated *Polynucleobacter* strains (Hahn, 2003; Wu & Hahn, 2006a) were more distantly related to *P. necessarius*; therefore, strains and environmental 16S rRNA gene sequences affiliated with the monophyletic *Polynucleobacter* lineage were sorted in operational taxonomic units called subclusters A (PnecA), B1 (PnecB1), B2 (PnecB2), C (PnecC) and D (PnecD) (Hahn, 2003; Wu & Hahn, 2006a). All members of PnecC and PnecD were preliminarily assigned to the species *P. necessarius* (Hahn *et al.*, 2009) and *Polynucleobacter cosmopolitanus* (Hahn *et al.*, 2010), respectively, and a single isolate affiliated with the phylogenetically diverse subcluster PnecA was described as *Polynucleobacter rarus* (Hahn *et al.*, 2011). While many strains affiliated with subclusters PnecC and PnecD could be cultivated (Hahn, 2003; Watanabe *et al.*, 2009), only a single strain affiliated with subcluster PnecB2 that is suitable for taxonomic characterization could be obtained so far. In this paper, we characterize this isolate, strain MWH-PoolGreenA3^T, and propose to establish for this strain the species *Polynucleobacter acidiphobus* sp. nov.

Abbreviations: FISH, fluorescent in situ hybridization; OD, optical density.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MWH-PoolGreenA3^T is FM208180.

A supplementary figure is available with the online version of this paper.

Isolation and characterization

Strain MWH-PoolGreenA3^T was isolated from a rock pool located in a streambed of a mountain brook in Corsica, France (geographical coordinates, 42° 10′ 21.05″ N 8° 53′ 54.54″ E). This rock pool represented a depression in the bedrock formed by the brook. At the time of sampling, the rock pool was isolated from the running water in the brook; however, brittle branches representing remains from a previous high-water event laying around the rock pool indicated that the pool was in contact with the brook at such an event. The brook receives water from the upstream-located Lake Creno. It is possible that the population represented by strain MWH-PoolGreenA3^T originates from this lake. The rock pool had a surface area of a few square metres and a depth of 20–30 cm. A heavy algal bloom resulted in a green water colour and made it impossible to see the bottom of the rock pool. The water temperature was 32 °C and the conductivity was 65 μS cm^{-1}.

Strain MWH-PoolGreenA3^T was isolated and cultivated by using the filtration acclimatization method and NSY medium (Hahn *et al.*, 2004). The strain can be maintained on NSY or R2A (Remel) medium with concentrations of 3 g l^{-1}; however, similar to all previously investigated *Polynucleobacter* strains, the biomass yield on these media was much lower than that observed for other members of the family *Burkholderiaceae*. Growth at different temperatures and growth under anoxic conditions in an anaerobic chamber were examined on NSY agar or on NSY medium supplemented with nitrate (0.8 mM). NaCl tolerance was determined using NSY agar supplemented with different NaCl concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0, 1.25, 1.5, 1.75 and 2.0 %, w/v). The temperature range supporting growth was tested on standard NSY agar plates exposed to different temperatures (5, 15, 20, 25, 30 and 35 °C). Utilization of various substrates was investigated in the same way as for previously described species of the genus *Polynucleobacter* (Hahn *et al.*, 2009; Hahn *et al.*, 2010, Hahn *et al.*, 2011). Briefly, growth enabled by utilization of a specific substrate was determined by comparison of optical density (OD) established in liquid one-tenth-strength NSY medium (0.3 g l^{-1}) with and without 0.5 g test substance l^{-1}. OD differences of <10 %, of 10–50 % and of >50 % of the OD established in the medium without test substance were scored after 10 days of growth as no utilization (−), weak utilization (w) and good utilization (+), respectively. Assimilation of urea and thiosulfate was tested in mineral medium IBM (Hahn *et al.*, 2004) lacking nitrogen or sulphur sources, respectively, supplemented with acetate (0.5 g l^{-1}) as sole carbon and energy source and urea (60 mg l^{-1}) or thiosulfate (67 mg l^{-1}), respectively.

Sequencing and phylogenetic analyses of 16S rRNA genes were performed as described previously (Hahn, 2003; Hahn *et al.*, 2005). Sequence similarity values were determined by using the software EzTaxon (Chun *et al.*, 2007). Neighbour-joining trees were calculated by using the software MEGA4 (Tamura *et al.*, 2007). The G+C content of DNA was determined as described by Tóth *et al.* (2008). Fatty acid methyl esters (FAMEs) were obtained as described by Kämpfer & Kroppenstedt (1996) and separated by a gas chromatograph (model 6890, Hewlett Packard). Peaks were automatically computed using the Microbial Identification standard software package (Sasser, 1990).

The results of the phenotypic and chemotaxonomic characterization of strain MWH-PoolGreenA3^T are presented in Tables 1 and 2. The strain differed from strains of *P. necessarius* subsp. *asymbioticus*, *P. rarus* and *P. cosmopolitanus* in its inability to utilize fumarate and galacturonic acid (Table 3). The G+C content of the DNA of strain MWH-PoolGreenA3^T was 48.3 mol%, which is substantially higher than those of *P. necessarius* strains (44–46 mol%), the *P. cosmopolitanus* type strain (44.9 mol%) and the *P. rarus* type strain (40.3 mol%). Whole-cell fatty acids of strain MWH-PoolGreenA3^T were dominated by the unsaturated components $C_{16:1}\omega7c$ (feature 3) and $C_{18:1}\omega7c$, and straight-chain $C_{16:0}$. As in *P. necessarius* subsp. *asymbioticus* and in *P. rarus*, considerable amounts of $C_{12:0}$ were detected which are lacking in *P. cosmopolitanus* (Table 2). Characteristic for MWH-PoolGreenA3^T in comparison to the other species of the genus *Polynucleobacter* were the absence of 11-methyl $C_{18:1}\omega7c$ and the presence of $C_{17:0}$ cyclo and $C_{14:0}$ 2-OH. The latter two components were not found in the other species of the genus *Polynucleobacter* but $C_{14:0}$ 2-OH was detected in several species of the related genus *Cupriavidus* (Fig. 1), namely *C. campinensis*, *C. necator* (earlier known as *Ralstonia eutropha*), *C. oxalaticus* (Goris *et al.*, 2001), *C. respiraculi* and *C. pampae* (Cuadrado *et al.*, 2010). Since every species of the genus *Polynucleobacter* contains a particular hydroxylated fatty acid, so far the content of hydroxylated fatty acids has turned out to be an effective measure to delineate the different species from each other.

At least some *in situ* grown cells of strain MWH-PoolGreenA3^T can pass through membrane filters with pore sizes of 0.2 μm. This trait is shared with several strains affiliated with *P. necessarius* subsp. *asymbioticus* and *P. cosmopolitanus*, as well as with strains affiliated with other taxa (Hahn, 2004).

Phylogeny

The phylogenetic analysis of the almost complete 16S rRNA gene sequence of strain MWH-PoolGreenA3^T demonstrated clustering within the genus *Polynucleobacter*, and indicated a close relationship with the three previously described species of this genus (Fig. 1). A more detailed phylogenetic analysis including the 16S rRNA gene and 16S–23S ITS sequences of strain MWH-PoolGreenA3^T was published previously (Hahn *et al.*, 2010). A further phylogenetic analysis (Supplementary Fig. S1, available in IJSEM Online) including environmental sequences revealed

Table 1. Traits characterizing strain MWH-PoolGreenA3^T and strains of the previously described taxa *P. necessarius* subsp. *asymbioticus*, *P. cosmopolitanus* and *P. rarus*

Taxa: 1, *P. acidiphobus* sp. nov. MWH-PoolGreenA3^T; 2, *P. necessarius* subsp. *asymbioticus* (n=4; data from Hahn *et al.*, 2009); 3, *P. cosmopolitanus* (n=5; Hahn *et al.*, 2010); 4, *P. rarus* MT-CBb6A5^T (Hahn *et al.*, 2011). Note that sufficient phenotypic and chemotaxonomic characterizations are lacking for the obligately endosymbiotic strains of the subspecies *P. necessarius* subsp. *necessarius*. All taxa are non-motile, positive for catalase and oxidase activities, grow anaerobically on NSY medium + 0.8 mM nitrate, assimilate pyruvic acid and do not assimilate L-serine. –, Negative; +, positive; w, weakly positive; +/–, some strains positive and some strains negative; +/w, some strains positive and some strains weakly positive; w/–, some strains weakly positive and some strains negative.

Characteristic	1	2	3	4
Cell morphology	Short curved rods	Straight or curved rods	Curved rods	Straight rods
Nucleoids visible (DAPI)	Rarely	Rarely	Rarely	Frequently
Cell length (μm)	0.5–1.4	0.5–2.9	0.4–1.4	0.8–1.8
Cell width (μm)	0.4–0.5	0.3–0.5	0.3–0.5	0.6–0.8
Growth at 5 °C	–	+	+/–	–
Growth at 35 °C	+	+/–	+	–
NaCl tolerance (%, w/v)*	0.3	0.3–0.5	0.3–0.5	0.3(w)
Anaerobic growth on NSY medium	+	+/–	+/–	–
Growth in mineral medium with acetic acid and B12	w	w/–	w/–	w
Assimilation of:				
Urea	–	+/–	+/–	–
Thiosulfate	–	+/–	+/–	–
Formic acid	–	w/–	–	–
Glyoxylic acid	–	w/–	w/–	+
Glycolic acid	–	–	–	w
Acetic acid	w	+	+	+
Oxalic acid	–	–	–	w
Propionic acid	–	+/–	+/w	–
Malonic acid	w	+/–	+/–	–
Oxaloacetic acid	+	+/–	+	–
Malic acid	w	+/w	+	+
Succinic acid	w	+	+	+
Fumaric acid	–	+/w	+	+
Levulinic acid	–	w/–	w/–	+
Citric acid	–	–	+/–	–
D-Mannose	–	w/–	w/–	w
D-Glucose	w	w/–	w/–	–
D-Galacturonic acid	–	w	+/w	+
D-Galactose	–	w/–	w	–
D-Lyxose	–	w/–	–	w
D-Fructose	–	w/–	–	w
D-Fucose	–	w/–	–	w
D-Sorbitol	–	w/–	–	–
L-Glutamate	–	+/–	w/–	–
L-Aspartate	–	+/–	–	–
L-Cysteine	+	+/w	+	w
L-Alanine	–	w/–	+/w	–
L-Asparagine	–	w/–	–	–
Betaine	–	w/–	–	–
DNA G+C content (mol%)	48.3	44–46	44.9	40.3

*Highest NaCl concentration (added to NSY medium) at which growth was observed.

the affiliation of strain MWH-PoolGreenA3^T with subcluster PnecB2 (Wu & Hahn, 2006a). This subcluster contains numerous environmental sequences retrieved from freshwater habitats located in Europe, Asia and North and Central America. Subcluster PnecB2 is characterized by an intracluster minimum 16S rRNA gene sequence similarity of 98.4 %, and the minimum similarity of 16S rRNA gene sequences between subclusters PnecB1 and PnecB2 is 97.8 %.

Table 2. Whole-cell fatty acid composition of strain MWH-Pool-GreenA3^T as compared with strains of *P. rarus*, *P. cosmopolitanus* and *P. necessarius* subsp. *asymbioticus*

Taxa: 1, *Polynucleobacter acidiphobus* sp. nov. MWH-Pool-GreenA3^T; 2, *P. rarus* MT-CBb6A5^T (data from Hahn *et al.*, 2011); 3, *P. cosmopolitanus* MWH-MoIso2^T (Hahn *et al.*, 2010); 4, *P. cosmopolitanus* (n=5; Hahn *et al.*, 2010); 5, *P. necessarius* subsp. *asymbioticus* QLW-P1DMWA-1^T (Hahn *et al.*, 2009); 6, *P. necessarius* (n=3; Hahn *et al.*, 2009). Values are percentages of the summed fatty acids named in the peak library of the MIDI system (listed are contents >0.2 %). Strains were grown on R2A agar plates for 3–5 days at 28 °C.

Fatty acid	1	2	3	4	5	6
Saturated						
$C_{12:0}$	3.8	3.0	–	–	3.4	3.4–5.5
$C_{14:0}$	0.9	0.4	0.7	0.6–2.3	0.9	0.3–1.2
$C_{15:0}$	–	–	–	–	0.3	–
$C_{16:0}$	24.2	19.8	15.4	11.0–15.4	22.2	15.5–33.5
$C_{17:0}$	–	0.3	–	–	–	0–0.5
$C_{18:0}$	0.5	0.5	0.8	0.5–1.1	1.2	0.5–2.6
Unsaturated						
$C_{14:1}\omega5c$	–	–	0.6	0–0.6	–	0–0.6
$C_{15:1}\omega8c$	–	–	–	–	–	–
$C_{15:1}\omega6c$	–	0.3	–	–	–	0–0.6
$C_{16:1}\omega5c$	0.4	0.5	0.3	0.3–1.1	0.9	0–0.9
$C_{17:1}\omega6c$	–	–	0.5	0–0.7	–	–
$C_{18:1}\omega9c$	–	–	0.3	0–2.0	–	0–0.4
$C_{18:1}\omega7c$	17.3	21.8	28.7	28.7–38.1	12.9	0.3–20.4
11-Methyl $C_{18:1}\omega7c$	–	7.5	3.7	0.4–3.7	3.1	0.2–8.1
10-Methyl $C_{19:0}$	–	0.4	0.7	0–0.7	–	–
Hydroxylated						
$C_{12:0}$ 2-OH	–	–	–	–	2.5	1.3–2.5
$C_{12:0}$ 3-OH	–	–	11.1	7.1–11.2	–	–
$C_{14:0}$ 2-OH	2.9	–	–	–	–	–
$C_{16:0}$ 3-OH	0.5	0.3	–	–	–	–
Cyclic						
$C_{17:0}$ cyclo	3.0	–	–	–	–	–
Summed features*						
1 ($C_{12:0}$ ALDE?)	–	1.8	–	–	0.4	0.4–2.1
2 (including $C_{14:0}$ 3-OH)	10.8	6.9	0.6	0.6–3.9	9.6	8.4–9.9
3 (including $C_{16:1}\omega7c$)	32.1	35.9	34.7	31.5–36.5	41.3	35.6–45.0
7 (including $C_{19:1}\omega6c$)	1.7	–	1.5	0–1.5	0.4	0.2–2.0

Table 3. Discriminative traits separating strain MWH-PoolGreenA3^T from the previously described species and subspecies of the genus *Polynucleobacter*

Taxa: 1, *Polynucleobacter acidiphobus* sp. nov. MWH-PoolGreenA3^T; 2, *P. necessarius* subsp. *asymbioticus*, *P. cosmopolitanus* and *P. rarus* (data from Hahn *et al.*, 2009; Hahn *et al.*, 2010; Hahn *et al.*, 2011). Note that a phenotypic characterization of the obligately endosymbiotic *P. necessarius* subsp. *necessarius* is lacking. FA, fatty acids.

Characteristic	1	2
Utilization of fumarate	–	+
Utilization of D-galacturonic acid	–	+
$C_{17:0}$ cyclo (% of summed FA)	3.0	Not detected
$C_{14:0}$ 2-OH (% of summed FA)	2.9	Not detected
DNA G+C content (mol%)	48.3	<46

Genotypic traits

Analysis of a collection of >300 publicly available 16S rRNA gene sequences representing cultured and uncultured strains affiliated with all four described subclusters (Hahn, 2003) revealed that members of subcluster PnecB2 are characterized by the presence of a combination of two diagnostic oligonucleotide sequences in the 16S rRNA gene. Sequence 5′-AGGTAAAAGCTCACCAAGGCGAT-3′ (*Escherichia coli* positions 258–280) is unique among *Polynucleobacter* PnecB2 strains; however, BLAST searches revealed that this sequence is present in some other members of the phylum *Proteobacteria* not affiliated with the genus *Polynucleobacter*. The second sequence, 5′-GGGAAGAAACASCRGCTC-3′ (*E. coli* positions 445–463), is present in almost all species of the genus *Polynucleobacter* (Hahn *et al.*, 2005) except three endosymbiotic strains, four environmental sequences and a single cultured strain. However, the combination of the two diagnostic sequences is exclusively found in the 16S rRNA sequences of strains affiliated with subcluster PnecB2.

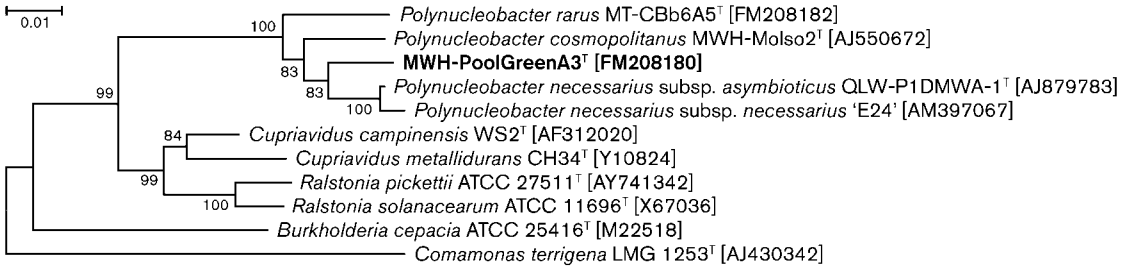

Fig. 1. Neighbour-joining tree based on almost complete 16S rRNA gene sequences, reconstructing the phylogenetic position of strain MWH-PoolGreenA3^T. Bar, 0.01 substitutions per nucleotide position. Note that previous analysis of the phylogeny of the genus *Polynucleobacter* based on 16S rRNA gene sequences and 16S–23S ITS sequences by the neighbour-joining and maximum-likelihood methods (Hahn *et al.*, 2010) confirmed the phylogenetic position of strain MWH-PoolGreenA3^T in the *Polynucleobacter* clade.

DNA–DNA reassociation experiments

The sequence similarities between 16S rRNA genes of strain MWH-PoolGreenA3^T and the type strain of *P. necessarius* subsp. *asymbioticus*, a sequence representing the endosymbiotic *P. necessarius* subsp. *necessarius* 'E24', the type strain of *P. cosmopolitanus* and the type strain of *P. rarus* were 97.8 %, 97.5 %, 97.3 % and 96.7 %, respectively. DNA–DNA reassociation experiments were performed in order to reveal if the new strain belonged to one of the two previously described species of the genus *Polynucleobacter* with type strains sharing >97 % 16S rRNA gene sequence similarity with strain MWH-PoolGreenA3^T (Stackebrandt & Goebel, 1994). Note that experiments with DNA of a representative of *P. necessarius* subsp. *necessarius* could not be performed due to the lack of pure cultures (Hahn *et al.*, 2009; Vannini *et al.*, 2007). Duplicated reassociation experiments with the DNA of the type strain of *P. necessarius* subsp. *asymbioticus* resulted in DNA–DNA similarity values of 37.3 % and 31.3 %, and duplicated experiments with the DNA of the type strain of *P. cosmopolitanus* in similarity values of 19.0 % and 17.3 %. These results indicate that the strain does not belong to one of the previously described species of the genus *Polynucleobacter* when the recommendation by the ad hoc committee of a threshold value of 70 % DNA–DNA similarity for delineation of prokaryotic species is considered (Wayne *et al.*, 1987).

Proposal of a novel species of the genus *Polynucleobacter*

Results from the phylogenetic analysis and chemotaxonomic investigations demonstrated the affiliation of strain MWH-PoolGreenA3^T to the genus *Polynucleobacter* (Tables 1 and 2, Fig. 1) but also revealed pronounced differences between this strain and strains affiliated with the three previously described species of this genus. The DNA–DNA reassociation experiments performed indicated that the novel strain does not belong to the previously described species *P. necessarius* and *P. cosmopolitanus*, and the <97 % 16S rRNA gene sequence similarity between strain MWH-PoolGreenA3^T and the type strain of *P. rarus* clearly indicated that the novel strain also does not belong to the latter species (Stackebrandt & Goebel, 1994). Strain MWH-PoolGreenA3^T can be discriminated from previously described species of the genus *Polynucleobacter* by phenotypic (Table 3) and genotypic traits. Based on these findings, we propose to establish the novel species *Polynucleobacter acidiphobus* sp. nov. with strain MWH-PoolGreenA3^T as the type strain. Furthermore, we propose to preliminarily include all strains phylogenetically affiliated with subcluster PnecB2 of the *Polynucleobacter* lineage (Wu & Hahn, 2006a) in this novel species. This subcluster represents a phylogenetically tight taxon separated from subcluster PnecB1 by sequence dissimilarities of 1.3–2.2 % (Supplementary Fig. S1), and members of this subcluster can be identified by genetic traits (see above). Further taxonomic investigations are necessary to clarify if subcluster PnecB2 (*P. acidiphobus* sp. nov.) and PnecB1 represent the same or distinct species.

Ecology and biogeography of *Polynucleobacter acidiphobus* sp. nov.

In contrast to the majority of free-living, non-pathogenic bacteria currently described as novel species, *Polynucleobacter acidiphobus* sp. nov. represents a taxon for which substantial amounts of environmental data are available. Bacteria sharing identical or almost identical 16S rRNA gene sequences with the type strain were detected in many habitats (Zwart *et al.*, 2002; Donachie *et al.*, 2004; Simpson *et al.*, 2004; Wu & Hahn, 2006a; Wu *et al.*, 2006; Shaw *et al.*, 2008), as well as in experimental systems (Horner-Devine *et al.*, 2003). Additional data (Wu & Hahn, 2006a, b; Salcher *et al.*, 2008; Alonso *et al.*, 2009) on *Polynucleobacter* subcluster PnecB, which is at least partially identical with the species proposed here, were established by using a PnecB-specific fluorescent in situ hybridization (FISH) probe (Wu & Hahn, 2006a). A survey of 65 freshwater lakes and ponds by using FISH detected members of the subcluster in 78 % of the habitats investigated (Wu & Hahn, 2006b). The relative PnecB abundance determined ranged

from 0.4 % to 13.2 % with a mean of 2.6 % of total bacterial numbers. The mean percentage corresponded to a mean abundance of 8×10^4 PnecB bacteria per ml. At two sites of a subtropical lagoon located in Uruguay, which differed in salinity, Alonso *et al.* (2009) observed relative abundances of PnecB bacteria of about 3 % (brackish water) and 5 % (freshwater) of total bacterial numbers. The highest PnecB numbers reported so far were observed in a Tibetan oligo-mesotrophic lake located at an altitude of 4987 m (Wu & Hahn, 2006b; Wu *et al.*, 2006). These investigations by using FISH revealed that PnecB (PnecB1 and/or PnecB2) bacteria possess a planktonic, free-living lifestyle. Importantly, all detections of PnecB bacteria by using FISH reported so far, as well as all sequence-based detections of PnecB2 bacteria reported so far, were restricted to circum-neutral and alkaline fresh and brackish waters (lakes, ponds, large rivers, estuaries). Bacteria affiliated with subclusters PnecB1 or PnecB2 were detected by cultivation independent methods in the estuaries Delaware Bay (NJ, USA) and Chesapeake Bay (MD, USA) (Shaw *et al.*, 2008). For the latter sample, a mild salinity of 3.5 parts per thousand was reported, which is roughly equal to the maximal NaCl concentration tolerated by strain MWH-PoolGreenA3^T in laboratory experiments (Table 1). As mentioned above, investigations by using FISH also detected PnecB bacteria in two parts of a subtropical coastal lagoon (Alonso *et al.*, 2009). Interestingly, no differences in activity (i.e. uptake of labelled substrates) of the two PnecB populations dwelling in water of different salinity (freshwater and brackish water) were observed. No detections have been reported from acidic freshwaters, saline waters of high salinity (marine environments or saline inland waters) or soil systems, which supports the notion that PnecB bacteria represent typical freshwater bacteria (Zwart *et al.*, 2002). Furthermore, indications for an endosymbiotic occurrence of PnecB bacteria are completely lacking. Wu & Hahn (2006b) suggested that PnecB bacteria depend on autochthonous rather than on allochthonous (imported) substrate sources. This is indicated by: (i) their numerous occurrence in large lakes with long water retention times, in which only small fractions of the organic carbon are of terrestrial origin; (ii) their depth distribution, which strongly resembles typical profiles of primary production in lakes; and (iii) the numerous occurrence of PnecB bacteria in mesocosms in which primary producers have been the dominating substrate source of bacteria (Horner-Devine *et al.*, 2003). Note that Salcher *et al.* (2008) did not observe a vertical distribution of PnecB bacteria in Piburger See lake similar to the above-mentioned vertical distribution in Lake Mondsee.

Currently, group-specific FISH probes discriminating between PnecB1 and PnecB2 (*P. acidiphobus* sp. nov.) are not available. Therefore, it is not known if there are group-specific differences in ecological adaptation between these two groups. Further ecological research is required to reveal potential differences.

Strains affiliated with subcluster PnecB2 were detected in habitats located in Europe (Zwart *et al.*, 2002; this study), Asia (Wu & Hahn, 2006a; Wu *et al.*, 2006), North America (Horner-Devine *et al.*, 2003; Simpson *et al.*, 2004; Shaw *et al.*, 2008), Central America (Shaw *et al.*, 2008), on an island (Hawaii) located in the central Pacific (Donachie *et al.*, 2004), and in East Africa (M. W. Hahn, unpublished data). The habitats of detection are located in temperate, subtropical and tropical climatic zones.

Description of *Polynucleobacter acidiphobus* sp. nov.

Polynucleobacter acidiphobus [a.ci.di.pho′bus. N.L. n. *acidum* (from L. adj. *acidus* sour) an acid; Gr. suff. *-phobos* (from Gr. n. *phobos* panic fear) having a horror; N.L. masc. adj. *acidiphobus* acid-hating, referring to the observation that this species was never detected in acidic waters].

Curved, non-motile rods, 0.5–1.4 μm in length and 0.4–0.5 μm in width. Chemo-organotrophic, aerobic and anaerobic growth. Planktonic, free-living lifestyle. Inhabits various freshwater habitats. Can be cultivated on NSY and R2A medium. Colonies grown on NSY agar are unpigmented, circular and convex with smooth surface. Mesophilic; no growth at 5 °C but growth at 35 °C. Grows without NaCl. Maximum NaCl concentration tolerated is 0.3 % (w/v). Oxidase- and catalase-positive. Utilizes acetate, pyruvate, malate, oxaloacetate, malonate, succinate, D-glucose and L-cysteine when these substrates are provided in a medium containing low amounts of NSY. Does not utilize formate, glyoxylate, glycolate, oxalate, propionate, fumarate, levulinate, citrate, D-mannose, D-galacturonic acid, D-galactose, D-lyxose, D-fructose, D-fucose, D-sorbitol, L-glutamate, L-aspartate, L-alanine, L-serine, L-asparagine or betaine. Whole-cell fatty acids are dominated by $C_{16:1}\omega7c$ (feature 3), $C_{18:1}\omega7c$ and straight chain $C_{16:0}$. The components $C_{12:0}$, $C_{14:0}$ 2-OH and $C_{17:0}$ cyclo are present, $C_{12:0}$ 2-OH, $C_{12:0}$ 3-OH and 11-methylated $C_{18:1}\omega7c$ are absent. The DNA G+C content of the type strain is 48.3 mol%. Tentatively, all strains affiliated with the genus *Polynucleobacter* and possessing the oligonucleotide sequence 5′-AGGTAAAAGCTCACCAAGG-CGAT-3′ (*E. coli* positions 258–280) within the 16S rRNA gene shall be assigned to the proposed species.

The type strain is MWH-PoolGreenA3^T (=DSM 21994^T =CIP 110079^T), isolated from a rock pool filled with freshwater located on the Mediterranean island Corsica, France.

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