Polynucleobacter rarus sp. nov., a free-living planktonic bacterium isolated from an acidic lake

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The heterotrophic, aerobic, facultatively anaerobic under denitrifying conditions, catalase- and oxidase-positive, non-motile strain MT-CBb6A5^T, which was isolated from an acidic lake located in Wisconsin (USA), was characterized. The strain grew on NSY medium over a temperature range of 15–30 °C and a NaCl range of 0.0–0.3 % (w/v). The predominant fatty acids were $C_{16:0}$, $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, feature 3 (including $C_{16:1}\omega7c$), and feature 2 (including $C_{14:0}$ 3-OH). The DNA G+C content of the strain was 40.3 mol%. Phylogenetic analysis as well as strong similarities in phenotypic and chemotaxonomic traits indicated the affiliation with the genus *Polynucleobacter*. 16S rRNA gene sequence similarity values with the two described species of the genus *Polynucleobacter* ranged from 95.6 to 96.0 %. The strain differs from the two described species of the genus *Polynucleobacter* in the ability to assimilate oxalic and glycolic acids, and in the presence of the fatty acids $C_{15:1}\omega8c$ and $C_{16:0}$ 3-OH as well as in quantitative differences in fatty acid composition. It has to be assumed that the strain shares with other free-living bacteria of the genus *Polynucleobacter* a planktonic lifestyle in the water column of freshwater habitats. Based on the phylogeny revealed and the chemotaxonomic and phenotypic differences from *Polynucleobacter necessarius* and *Polynucleobacter cosmopolitanus*, we propose to establish the novel species *Polynucleobacter rarus* sp. nov. with the type strain MT-CBb6A5^T (=DSM 21648^T =CIP 109928^T).

K. Heckmann and H.-J. Schmidt described the genus *Polynucleobacter* to accommodate bacteria living as obligate endosymbionts in cells of freshwater ciliates affiliated with the genus *Euplotes*, and the species *Polynucleobacter necessarius* for obligate endosymbionts of *Euplotes aediculatus* (Heckmann & Schmidt, 1987). Recently, a close phylogenetic relationship between such obligate endosymbionts and obligately free-living strains was demonstrated (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). Due to the very pronounced differences in lifestyle of the closely related obligately endosymbiotic and obligately free-living strains, the placement of these organisms 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) was proposed (Hahn *et al.*, 2009). Furthermore, the species *Polynucleobacter cosmopolitanus* representing the second species within the genus *Polynucleobacter* was described recently (Hahn *et al.*, 2010). This species accommodates, so far, exclusively free-living strains. The obligately free-living bacteria affiliated with these two species represent aerobic, chemo-organotrophic, non-motile bacteria. Investigations employing fluorescent in situ hybridization (FISH) probes specific for *P. necessarius* or *P. cosmopolitanus* demonstrated that the free-living strains representing these two taxa possess a planktonic lifestyle and contribute significantly to bacterioplankton in freshwater habitats (Hahn *et al.*, 2005; Wu & Hahn, 2006; Salcher *et al.*, 2008). The highest contributions of *P. necessarius* and *P. cosmopolitanus* reported so far were about 60 % and 8 %, respectively, of total bacterial cell numbers (Hahn *et al.*, 2005, 2010). Numerous studies employing cultivation-independent methods for the exploration of bacterial diversity in freshwater, marine and terrestrial habitats revealed that bacteria of the genus *Polynucleobacter* are present in a broad variety of freshwater habitats (e.g. Hiorns *et al.*, 1997; Crump *et al.*, 1999; Crump & Hobbie, 2005; Zwart *et al.*, 2002; Burkert *et al.*, 2003; Hahn

Abbreviation: OD, optical density.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MT-CBb6A5^T is FM208182.

et al., 2005; Grossart *et al.*, 2008) but reports on their presence at off-shore marine and terrestrial sites are lacking.

In previous studies, a monophyletic cluster of strains (minimal 16S rRNA gene sequence similarity of 95.7 %, Hahn, 2003) including a sequence of an endosymbiotic *P. necessarius* strain (Springer *et al.*, 1996) was described as '*Polynucleobacter necessarius* cluster' (Zwart *et al.*, 2002), which was later subdivided in four monophyletic subclusters designated A to D (Hahn, 2003). The previously emended species *P. necessarius* is equivalent to subcluster C (also known as subcluster PnecC), and the recently described species *P. cosmopolitanus* is equivalent to subcluster D (PnecD). Here we describe a strain affiliated with

Table 1. Traits characterizing strain MT-CBb6A5^T and strains of the previously described taxa *P. necessarius* subsp. *asymbioticus* and *P. cosmopolitanus*

Taxa: 1, *Polynucleobacter rarus* sp. nov. MT-CBb6A5^T; 2, *P. necessarius* subsp. *asymbioticus* (n=4; data from Hahn *et al.*, 2009); 3, *P. cosmopolitanus* (n=5; Hahn *et al.*, 2010). Note that the subspecies *P. necessarius* subsp. *necessarius* lacks a sufficient phenotypic and chemotaxonomic description due to the lack of pure cultures. 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
Cell morphology	Straight rods	Straight or curved rods	Curved rods
Nucleoids visible (DAPI)	Frequently	Rarely	Rarely
Cell length (μm)	0.8–1.8	0.5–2.9	0.4–1.4
Cell width (μm)	0.6–0.8	0.3–0.5	0.3–0.5
Growth at 5 °C	−	+	+/−
Growth at 35 °C	−	+/−	+
NaCl tolerance (%, w/v)	0.3(w)	0.3–0.5	0.3–0.5
Anaerobic growth on NSY medium	−	+/−	+
Growth in mineral medium with acetic acid and B12	w	w/−	w/−
Assimilation of:			
Urea	−	+/−	+/−
Thiosulfate	−	+/−	+/−
Formic acid	−	w/−	−
Glyoxylic acid	+	w/−	w/−
Glycolic acid	w	−	−
Acetic acid	+	+	+
Oxalic acid	w	−	−
Propionic acid	−	+/−	+/w
Malonic acid	−	+/−	+/−
Oxaloacetic acid	−	+/−	+
Malic acid	+	+/w	+
Succinic acid	+	+	+
Fumaric acid	+	+/w	+
Levulinic acid	+	w/−	w/−
Citric acid	−	−	+/−
D-Mannose	w	w/−	w/−
D-Glucose	−	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%)	40.3	44–46	44.9

subcluster A (PnecA) and propose to establish for this strain the species *Polynucleobacter rarus* sp. nov. Note that this new species represents only a fraction of the highly diverse subcluster A of the '*Polynucleobacter necessarius* cluster'.

Isolation and characterization

Strain MT-CBb6A5^T was isolated from Crystal Bog Lake (Newton *et al.*, 2006) by using the filtration-acclimatization method (Hahn, 2003; Hahn *et al.*, 2004). This isolation method includes a step of filtration through 0.2 μm filters, which seems to contradict the cell dimensions of the cultivated strain presented below. However, variations of pore sizes of the filter or much smaller *in situ* cell sizes of the strain may be responsible for the passing of the ancestor cell through the filter. At the time of writing, only a single strain affiliated with subcluster A of the '*P. necessarius* cluster' has been isolated, while >150 and >50 strains of subcluster C (*P. necessarius*) and subcluster D (*P. cosmopolitanus*), respectively, have been isolated (Hahn, 2003; Hahn *et al.*, 2005, 2010; Watanabe *et al.*, 2009). The isolation method for bacteria of the genus *Polynucleobacter* developed by Watanabe *et al.* (2009) uses a 0.8 μm filtration step, whereas, in several isolation experiments, Hahn *et al.* (2005, 2009) replaced the filtration step by a dilution step (dilution-acclimatization method); thus, the singular isolation of a *Polynucleobacter* strain affiliated with subcluster A cannot exclusively be explained by the utilization of 0.2 μm filters.

Initially, pure cultures of the strain in liquid NSY medium (Hahn *et al.*, 2004) showed formation of flocks; however, this trait disappeared after some subcultivation steps. The investigated strain was routinely grown on NSY and R2A (Remel) medium with concentrations of 3 g l^{-1}; however, similar to previously investigated *Polynucleobacter* strains, the biomass yield of strain MT-CBb6A5^T on these media was much lower than that observed for other members of the family *Burkholderiaceae*. Growth at different

Table 2. Whole-cell fatty acid composition of strain MT-CBb6A5^T as compared with strains of *P. necessarius* subsp. *asymbioticus* and *P. cosmopolitanus*

Taxa: 1, *Polynucleobacter rarus* MT-CBb6A5^T; 2, *P. necessarius* subsp. *asymbioticus* QLW-P1DMWA-1^T (data from Hahn *et al.*, 2009); 3, *P. necessarius* (n=3; Hahn *et al.*, 2009); 4, *P. cosmopolitanus* MWH-MoIso2^T (Hahn *et al.*, 2010); 5, *P. cosmopolitanus* (n=5; Hahn *et al.*, 2010). Values are percentages of the summed fatty acids named in the peak library of the MIDI system (contents ⩾0.1 %). Strains were grown on R2A agar plates for 3–5 days at 28 °C. tr, Trace, may be detected or not in independent experiments.

Fatty acid	1	2	3	4	5
Saturated					
$C_{12:0}$	3.0	3.4	3.4–5.5	–	–
$C_{14:0}$	0.4	0.9	0.3–1.2	0.7	0.6–2.3
$C_{15:0}$	tr	0.3	tr	0.2	tr
$C_{16:0}$	19.8	22.2	15.5–33.5	15.4	11.0–15.4
$C_{17:0}$	0.3	–	tr	–	tr
$C_{18:0}$	0.5	1.2	0.5–2.6	0.8	0.5–1.1
Unsaturated					
$C_{14:1}\omega5c$	0.2	–	0–0.6	0.6	0–0.6
$C_{15:1}\omega8c$	0.2	–	–	–	–
$C_{15:1}\omega6c$	0.3	–	0–0.6	–	–
$C_{16:1}\omega5c$	0.5	0.9	0–0.9	0.3	0.3–1.1
$C_{17:1}\omega6c$	tr	–	–	0.5	0–0.7
$C_{18:1}\omega9c$	tr	–	0–0.4	0.3	0–2.0
$C_{18:1}\omega7c$	21.8	12.9	0.25–20.4	28.7	28.7–38.1
$C_{18:1}\omega5c$	–	–	–	0.2	tr
11-Methyl $C_{18:1}\omega7c$	7.5	3.1	0.2–8.1	3.7	0.4–3.7
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
$C_{16:0}$ 3-OH	0.3	–	–	–	–
Summed features					
1 ($C_{12:0}$ ALDE?)	1.8	0.4	0.4–2.1	0.1	tr
2 (including $C_{14:0}$ 3-OH)	6.9	9.6	8.4–9.9	0.6	0.6–3.9
3 (including $C_{16:1}\omega7c$)	35.9	41.3	35.6–45.0	34.7	31.5–36.5
7 (including $C_{19:1}\omega6c$)	–	0.4	0.2–2.0	1.5	0–1.5

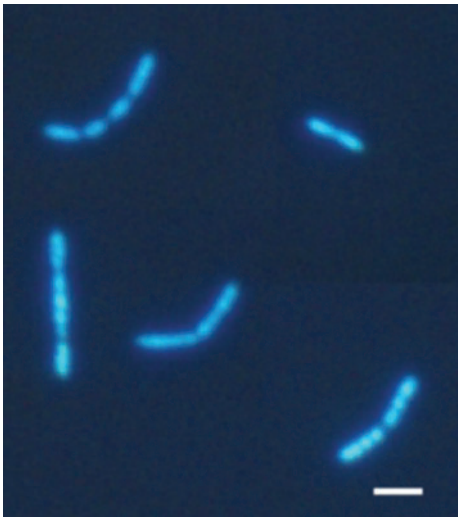

Fig. 1. Epifluorescence microscopic images of cells of strain MT-CBb6A5^T stained with the nucleic acid dye 4′,6-diamidino-2-phenylindole (DAPI). Note that the image shows a collection of cells showing multiple nucleoids, which is a trait not shared by all cells present in cultures of the strain. The fraction of cells showing this trait is variable and seems to be influenced by the age of the culture. Bar, 2 μm.

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 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, 2010). 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 on the medium without test substance were scored after 10 days of growth as no utilization (−), weak utilization (w) and good utilization (+), respectively.

Sequencing and phylogenetic analyses of 16S rRNA genes were performed as described previously (Hahn, 2003; Hahn *et al.*, 2005). Neighbour-joining trees were calculated by using the software MEGA4 (Tamura *et al.*, 2007) and maximum-likelihood trees were generated by using the RaxML web server (Stamatakis *et al.*, 2008). 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).

Table 3. Discriminative characteristics separating strain MT-CBb6A5^T from *P. necessarius* subsp. *asymbioticus* and *P. cosmopolitanus*

Taxa: 1, *Polynucleobacter rarus* sp. nov. MT-CBb6A5^T; 2, *P. necessarius* subsp. *asymbioticus* [*n*=3 (fatty acids) or 4 (phenotypic features); data from Hahn *et al.*, 2009]; 3, *P. cosmopolitanus* [n=3 (phenotypic features) or 5 (fatty acids); Hahn *et al.*, 2010]. *P. rarus* sp. nov. differs from the obligately endosymbiotic *P. necessarius* subsp. *necessarius* in its free-living lifestyle. Note that only an incomplete phenotypic and chemotaxonomic description of *P. necessarius* subsp. *necessarius* is available because of the lack of pure cultures. –, Negative; +, positive.

Characteristic	1	2	3
Nucleoids visible (DAPI)*	Frequently	Rarely	Rarely
Cell width (μm)	0.6–0.8	0.3–0.5	0.3–0.5
Utilization of glycolic acid	+	−	−
Utilization of oxalic acid	+	−	−
$C_{12:0}$	+	+	−
$C_{15:1}\omega 8c$	+	−	−
$C_{12:0}$ 2-OH	−	+	−
$C_{16:0}$ 3-OH	+	−	−
DNA G+C content (mol%)	40.3	44–46	44.9

*Staining with a fluorescent nucleic acid dye (e.g. DAPI) and epifluorescence microscopic observation.

The results of the phenotypic and chemotaxonomic characterization of strain MT-CBb6A5^T are presented in Tables 1 and 2. The strain differs from strains of *P. necessarius* subsp. *asymbioticus* and *P. cosmopolitanus* in the relatively large cell widths, and in the frequently observable presence of multiple nucleoid structures (Fig. 1). Such structures were reported for endosymbiotic strains recently assigned to the subspecies *P. necessarius* subsp. *necessarius* and the genus name refers to them (Heckmann & Schmidt, 1987); however, this feature was only rarely observed in other free-living *Polynucleobacter* strains (Hahn, 2003; Hahn *et al.*, 2009, 2010). Furthermore, the investigated strain differed from both previously described taxa in the ability to utilize oxalic and glycolic acids (Table 3). Strain MT-CBb6A5^T showed visible growth under anoxic conditions only on NSY medium supplemented with increased concentrations of nitrate and not on standard NSY medium. This observation could result from nitrate respiration.

The G+C content of the DNA of strain MT-CBb6A5^T was 40.3 mol%, which is substantially lower than those of *P. necessarius* strains (44–46 mol%) and the *P. cosmopolitanus* type strain (44.9 mol%). Whole-cell fatty acids of strain MT-CBb6A5^T were dominated by the unsaturated components $C_{16:1}\omega 7c$ (feature 3), $C_{18:1}\omega 7c$ and 11-methyl $C_{18:1}\omega 7c$ and straight chain $C_{16:0}$. Relatively high amounts

of feature 2, including $C_{14:0}$ 3-OH, were also detected. The pattern, consisting of these major components and a high number of minor compounds, resembled those of the two other species of the genus *Polynucleobacter*, confirming the membership of the strain in this genus. The novel strain contained $C_{12:0}$, a feature differentiating it from *P. necessarius* subsp. *asymbioticus*. Furthermore, strain MT-CBb6A5^T was characterized by the presence of low amounts of $C_{16:0}$ 3-OH and $C_{15:1}\omega 8c$, which were lacking in the other *Polynucleobacter* strains. Instead, strain MT-CBb6A5^T was devoid of $C_{12:0}$ 2-OH and $C_{12:0}$ 3-OH, which are present in *P. necessarius* subsp. *asymbioticus* and *P. cosmopolitanus*, respectively.

Phylogeny

The phylogenetic analysis of the almost complete 16S rRNA gene sequence of strain MT-CBb6A5^T demonstrated a close relationship with the two previously described species of the genus *Polynucleobacter* but also demonstrated a clustering separate from those two taxa (Fig. 2). Note that a more detailed phylogenetic analysis including the 16S rRNA gene and the 16S–23S ITS sequences of strain MT-CBb6A5^T was published previously (Hahn *et al.*, 2010). The sequence similarities between 16S rRNA genes of strain MT-CBb6A5^T and the type strain of *P. necessarius* subsp. *asymbioticus*, a sequence representing *P. necessarius* subsp. *necessarius* 'E24', and the type strain of *P. cosmopolitanus* were 96.0 %, 95.6 % and 96.0 %, respectively.

Ecology of *Polynucleobacter rarus* sp. nov.

Despite the lack of both quantitative *in situ* data and systematic surveys on the distribution of *P. rarus* sp. nov., it seems that this taxon represents, in contrast to *P. necessarius* subsp. *asymbioticus* and *P. cosmopolitanus*, a rare species. A BLAST search with the almost complete 16S rRNA gene sequence of strain MT-CBb6A5^T as a query resulted in only nine hits (all affiliated with subcluster A of the '*Polynucleobacter* cluster') with sequence similarities >97 %. Most of these hits represent environmental sequences of uncultured bacteria. The closest hit (accession number FR667321), representing a bacterium from a high mountain lake located in the Pyrenees, Spain (M. Bartrons, unpublished data), shares with *P. rarus* sp. nov. a sequence similarity of only 99.5%. The other eight sequences originate from the Adirondack lakes, New York (accession number EF520438, Percent *et al.*, 2008), the estuaries Chesapeake Bay and Delaware Bay (accession numbers EU801586 and EU800645, Shaw *et al.*, 2008), from Arctic Toolik Lake (accession number AF534432, Crump *et al.*, 2003), from Lake Shirakoma, Japan (accession numbers AB599841 and AB599847, K. Watanabe, Y. Ishii, N. Komatsu, T. Honma, R. Miyata, N. Noda, Y. Sekiguchi, S. Hayashi and A. Imai, unpublished data) and from Crystal Bog Lake (two identical sequences with accession numbers AY792238 and AY792240, Newton *et al.*, 2006). The latter two sequences share with strain MT-CBb6A5^T the same origin but only sequence similarities of 97.7 %. Similar BLAST searches with the type strains of of *P. cosmopolitanus* and *P. necessarius* subsp. *asymbioticus* resulted in >100 hits representing environmental sequences and cultivated strains with sequence similarities ranging from 98.5 % to 100 %, respectively. Thus, the under representation of strains affiliated to subcluster A relative to members of other subclusters in *Polynucleobacter* culture collections seems to result mainly from a relative rareness of the taxon in the environment.

The origin of strain MT-CBb6A5^T and the closest related taxa from the water column of freshwater lakes or from rivers and estuaries receiving water from freshwater lakes seems to indicate that strains affiliated with *P. rarus* sp. nov. dwell in the water column of freshwater systems as free-living planktonic bacteria. Such a lifestyle was demonstrated for *P. necessarius* subsp. *asymbioticus* and *P. cosmopolitanus* previously (Hahn *et al.*, 2005; Wu & Hahn, 2006). On the other hand, the initial observation of flock formation in liquid cultures could hint at an attached lifestyle of the strain. Dystrophic Crystal Bog Lake, from

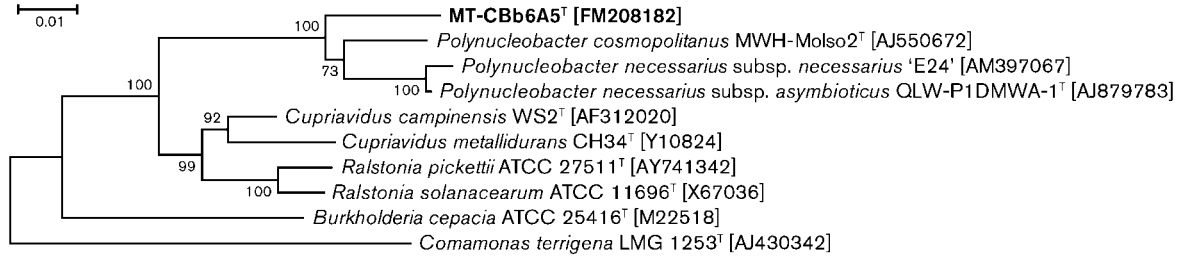

Fig. 2. Neighbour-joining tree based on almost complete 16S rRNA gene sequences, reconstructing the phylogenetic position of strain MT-CBb6A5^T. Bar, 0.01 substitutions per nucleotide position. Note that previous analysis of the phylogeny of the genus *Polynucleobacter*, which analysed 16S rRNA gene sequences and 16S–23S ITS sequences by the neighbour-joining and maximum-likelihood method (Hahn *et al.*, 2010), confirmed the phylogenetic position of strain MT-CBb6A5^T in the *Polynucleobacter* clade.

which the type strain of *P. rarus* sp. nov. was isolated, is a shallow, acidic lake strongly influenced by the surrounding bog, which results in a relatively high concentration of allochtonous humic substances in the water of the lake (Newton *et al.*, 2006). A preference of *P. rarus* sp. nov. for acidic habitats is likely; however, the small dataset available for this taxon enables only preliminary conclusions.

Results from the phylogenetic analysis and chemotaxonomic investigations demonstrated the affiliation of strain MT-CBb6A5^T to the genus *Polynucleobacter* (Tables 1 and 2, Fig. 2) but also revealed pronounced differences between this strain and strains affiliated with previously described species of the genus *Polynucleobacter* (Table 3). The 16S rRNA gene sequence similarities determined between strain MT-CBb6A5^T and strains representing the two *P. necessarius* subspecies as well as *P. cosmopolitanus* of <97 % clearly indicate that the investigated strain represents a new species of the genus *Polynucleobacter*. Therefore, we propose to establish the species *Polynucleobacter rarus* sp. nov. with strain MT-CBb6A5^T as the type strain. Note that we do not propose to tentatively include all strains of the so-called subcluster A (PnecA) in this new species. This subcluster represents, in contrast to subclusters PnecC (*P. necessarius*) and PnecD (*P. cosmopolitanus*), a phylogenetically more diverse subcluster, which makes it more likely than the other two subclusters to consist of more than one species.

Description of *Polynucleobacter rarus* sp. nov.

Polynucleobacter rarus (ra′rus. L. masc. adj. *rarus* rare, referring to the observation that this species represents a rare species, in contrast to the previously described species of the genus *Polynucleobacter*).

Straight, non-motile rods, 0.8–1.8 μm in length and 0.6–0.8 μm in width. Staining with nucleic acid dyes results in the microscopical visibility of multiple nucleoids in a significant fraction of cells. Chemo-organotrophic and aerobic; anaerobic growth in the presence of nitrate. 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 or 35 °C. Grows without NaCl. Maximum NaCl concentration tolerated is 0.3 % (w/v). Oxidase- and catalase-positive. Utilizes glyoxylate, glycolate, acetate, oxalate, pyruvate, malate, succinate, fumarate, levulinate, D-mannose, D-galacturonic acid, D-lyxose, D-fructose, D-fucose and L-cysteine when these substrates are provided in a medium containing low amounts of NSY. Does not utilize formate, propionate, malonate, oxaloacetate, citrate, D-glucose, D-galactose, D-sorbitol, L-glutamate, L-aspartate, L-alanine, L-serine, L-asparagine or betaine. Major cellular fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, feature 3 (including $C_{16:1}\omega7c$), and feature 2 (including $C_{14:0}$ 3-OH). The DNA G+C content of the type strain is 40.3 mol%. The sole cultivated strain currently representing this species is a free-living strain.

The type strain is MT-CBb6A5^T (=DSM 21648^T =CIP 109928^T), isolated from the water column (pelagic zone) of Crystal Bog Lake in Wisconsin, USA.

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