Telmatobacter bradus gen. nov., sp. nov., a cellulolytic facultative anaerobe from subdivision 1 of the *Acidobacteria*, and emended description of *Acidobacterium capsulatum* Kishimoto *et al.* 1991

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A Gram-negative, facultatively anaerobic, chemo-organotrophic, non-pigmented, slow-growing bacterium was isolated from acidic *Sphagnum* peat and designated strain TPB6017^T. Cells of this strain were long rods that multiplied by normal cell division and were motile by means of a single flagellum. Cells grew under reduced oxygen tension and under anoxic conditions and were able to ferment sugars and several polysaccharides, including amorphous and crystalline cellulose. Strain TPB6017^T was a psychrotolerant acidophile capable of growth between pH 3.0 and 7.5 (optimum 4.5–5.0) and at 4–35 °C (optimum 20–28 °C). It was extremely sensitive to salt stress; growth was inhibited at NaCl concentrations above 0.1 % (w/v). The major fatty acids were iso-C_{15:0} and iso-C_{17:1ω9c}; the polar lipids were phosphatidylethanolamine and a number of phospholipids and aminophospholipids with an unknown structure. The quinone was MK-8. The DNA G + C content was 57.6 mol%. Comparative 16S rRNA gene sequence analysis revealed that strain TPB6017^T was a member of subdivision 1 of the phylum *Acidobacteria* and belonged to a phylogenetic lineage defined by the acidophilic aerobic chemo-organotroph *Acidobacterium capsulatum* (92.3 % sequence similarity). However, cell morphology, type of flagellation, the absence of pigment, differences in fatty acid and polar lipid composition, possession of a cellulolytic capability, inability to grow under fully oxic conditions and good growth in anoxic conditions distinguished strain TPB6017^T from *A. capsulatum*. Therefore, it is proposed that strain TPB6017^T represents a novel acidobacterium species in a new genus, *Telmatobacter bradus* gen. nov., sp. nov.; strain TPB6017^T (=DSM 23630^T=VKM B-2570^T) is the type strain.

At present, the list of taxonomically characterized microorganisms in subdivision 1 of the phylum *Acidobacteria* contains only four genera, i.e. *Acidobacterium* (Kishimoto *et al.*, 1991), *Terriglobus* (Eichorst *et al.*, 2007), *Edaphobacter* (Koch *et al.*, 2008) and *Granulicella* (Pankratov & Dedysh, 2010). All described members of these genera are acidophilic, strictly aerobic, chemo-organotrophic bacteria that utilize sugars and some polysaccharides but not cellulose or chitin. 16S rRNA gene sequences of subdivision 1 acidobacteria have been recovered from anoxic freshwater sediments (Barns *et al.*, 1999), anaerobic methanogenic enrichments (Sizova *et al.*, 2003) and anoxic peat layers

(Juottonen *et al.*, 2005; Jackson *et al.*, 2009), as well as from oxic/anoxic interfaces of ombrotrophic peat bogs (Dedysh *et al.*, 2006), suggesting the occurrence of as-yet-uncultured anaerobic representatives in this group. Our knowledge about the hydrolytic capabilities of these bacteria also appears to be incomplete. Though the recently sequenced genomes of *Acidobacterium capsulatum* and ‘*Koribacter versatilis*’ Ellin345 were shown to contain candidate cellulases from glycoside hydrolase family 9 (Ward *et al.*, 2009), the ability to degrade cellulose has not been proven for acidobacteria. In this paper, we describe a member of this phylum, strain TPB6017^T, that possesses both phenotypic traits, i.e. the ability to grow anaerobically and the ability to degrade cellulose.

Strain TPB6017^T was isolated from a peat soil (pH 4.0) sampled at the oxic/anoxic interface (depth of 10 to 20 cm) of the *Sphagnum* peat bog Bakchar, Tomsk region, West

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Telmatobacter bradus* strain TPB6017^T is AM887760.

Four supplementary figures and two supplementary tables are available with the online version of this paper.

Siberia (56° 51' N 82° 50' E) as reported by Pankratov *et al.* (2008). The isolation was performed using basal medium MM1 solidified with gellan gum (Fluka) and containing (g l⁻¹ distilled water): MgSO₄·7H₂O, 0.04; CaCl₂·2H₂O, 0.02; yeast extract, 0.05; and pectin, 0.5, as the carbon source. Humic acid (dialysed, prepared as described by Pansu & Gautheyrou, 2006; 1 ml), galacturonic acid sodium salt (30 mg), glucuronic acid (30 mg) and homoserine (10 mg) were included in medium MM1 as additional components. To avoid increasing ionic strength, medium pH was adjusted to 4.5 with alginic acid (~30 mg l⁻¹).

A number of small (0.5–1.0 mm in diameter), pink-pigmented colonies developed on this medium after 10 days of incubation under aerobic conditions. Microscopic examination showed that these colonies consisted of morphologically different cells, numerically predominant thick short rods and less abundant thin long rods. To identify these bacteria, we applied whole cell hybridization with Cy3-labelled, 16S rRNA-targeted, phylum-specific oligonucleotide probes. Thick rods hybridized to the *Alphaproteobacteria*-specific probe mix ALP1b + ALP968 (Manz *et al.*, 1992) (not shown), whereas thin rods were brightly stained with probe HoAc1402 (Juretschko *et al.*, 2002) with reported group specificity for members of the phylum *Acidobacteria* (Supplementary Fig. S1, available in IJSEM Online). Separation of these two groups was achieved by spread-planting of cell material from the original colonies onto solid MM1 medium and extended aerobic incubation. Pink-pigmented colonies of the alphaproteobacterial isolate developed after 7 days of incubation, whereas very small (<0.5 mm in diameter), milk-white colonies of acidobacteria were visible with the naked eye only after 5 weeks of incubation. These colonies were picked and respread onto fresh MM1 plates. This procedure was repeated several times until a pure culture, designated strain TPB6017^T, was obtained. This isolate was further maintained in liquid basal medium MM1 with pectin, pH 4.5–5.0, and was subcultured at 4–6 week intervals.

The 16S rRNA gene sequence of strain TPB6017^T was determined previously (Pankratov *et al.*, 2008). Phylogenetic analysis was carried out using the ARB program package (Ludwig *et al.*, 2004). This analysis showed that strain TPB6017^T is a member of subdivision 1 of the *Acidobacteria* and belongs to a phylogenetic lineage defined by *A. capsulatum* (92.3 % 16S rRNA gene sequence similarity to the type strain) (Fig. 1). Sequence similarities to members of the genera *Edaphobacter*, *Granulicella* and *Terriglobus* were 92.1–92.7, 91.5–92.3 and 91.7 %, respectively. Notably, the 16S rRNA gene sequence of strain TPB6017^T formed a common cluster with a number of 16S rRNA gene clones (P-48, PC-20-32, PC-20-113 and PC-10-50) that were retrieved from acidic *Sphagnum*-derived peat collected from North European ombrotrophic peat bog Obukhovskoye and from cellulolytic enrichment cultures obtained from this peat (Pankratov *et al.*, 2011). In treeing analyses, strain TPB6017^T and these clones always clustered together irrespective of the algorithm (neighbour-joining, maximum-likelihood or

maximum-parsimony) used for the phylogenetic tree construction (Fig. 1). Since *A. capsulatum* was the closest taxonomically described relative of our novel isolate, the type strain of this species, DSM 11244^T, was used as a reference organism in our study. It was maintained on medium 269, recommended by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) for cultivation of *A. capsulatum*, but in all comparative tests it was grown on medium MM1 under the same conditions as strain TPB6017^T.

Morphological observations and cell size measurements were made with an Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). Cells of strain TPB6017^T were non-spore-forming rods, 0.4–0.6 µm in width and 2–10 µm in length (Fig. 2a). They occurred singly and multiplied by normal cell division. Cells stained Gram-negative using Gram differential staining kit (Deltalab). Younger (3- to 5-day-old) cultures contained many elongated (up to 100–150 µm long) cells. In contrast, most cells in 2- to 3-week-old cultures were relatively short (0.8–1.5 µm).

Cells in young cultures of strain TPB6017^T were highly motile. Cell suspensions from these cultures were dried onto grids and treated with 1 % (w/v) phosphotungstic acid solution. Negatively stained cells were examined with a JEM-100C electron microscope (JEOL) at an 80 kV accelerating voltage. As revealed by the analysis, cell motility was due to possession of a single, thick and long (30 nm by 2.0–3.5 µm) lateral flagellum (Fig. 2b).

Under aerobic conditions and on a solid medium, strain TPB6017^T formed very small (0.3–1.5 mm in diameter after 6–8 weeks of incubation), opaque colonies with winding profile, wavy edges and homogeneous consistency. The colony colour was milk-white or light beige depending on growth conditions. No pigments were detected in this bacterium by examination of absorption spectra of methanol and chloroform cell extracts between 200 and 1100 nm using a Cary-50 spectrophotometer (Varian).

In our original report on isolation of strain TPB6017^T (Pankratov *et al.*, 2008), we characterized this bacterium as a strictly aerobic chemo-organotroph. Indeed, for several months after isolation we maintained strain TPB6017^T in aerobic conditions either on solid medium MM1 or in 160 ml serum flasks containing 30–40 ml liquid medium MM1. We noticed, however, that growth under these conditions was very poor (specific growth rate below 0.005 h⁻¹). Moreover, growth was completely inhibited if liquid cultures were incubated on a shaker. Inoculation of cells into Hungate test tubes containing 10 ml semi-solid (0.05 % gellan gum) medium MM1 and further incubation under aerobic conditions resulted in formation of growth rings at a depth of 1 cm below the medium surface and small gas bubbles. All these observations suggested that strain TPB6017^T grows better at a reduced oxygen tension and, possibly, also grows under anoxic conditions. To verify this assumption, the effect of oxygen on the growth

Fig. 1. 16S rRNA gene-based neighbour-joining tree (Jukes–Cantor correction) showing the phylogenetic relationship of strain TPB6017^T to taxonomically characterized representatives of the phylum *Acidobacteria*, and some environmental 16S rRNA gene sequences retrieved from *Sphagnum*-derived peat and cellulolytic enrichment cultures obtained from peat (Pankratov *et al.*, 2011). The tree was calculated based on 1329 nt positions using the filter implemented in ARB for the phylum *Acidobacteria*. The significance levels of interior branch points obtained in neighbour-joining analysis were determined by bootstrap analysis (1000 data resamplings) using PHYLIP (Felsenstein, 1989). Bootstrap values (1000 data resamplings) of >50% are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. Six members of the *Planctomycetes*, i.e. *Isosphaera pallida* (AJ231193), *Gemmata obscuriglobus* (X54522), *Planctomyces brasiliensis* (AJ231190), *Planctomyces maris* (AJ231184), *Schlesneria paludicola* (AM162407) and *Singulisphaera acidiphila* (AM850678) were used as an outgroup (not shown). Bar, 0.1 substitutions per nucleotide position.

rate of strain TPB6017^T was studied using tightly closed 160 ml serum flasks containing 50 ml liquid medium MM1. Before autoclaving, these flasks were flushed for 5 min with a mixture of CO₂ and N₂ (7:93) and then different amounts of air (20% O₂) were injected into the headspace using a syringe to create a range of O₂ concentrations between 5 and 95% full oxygen saturation. To create anaerobic conditions, titanium (III) citrate (final concentration 0.6 mM) as a reducing agent and resazurin as an oxidation/reduction indicator (1 mg l⁻¹) were added to flasks as described by Sizova *et al.* (2003). Concentration of O₂ in the flask headspace was determined at the beginning and end of this experiment using a Crystall 5000 GC (Chromatec) equipped with a katharometer detector. Dissolved O₂ concentrations were determined using an oximeter (HACH LDO). Experimental flasks were shaken vigorously and growth of strain TPB6017^T was assessed by measuring OD₆₀₀ after 1 day of incubation. Since this isolate grows very slowly, pO₂ did not decrease during the course of the experiment. We found that growth was nearly absent in flasks with atmospheric oxygen concentrations and was very slow ($\mu=0.004$ h⁻¹) at 80% full oxygen saturation (~ 7.5 mg O₂ l⁻¹ at 25 °C). At 55–75% full oxygen saturation, the specific growth rate of

strain TPB6017^T increased up to 0.03–0.05 h⁻¹ and remained the same with further decreases in O₂ concentration up to fully anoxic conditions. Strain TPB6017^T, therefore, is a facultatively anaerobic organism that can grow only under reduced oxygen tension. Isolation of this organism during aerobic incubation became possible due to its tight association with an unidentified aerobic alphaproteobacterium, which reduced oxygen during active growth.

We also re-examined the ability of *A. capsulatum* DSM 11244^T to grow under anoxic conditions. The original description of this bacterium by Kishimoto *et al.* (1991) characterizes this organism as a strict aerobe. Later studies, however, reported its ability to grow slowly under micro-oxic and anoxic conditions (Blöthe *et al.*, 2008; Coupland & Johnson, 2008). Our experiments confirmed these findings. *A. capsulatum* DSM 11244^T grew relatively well under reduced oxygen tension in 120 ml serum flasks containing 50–100 ml liquid medium MM1 and it also showed weak growth (max. OD₆₀₀ of ~ 0.2) under strictly anoxic conditions, though the optimum growth of this bacterium occurred under fully oxic conditions. In this respect, the relation of *A. capsulatum* DSM 11244^T to oxygen was opposite to that of strain TPB6017^T, which did

Fig. 2. Cell morphology of strain TPB6017^T. Phase-contrast micrograph (a; bar, 10 μ m) and electron micrograph (b; bar, 1 μ m) of a negatively stained cell.

not grow under fully oxic conditions and grew well in strictly anoxic conditions (max. OD₆₀₀ of up to 0.8).

Further tests were performed using batch cultures in 120 ml serum flasks containing 50 ml liquid medium MM1 and under reduced oxygen tension conditions appropriate for both strain TPB6017^T and *A. capsulatum* DSM 11244^T. Temperature and pH growth range of strain TPB6017^T, as well as its sensitivity to different salts, were examined using liquid medium MM1 with pectin. The OD₆₀₀ was measured in a Cary-50 spectrophotometer at 3-day intervals for 4 weeks. Strain TPB6017^T grew in the pH range 3.0–7.5, with optimum growth at pH 4.5–5.0. The temperature range for growth was 4–35 °C, with optimum growth at 20–28 °C. As a true inhabitant of an ombrotrophic bog, which is strongly depleted in mineral nutrients, strain TPB6017^T was highly sensitive to salt stress. Growth of this isolate was inhibited at NaCl concentrations above 0.06 % (w/v). The same was true for KCl and Na₂SO₄. Inhibition by phosphates was observed at concentrations above 1 % (w/v).

The range of potential growth substrates of strain TPB6017^T was examined by replacing pectin in medium

MM1 with different carbon sources (0.05 %, w/v). Since strain TPB6017^T had an obligate requirement for the presence of growth factors (50 mg yeast extract l⁻¹) in cultivation media, utilization of a given carbon compound was assumed to have occurred when growth was distinctly improved in its presence compared with basal medium MM1 alone. The capability to degrade different biopolymers was examined by measuring the rate of CO₂ production in tightly closed 120 ml bottles containing 50 ml liquid medium MM1 with 0.05 % (w/v) of the corresponding polymer substrate for 3 weeks at 20 °C. Control incubations were run in parallel under the same conditions but without substrate. These experiments were performed in triplicate. CO₂ concentration was measured with a non-dispersive infrared gas-analyser ‘Infralit’ as described previously (Pankratov *et al.*, 2011). H₂ and O₂ were measured using a Crystall 5000 GC (Chromatec) with a katharometer detector. Cellulolytic capabilities of strain TPB6017^T were determined using crystalline cellulose (Sigma), amorphous phosphate cellulose and acetate cellulose (0.2 %, w/v) as carbon sources. Several additional procedures including the test with carboxymethyl-cellulose (0.05 %, w/v) and Congo Red (Wood *et al.*, 1988), as well as ostazin brilliant red H-3B hydroxyethyl cellulose (Sigma) discoloration (Farkaš *et al.*, 1985), were applied to identify the presence of cellulolytic enzymes in this acidobacterium.

The preferred growth substrates of strain TPB6017^T were sugars and polysaccharides. Under reduced oxygen tension, optimum growth was observed on pectin, raffinose, rhamnose, sucrose, xylose, maltose, melibiose and galactose. Carboxylic acids and most alcohols were not utilized. Weak growth occurred on inulin and arbutin. Strain TPB6017^T was able to degrade starch, xylan, gellan gum (polysaccharide from *Alteromonas* sp.) and laminarin, but not agar-agar, alginate, chitin, chitosan, chondroitin, fucoidan, heparin, lichenan or pullulan. As shown by CO₂ evolution dynamics, our novel isolate was also capable of degrading crystalline and amorphous (phosphate) cellulose (Supplementary Fig. S2, available in IJSEM Online). The presence of cellulolytic activity in strain TPB6017^T was also evident during its growth on cellulose-containing agar plates. Clear zones that formed around the colonies indicated destruction of cellulose (Supplementary Fig. S3, available in IJSEM Online). The specific growth rate of strain TPB6017^T on crystalline cellulose in liquid medium determined by direct cell counts was 0.007 h⁻¹. Cellulose acetate, carboxymethyl-cellulose and carboxyethyl-cellulose were not hydrolysed by strain TPB6017^T. We re-examined the presence of a cellulolytic potential in *A. capsulatum* DSM 11244^T, but the results were negative. None of the previously described representatives of the *Acidobacteria* are capable of hydrolysing cellulose. Two acidobacterial isolates obtained from agricultural grassland soils, strains KBS 83 and KBS 96, were recently reported to be cellulolytic, but their cellulose-degrading capability was not demonstrated in detail (Eichorst *et al.*, 2011). Therefore, strain TPB6017^T is the first taxonomically characterized cellulolytic acidobacterium.

Anaerobic growth of strain TPB6017^T occurred by means of fermenting sugars and polysaccharides. Best growth was observed with galactose, glucose, maltose, melibiose, raffinose, rhamnose and sucrose. Other substrates were cellobiose, cellulose, fructose, sodium galacturonate, lactose, mannose and pectin. All of these substrates were fermented with accumulation of acetate and hydrogen. Other fermentation products were 2-methylbutyrate, 3-methylacetate and ethanol (Supplementary Table S1, available in IJSEM Online). Significant amounts of ethanol were observed during fermentation of galactose, glucose and rhamnose. The products of cellulose degradation under anoxic conditions were acetate and hydrogen. Our experiments with *A. capsulatum* DSM 11244^T confirmed the data of Blöthe *et al.* (2008) indicating that anaerobic growth of this bacterium occurs by means of fermentation. Since this growth was very weak, only acetate was detected in cultivation medium and hydrogen production was not observed. The fermentation balances of strain TPB6017^T and *A. capsulatum* DSM 11244^T with glucose as a source of carbon are shown in Supplementary Table S2 (available in IJSEM Online).

Nitrogen sources were tested in liquid medium MM1 containing 0.0025 % (w/v) yeast extract and 0.02 % (w/v) of the respective compound. The preferred nitrogen source of strain TPB6017^T was ammonium. Other nitrogen sources were Casamino acids, peptone and yeast extract. Amino acids (L-glutamine, DL-tyrosine, DL-valine, DL-tryptophan, L-methionine, glycine, DL-threonine, L-proline, L-arginine, L-asparagine, L-cysteine), nitrate and N₂ were not utilized.

Strain TPB6017^T was unable to grow by anaerobic respiration with nitrate (1 mM) as the electron acceptor and glucose or fructose (0.05 %, w/v) as the sole carbon and energy source.

Analyses of enzymic profiles, urease hydrolysis, indole production and the Hugh–Leifson test were made with API 20NE and API ZYM kits (bioMérieux). Catalase, oxidase, reduction of thiosulphate to hydrogen sulphide, and nitrate and nitrite reduction tests were carried out by standard methods (Gerhardt *et al.*, 1981). API ZYM tests revealed the presence of acid phosphatase and esterase as well as activities of hydrolytic enzymes such as α - and β -galactosidase, α - and β -glucosidase, and α -fucosidase. Urease and protease were not detected. Oxidase was also absent. Catalase activity was weak. Reduction of thiosulphate to hydrogen sulphide and indole production were negative. The Hugh–Leifson test was positive.

Susceptibility to antibiotics was determined on solid medium MM1 using discs (Oxoid) containing the following antibiotics (μ g): ampicillin (10), gentamicin (10), kanamycin (30), neomycin (10), novobiocin (30), streptomycin (10), chloramphenicol (30) and lincomycin (10). Plates were incubated in a desiccator with reduced oxygen concentration (approx. 5 % by vol.). Strain TPB6017^T was resistant to streptomycin, kanamycin, gentamicin and neomycin, but

susceptible to ampicillin, lincomycin, chloramphenicol and novobiocin.

Cell biomass for cellular fatty acid and isoprenoid quinone analyses and for DNA extraction was obtained from batch cultures of strain TPB6017^T grown in liquid medium MM1 at 20 °C for 2 weeks. For comparison of fatty acid composition, *A. capsulatum* DSM 11244^T was grown in parallel on the same medium under identical growth conditions. Fatty acid profiles of both strains were analysed at the Identification Service of the DSMZ (Braunschweig, Germany) as described by Kämpfer & Kroppenstedt (1996) using an Agilent 6890N GC, Sherlock MIS version 4.5 and the library TSBA50 5.00. The major fatty acids of strain TPB6017^T were iso-C_{15:0} and iso-C_{17:1} ω 9c. The latter fatty acid was absent from the profile of *A. capsulatum* DSM 11244^T, which contained iso-C_{15:0}, C_{18:1} ω 9c and C_{16:0} as the major components (Table 1). Therefore, the fatty acid profile of strain TPB6017^T differed from that in *A. capsulatum* DSM 11244^T by the presence of iso-C_{17:1} ω 9c, the absence of C_{18:1} ω 9c and low C_{16:0} content.

Polar lipids were extracted from cell biomass with chloroform/methanol (1:2, v/v) by stirring for 1 h in an ice bath, followed with centrifugation at 5000 $\times g$ for 20 min; during this time, two phases were separated. The extraction procedure was performed twice and the upper phases were collected and combined; 2 ml chloroform and 2 ml distilled water were added to the supernatant and the resulting mixture was subjected to careful shaking for

Table 1. Whole-cell fatty acid compositions (%) of strain TPB6017^T and *Acidobacterium capsulatum* DSM 11244^T

Strains: 1, strain TPB6017^T; 2, *A. capsulatum* DSM 11244^T. Both strains were grown in parallel on medium MM1. Major fatty acids are shown in bold. –, Not detected.

Fatty acid	1	2
Saturated		
C _{14:0}	0.1	2.3
C _{16:0}	1.5	16.3
C _{17:0}	0.2	3.2
C _{18:0}	0.5	6.6
Unsaturated		
C _{17:1} ω 8c	–	1.9
C _{18:1} ω 9c	–	17.3
Methyl-branched		
iso-C _{15:0}	69.8	42.6
iso-C _{15:1} F	1.6	–
iso-C _{17:1} ω 9c	14.8	–
iso-C _{17:0}	5.4	3.1
anteiso-C _{17:0}	1.1	–
Hydroxy		
C _{13:0} 3-OH	0.4	–
Summed feature		
C _{16:1} ω 7c and/or iso-C _{15:0} 2-OH	0.4	3.1
iso-C _{15:1} I and/or C _{13:0} 3-OH	1.9	3.7

15 min in an ice bath. Then, the mixture was centrifuged and, during this time, three phases were separated. The lower phase with polar lipids was withdrawn, evaporated at 30 °C and dissolved in 200 µl chloroform. Polar lipids were separated by two-dimensional TLC (Kieselgel 60, 10 × 10 cm; Merck) using chloroform:methanol:water (60:25:4) in the first direction, followed by chloroform:acetic acid:methanol:water (85:15:12:4) in the second direction. Plates were sprayed with various specific reagents for detection of different phospholipids (Kates, 1972). Phospholipid standards (Sigma) were used during comparative analysis. Polar lipid profiles of strain TPB6017^T and *A. capsulatum* DSM 11244^T contained phosphatidylethanolamine as a major component and a number of unknown phospholipids and aminophospholipids (Supplementary Fig. S4, available in IJSEM Online). Strain TPB6017^T differed from *A. capsulatum* DSM 11244^T by the absence of several unidentified phospholipids (PL4–6) and aminophospholipids (APL3–4).

Isoprenoid quinones were extracted according to Collins (1985) and analysed using a tandem-type MS LCQ ADVANTAGE MAX and ionization MS Finnigan Mat 8430 with atmospheric pressure chemical ionization. The mass spectra were first recorded in MS mode and then analysed using MS/MS mode. Similarly to all currently described subdivision 1 acidobacteria, strain TPB6017^T contained menaquinone MK-8 as the predominant isoprenoid quinone.

The DNA G+C content was determined by thermal denaturation using a Cary 100 UV-VIS spectrophotometer (Varian) at a heating rate of 0.5 °C min⁻¹. The DNA G+C content was calculated according to Owen *et al.* (1969). DNA of *Escherichia coli* K-12 (DNA G+C content 51.7 mol%) was used as the standard. This analysis showed that DNA G+C content of strain TPB6017^T is 57.6 mol%.

In summary, strain TPB6017^T can be characterized as a moderately acidophilic and psychrotolerant organism. A facultatively anaerobic lifestyle allows this bacterium to thrive at the oxic/anoxic interface of freshwater wetlands. High cell motility provides a way to follow the water level fluctuations and to occupy the most favourable eco-niche. Another ecologically important trait of this bacterium is its ability to degrade cellulose, the major component of *Sphagnum*-derived litter in northern wetlands. By degrading plant-derived biopolymers under micro-oxic or anoxic conditions, strain TPB6017^T avoids competition with peat-inhabiting fungi, since their hydrolytic activity strongly depends on oxygen availability. Production of acetate and H₂ during cellulose degradation makes strain TPB6017^T an important member of the anaerobic community, which is responsible for providing substrates for methanogenic peat-inhabiting *Archaea*.

The characteristics that differentiate strain TPB6017^T from other described members of subdivision 1 of the *Acidobacteria* are summarized in Table 2. Cells of our novel isolate are longer than those of members of the genera

Table 2. Major characteristics of the genus *Telmatobacter* gen. nov. and related genera

Genera: 1, *Telmatobacter* gen. nov.; 2, *Acidobacterium* (Kishimoto *et al.*, 1991); 3, *Granulicella* (Pankratov & Dedysh, 2010); 4, *Terriglobus* (Eichorst *et al.*, 2007); 5, *Edaphobacter* (Koch *et al.*, 2008). ND, Not determined; w, weak.

Characteristic	1	2	3	4	5
Source of isolation	<i>Sphagnum</i> peat	Acidic mineral environment	<i>Sphagnum</i> peat and <i>Cladonia</i> sp.	Soil and termite hindgut	Alpine and forest soil
Cell length (µm)	2–10	1.1–2.3	1.5–15.0	1.0–1.2	1.0–2.1
Motility	+	+	–	–	+
Copious EPS production†	–	–	+	+	–
Pigment	None	Orange	Pink to red	Pink or none	None
pH range	3.0–7.5	3.0–6.0	3.0–7.5	5.0–7.0	4.0–7.0
pH optimum	4.5–5.0	ND	3.8–4.5	5.0–6.0	5.5
Growth in/at:					
0.1 % NaCl	–	+	+	+	+
3.5 % NaCl	–	–	+	–	–
37 °C	–	+	–	–	+
4 °C	+	ND	+	–	–
Anaerobic growth	+	w	–	–	–
Cellulose degradation	+	–	–	–	–
iso-C _{17:1ω9c} as a major fatty acid	+	–	–	–	–
DNA G+C content (mol%)	57.6	59.9–60.8	59.1–61.3	59.8 ± 0.5	55.8–56.9

*Negative only for strain Jbg-1^T (Koch *et al.*, 2008).

†EPS, Extracellular polymeric substance.

Terriglobus, *Edaphobacter* and *Acidobacterium*. The absence of pigments distinguishes strain TPB6017^T from members of the genera *Acidobacterium* and *Granulicella*. Motility by means of a single lateral flagellum, inability to grow under fully oxic conditions, good growth under strictly anoxic conditions, cellulolytic capabilities and inability to grow at NaCl concentrations above 0.1% differentiate strain TPB6017^T from all previously described members of this subdivision. Therefore, it is proposed that this isolate represents a novel species in a new genus, *Telmatobacter bradus* gen. nov., sp. nov.

Description of *Telmatobacter* gen. nov.

Telmatobacter [Tel.ma.to.bac'ter. Gr. n. *telma* -atos swamp, bog; N.L. masc. n. *bacter* (equivalent of Gr. neut. n. *baktron*) a short rod; N.L. masc. n. *Telmatobacter* rod-shaped bog-inhabiting bacterium].

Gram-negative, non-spore-forming, motile rods that occur singly. Reproduce by normal cell division. Facultatively anaerobic chemo-organotrophs. Sugars and pectin are the preferred growth substrates. Capable of fermenting sugars and several polysaccharides, including crystalline cellulose. Acidophilic and mesophilic. Liquid medium is superior to solid agar medium. Do not produce H₂S from thiosulfate or indole from tryptophan. NaCl inhibits growth at concentrations above 0.1% (w/v). Major fatty acids are iso-C_{15:0} and iso-C_{17:1ω9}; the polar lipids are phosphatidylethanolamine and a number of phospholipids and aminophospholipids with an unknown structure. The quinone is MK-8. Found in acidic wetlands, specifically *Sphagnum* peat bogs. The type species is *Telmatobacter bradus*.

Description of *Telmatobacter bradus* sp. nov.

Telmatobacter bradus [bra'dus. N.L. masc. adj. *bradus* (from Gr. masc. adj. *bradys*) slow, referring to the slow growth of this bacterium].

The description is as for the genus but with the following additional traits. Cells are 0.4–0.6 µm wide and 2.0–10.0 µm long; sometimes, cells can be up to 180 µm long. Colony colour varies from milk-white to beige. Cells are motile by means of a single subpolar flagellum. Carbon sources (0.05%, w/v) utilized include D-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, lactose, D-lactulose, maltose, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, sucrose, D-xylose, D-salicin, D-galacturonate, D-glucuronate, pyruvate, arbutin and inulin. Does not utilize N-acetylglucosamine, D-fucose, D-leucrose, D-ribose, L-sorbose, trehalose, acetate, butyrate, capronate, citrate, malate, lactate, formate, fumarate, oxalate, propionate, succinate, valerate, adonitol, arabitol, mannitol, ethanol, dulcitol, *myo*-inositol, methanol or sorbitol. Hydrolyses aesculin, laminarin, pectin, starch, xylan and cellulose but not sodium alginate, carboxymethyl-cellulose, chitin, chitosan, chondroitin sulphate, heparin, fucoidan, lichenan

or pullulan. Nitrogen sources utilized are ammonium, Casamino acids, peptone and yeast extract but not nitrate, nitrite, L-glutamine, DL-tyrosine, DL-valine, DL-tryptophan, L-methionine, glycine, DL-threonine, L-proline, L-arginine, L-asparagine or L-cysteine. The following enzyme activities are present: acid phosphatase, esterase (C4), α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-fucosidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase. Urease, trypsin, alkaline phosphatase, α-mannosidase, protease, lipase (C14), leucine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin and esterase (C8) are absent (API ZYM test). Oxidase-negative. Catalase activity is weak. Capable of growth at pH 3.0–7.5 (optimum pH 4.5–5.0) and at 4–35 °C (optimum at 20–28 °C). Sensitive to sodium chloride, sodium sulphate and potassium chloride (but not potassium phosphate) at concentrations above 0.06% (w/v). Resistant to streptomycin, kanamycin, gentamicin and neomycin, but susceptible to ampicillin, lincomycin, chloramphenicol and novobiocin. Anaerobic growth is possible via fermentation. Glucose, fructose, sucrose, cellobiose, maltose, melibiose, cellulose, raffinose, xylose, galactose and galacturonic acid sodium salt are substrates of fermentation. Does not ferment starch, leucrose or melezitose. Products of fermentation are acetate and hydrogen, plus traces of 2-methylbutyrate and 3-methylacetate. Production of ethanol and butanol is variable depending on substrate used.

The type strain is TPB6017^T (=DSM 23630^T=VKM B-2570^T), isolated from the *Sphagnum* peat bog Bakchar, Tomsk region, West Siberia. The DNA G+C content of the type strain is 57.6 mol%.

Emended description of *Acidobacterium capsulatum* Kishimoto *et al.* 1991

The description of the species *Acidobacterium capsulatum* is as given by Kishimoto *et al.* (1991) with the following modifications. Grows under fully oxic and micro-oxic conditions. Weak growth by means of fermentation also occurs in anoxic conditions. Polar lipids are phosphatidylethanolamine and a number of phospholipids and aminophospholipids with an unknown structure. Major fatty acids are iso-C_{15:0} and C_{18:1ω9c}.

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