Acidisoma tundrae gen. nov., sp. nov. and *Acidisoma sibiricum* sp. nov., two acidophilic, psychrotolerant members of the *Alphaproteobacteria* from acidic northern wetlands

Svetlana E. Belova,¹ Timofei A. Pankratov,¹ Ekaterina N. Detkova,¹
Elena N. Kaparullina² and Svetlana N. Dedysh¹

Correspondence
Svetlana N. Dedysh
dedysh@mail.ru

¹S.N. Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letya Otyabrya 7/2, Moscow 117312, Russia

²G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow region 142292, Russia

Three obligately aerobic, heterotrophic bacteria, designated strains WM1^T, TPB606^T and TPB621, were isolated from acidic *Sphagnum*-dominated tundra and Siberian wetlands in Russia. Cells of these isolates were Gram-negative, non-motile coccobacilli that occurred singly, in pairs or in chains, and were covered by large capsules. The novel strains were moderately acidophilic and psychrotolerant organisms capable of growth at pH 3.0–7.6 and 2–30 °C. Cells contained numerous intracellular poly- β -hydroxybutyrate granules (3–4 per cell). The major cellular fatty acid was cyclo C_{19:0 ω 8c} and the predominant quinone was Q-10. Strains TPB606^T and TPB621, isolated from Siberian wetland, possessed almost identical 16S rRNA gene sequences and shared 97.2% sequence similarity with tundra strain WM1^T. The three strains were shown to belong to the *Alphaproteobacteria*, but were related only distantly to the type strains of acidophilic bacteria *Acidisphaera rubrifaciens* (93.4–94.3% 16S rRNA gene sequence similarity), *Rhodopila globiformis* (92.2–93.3%), and members of the genera *Acidiphilium* (91.3–93%) and *Acidocella* (91.8–92.4%). The DNA G+C contents of the novel strains were 60.5–61.9 mol%. The low levels of DNA–DNA relatedness (37%) and a number of phenotypic differences between the Siberian strains TPB606^T and TPB621 and the tundra strain WM1^T indicated that they represent two separate species. As the three isolates are clearly distinct from all recognized acidophilic members of the *Alphaproteobacteria*, they are considered to represent two novel species of a new genus, for which the names *Acidisoma tundrae* gen. nov., sp. nov. and *Acidisoma sibiricum* sp. nov. are proposed. The type strain of *Acidisoma sibiricum* is TPB606^T (=DSM 21000^T=VKM B-2487^T) and the type strain of *Acidisoma tundrae* is WM1^T (=DSM 19999^T=VKM B-2488^T).

Representatives of the class *Alphaproteobacteria* are common inhabitants of acidic aquatic and terrestrial environments, such as acidic hot springs, mine waters, acidic soils and *Sphagnum*-dominated wetlands (Harrison, 1981; Kishimoto *et al.*, 1995; Hiraishi *et al.*, 1998, 2000; Nogales *et al.*, 2001; Dedysh *et al.*, 2006; García-Moyano

et al., 2007). In acidic peat bogs, the *Alphaproteobacteria* were detected by fluorescence *in situ* hybridization to be the most abundant bacterial population (Dedysh *et al.*, 2006). Few of the alphaproteobacterial 16S rRNA gene sequences retrieved from this acidic habitat were affiliated with the phylogenetic lineage defined by the genera *Acidisphaera* and *Acidiphilium*. Here, the isolation of three representative members of this phylogenetic lineage from acidic *Sphagnum* peat is reported. The characteristics of these novel isolates are described, on the basis of which they are considered to represent two novel species of a new genus within the *Alphaproteobacteria*.

Strain WM1^T was isolated from acidic *Sphagnum* peat (pH 4.5) collected in July 2003 from the upper (0–10 cm)

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains WM1^T, TPB606^T and TPB621 are AM947652, AM947653 and AM947654, respectively.

Phase-contrast micrographs of cells of strains WM1^T and TPB606^T, a figure showing the influence of the pH of the medium on growth of strain WM1^T, and two-dimensional thin-layer chromatographs of polar lipid extracts obtained from strains TPB606^T and WM1^T are available as supplementary material with the online version of this paper.

oxic layer of the unnamed oligotrophic tundra wetland located in the Vorkuta region of northern Russia (67° N 63° E). Isolation was by spread-plating serially diluted peat suspension onto the surface of medium MB comprising (per litre distilled water): 0.5 g glucose, 0.1 g KH_2PO_4 , 0.25 g KNO_3 , 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 g yeast extract and 18 g agar-agar (Difco). After sterilization, the pH of the medium was adjusted to 3.5 with 0.1 M H_3PO_4 . Strains TPB606^T and TPB621 were isolated from acidic peat soil (pH 4.2) sampled in August 2006 at a depth of 10–20 cm of the *Sphagnum* peat bog Bakchar, Tomsk region, western Siberia (56° 51' N 82° 50' E). The medium used comprised (per litre distilled water): 0.5 g laminarin (for isolation of strain TPB606^T) or xylan (for isolation of strain TPB621), 0.04 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05 g yeast extract, 0.03 g galacturonic acid sodium salt, 0.03 g glucuronic acid and 1 ml of humic acid dialysate. The pH of the medium was adjusted to 4.2. White or cream-coloured, raised, smooth, slimy colonies developed on plates inoculated with the terminal dilutions of these peat suspensions. Colonies consisted of coccobacilli that possessed large polysaccharide capsules. Partial sequencing of the 16S rRNA genes from these isolates showed that they were affiliated with the *Alphaproteobacteria* but were related only distantly (91–94 % sequence similarity) to members of the genera *Acidisphaera*, *Rhodopila*, *Acidiphilium* and *Acidocella*. Thus, the goal of the present study was to describe the characteristics of the newly isolated strains and to determine their taxonomic position.

The isolates were maintained on agar medium containing (per litre distilled water): 0.5 g sodium gluconate, 0.1 g KH_2PO_4 , 0.25 g $(\text{NH}_4)_2\text{SO}_4$, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.2 g NaCl, 0.1 g yeast extract and 18 g agar-agar (Difco). Morphological observations and cell size measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). Preparation of ultrathin sections for electron microscopy was performed as described by Kulichevskaya *et al.* (2006). Absorption spectra of acetone/methanol (7 : 2, v/v) extracts of cells of the new isolates were determined between 250 and 1000 nm with an SPh-56 spectrophotometer (Lomo-Spectrum). Physiological tests were carried out in liquid medium MB containing sodium gluconate instead of glucose. Growth of the novel strains was monitored by nephelometry at 410 nm by using a Specol spectrophotometer (Carl Zeiss) for 14 days under various conditions, including temperatures of 2–37 °C, pH 2.8–8.0 and NaCl concentrations of 0–3.0 % (w/v). Variations in pH were achieved by mixing 0.1 M solutions of H_3PO_4 , KH_2PO_4 , K_2HPO_4 and K_3PO_4 to create media with the same ionic strength. The range of potential growth substrates for the novel strains was examined by replacing glucose in medium MB with single carbon sources. Growth in the presence of nitrogen sources was tested by using liquid medium MB, in which KNO_3 was replaced with one of the following compounds at a concentration of 0.05 % (w/v): $(\text{NH}_4)_2\text{SO}_4$, NH_4 formate, NaNO_2 , $\text{NH}_2\text{OH-HCl}$, urea or

one of the amino acids listed in Table 1. Except for growth temperature experiments, batch cultures were incubated on a rotary shaker at 180 r.p.m. and at 24 °C. The capacity to degrade different biopolymers was examined by measuring the rate of CO_2 production in tightly closed 120-ml serum bottles containing 20 ml liquid MB medium with 0.05 % (w/v) of the corresponding polymer substrate for 1 month at 20 °C. Control incubations were run in parallel under the same conditions but without substrate. Oxidative and fermentative utilization of carbohydrates was determined as described for the Hugh–Leifson test (Gerhardt *et al.*,

Table 1. Differential characteristics of strains WM1^T (*Acidisoma tundrae* gen. nov., sp. nov.) and TPB606^T and TPB621 (*Acidisoma sibiricum* gen. nov., sp. nov.)

The two strains utilized arabinose, fructose, galactose, xylose, glucose, glycerol, mannitol, sorbitol, *myo*-inositol, sorbose, dulcitol, gluconate, pyruvate, galacturonate, yeast extract, Casamino acids, peptone and asparagine. They were capable of hydrolysing starch, xylan, heparin and aesculin. Neither of the strains utilized maltose, raffinose, methanol, butanol, propanol, fumarate, oxalate, succinate, acetate, citrate, formate, propionate, capronate, benzoate, glucuronic acid, alanine, aspartate or leucine. Neither strain was capable of hydrolysing alginate, chondroitin sulfate, carboxymethylcellulose, pullulan, casein or Tween 80, or of gelatin liquefaction. The two strains utilized ammonia, urea, nitrate, yeast extract, glutamine, proline, histidine HCl and methionine as nitrogen sources. Neither strain utilized nitrite, $\text{NH}_2\text{OH-HCl}$, lysine, arginine, glycine, serine or NH_4 formate as nitrogen sources. Neither strain was capable of dissimilatory nitrate reduction. +, Positive; –, negative, v, variable.

Characteristic	Strain WM1 ^T	Strains TPB606 ^T and TPB621
Cell size (µm)	0.8–1.5 × 1.9–4.1	0.7–1.2 × 1.4–3.1
Colony colour	White, ivory	White, cream, pinkish
Temperature range (°C)	2–30	2–30
Temperature optimum (°C)	15–22	20–25
pH growth range	3.0–7.5	3.7–7.6
pH optimum	4.5–5.7	5.0–6.5
NaCl tolerance (% w/v)	<1.5	<2.5
Carbon source utilization		
Lactose	–	v
Mannose	+	v
Sucrose	–	v
Trehalose	+	v
Cellobiose	–	v
Ethanol	+	–
Lactate, malate, butyrate	+	–
Pectin	+	–
Fucoidan	+	v
Laminarin	–	v
Nitrogen source utilization		
Asparagine	–	v
Alanine	+	–
DNA G + C content (mol%)	61.9	60.5–61.3

1981) and by using the API 20 NE kit (bioMérieux). Enzyme activities were examined by using the API ZYM kit (bioMérieux). Gelatin liquefaction was assessed according to method 1 of Gerhardt *et al.* (1981). Cultures were tested for growth under anaerobic conditions in anaerobic jars by using AnaeroGen anaerobic system envelopes (Oxoid) as well as in liquid media recommended for cultivation of phototrophic purple *Alphaproteobacteria* (Imhoff, 2006). Susceptibility to antibiotics was determined on MB agar plates by using discs (Oxoid) containing the following antibiotics: ampicillin (10 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (10 µg), novobiocin (30 µg), streptomycin (10 µg), chloramphenicol (30 µg) and lincomycin (10 µg).

Cell biomass for cellular fatty acid, polar lipid and isoprenoid quinone analyses and for DNA extraction was obtained from batch cultures grown in liquid medium MB at 24 °C for 10 days. Fatty acid profiles were analysed at the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) as described by Kämpfer & Kroppenstedt (1996). Isoprenoid quinones were extracted according to Collins (1985) and were analysed by using an LCQ ADVANTAGE MAX tandem-type mass spectrometer and Finnigan Mat 8430 ionization mass spectrometer. 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 g for 20 min; two phases were separated during this period. The extraction procedure was performed twice and the upper phases were collected and combined. Then, 2 ml chloroform and 2 ml distilled water were added to the supernatant and the resulting mixture was subjected to careful shaking for 15 min in an ice bath. The mixture was then centrifuged, during which three phases were separated. The lowest 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) by using chloroform/methanol/7 M sodium hydroxide (60:30:4) in the first direction, followed by chloroform/methanol/acetic acid/water (85:12.5:12.5:3) in the second direction. Plates were sprayed with various specific reagents for detection of different polar lipids (Kates, 1972). Phospholipid standards from Sigma were used during comparative analysis.

The DNA base composition of the novel strains was determined by using thermal denaturation with a Unicam SP1800 spectrophotometer at a heating rate of 0.5 °C min⁻¹. The DNA G + C content was calculated according to Owen *et al.* (1969). DNA–DNA hybridization experiments were performed as described by De Ley *et al.* (1970). PCR-mediated amplification of the 16S rRNA gene was performed by using primers 9f and 1492r and the reaction conditions described by Weisburg *et al.* (1991). 16S rRNA gene amplicons were purified by using the Wizard PCR Preps DNA purification system (Promega) and were sequenced on an ABI Prism 377 DNA sequencer (PE

Applied Biosystems). Phylogenetic analysis was carried out by using the ARB program package (Ludwig *et al.*, 2004). The newly obtained 16S rRNA gene sequences were added to the ARB database SSU_04 and were aligned manually. Phylogenetic trees were constructed by using distance-based (neighbour-joining), maximum-likelihood (DNAML) and maximum-parsimony methods. Significance levels of interior branch points obtained in the neighbour-joining analysis were determined by bootstrap analysis (1000 data resamplings) by using the PHYLIP program (Felsenstein, 1989).

On agar medium MB, strains WM1^T, TPB606^T and TPB621 formed circular, convex, slimy colonies with an entire margin and smooth surface. Colony colour varied from white to ivory for strain WM1^T and from white to cream or pinkish for strains TPB606^T and TPB621. Two-week-old colonies of these bacteria were 2–5 mm in diameter. Cells were non-spore-forming, non-motile coccobacilli that stained Gram-negative and occurred singly, in pairs or in chains (see Supplementary Fig. S1, available in IJSEM Online), and were covered with large polysaccharide capsules. Cells multiplied by binary fission and exhibited pleomorphism under variable growth conditions. Cells of tundra strain WM1^T were slightly larger (0.8–1.5 × 1.9–4.1 µm) than those of Siberian strains TPB606^T and TPB621 (0.7–1.2 × 1.4–3.1 µm) (Table 1). Electron microscopy of ultrathin sections revealed a Gram-negative cell-wall structure and the presence of numerous poly-β-hydroxybutyrate granules (3 or 4 per cell) (Fig. 1). Internal photosynthetic membranes were not found. Bacteriochlorophyll *a* was not detected under any experimental conditions. Low absorption maxima at 480 and 534 nm were detected in the acetone/methanol extracts of cream-pigmented strains TPB606^T and TPB621, suggesting the presence of a trace amount of the carotenoid spirilloxanthin. No pigments were revealed in cells of strain WM1^T. None of the new isolates was capable of anaerobic growth in the light. Stimulation of aerobic growth by continuous incandescent illumination as

Fig. 1. Electron micrograph of an ultrathin section of a cell of strain WM1^T. Large spherical bodies are granules of poly-β-hydroxybutyrate. Bar, 1 µm.

described for *Acidisphaera rubrifaciens* (Hiraishi *et al.*, 2000) was not detected for the novel strains.

The novel strains from *Sphagnum* peat were obligately aerobic chemoheterotrophs. They had an absolute requirement for the presence of growth factors (50–100 mg yeast extract l⁻¹) in cultivation media. Thus, utilization of a given carbon compound was assumed to have occurred when growth was distinctly improved in its presence compared with in basal MB medium alone. The list of carbon substrates tested and the results are given in the two species descriptions below and are shown in Table 1. Most sugars, polyalcohols and some organic acids were preferable growth substrates. Strain WM1^T differed from strains TPB606^T and TPB621 by its ability to utilize ethanol, lactate, malate and butyrate. All the novel strains were able to hydrolyse starch, xylan, heparin and aesculin, whereas pectin was hydrolysed only by tundra strain WM1^T. All three strains displayed catalase and cytochrome oxidase activities. Dissimilatory nitrate reduction was negative. Ammonia, nitrate, urea, Bacto yeast extract, glutamine, proline, histidine and methionine were utilized as nitrogen sources. Growth yield on media with nitrate was significantly lower than that on media with ammonia. None of the strains utilized nitrite, NH₂OH-HCl, lysine, arginine, glycine, serine or NH₄-formate as nitrogen sources. All strains were capable of slow growth in liquid nitrogen-free medium MB. The following enzymic activities (API ZYM) were detected in all of the strains: acid phosphatase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, valine arylamidase and phosphohydrolase. The following enzymic activities were absent: lipase, trypsin, chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase and arginine dihydrolase. Alkaline phosphatase and urease were detected only in Siberian strains TPB606^T and TPB621, whereas α -glucosidase was detected only in tundra strain WM1^T.

Strain WM1^T grew in the pH range 3.0–7.5, with optimum growth at pH 4.5–5.7 (Supplementary Fig. S2, in IJSEM Online). Strains TPB606^T and TPB621 grew at slightly higher pH, 3.7–7.6, with optimum growth at pH 5.0–6.5. The temperature range for growth was 2–30 °C, with optimum growth at 15–22 °C for tundra strain WM1^T and 20–25 °C for Siberian strains TPB606^T and TPB621. Growth of strain WM1^T was completely inhibited at NaCl concentrations above 1.5 % (w/v), but was stimulated at low concentrations (0.05–0.1 %, w/v). Strains TPB606^T and TPB621 were able to tolerate higher salinities and were able to grow in media containing up to 2.5 % (w/v) NaCl. Under optimal growth conditions, the generation time of these bacteria was in the range 6–10 h. All three strains were susceptible to kanamycin and gentamicin, but resistant to ampicillin and lincomycin. Novobiocin, streptomycin, chloramphenicol and neomycin inhibited growth of strain WM1^T but did not influence growth of strains TPB606^T and TPB621.

Similar to phylogenetically related acidophilic bacteria of the genera *Acidisphaera*, *Acidiphilium*, *Acidocella* and *Rhodopila* (Hiraishi *et al.*, 2000; Hiraishi & Imhoff, 2005; Hiraishi, 2005a, b; Madigan & Imhoff, 2005), the new isolates contained ubiquinone with ten isoprene units (Q-10) as the predominant isoprenoid quinone. The major component of the cellular fatty acid profiles of strains WM1^T, TPB606^T and TPB621 was cyclopropane C_{19:0} ω 8c, which constituted 44.4–55.4 % of the total fatty acids (Table 2). Interestingly, the presence of this fatty acid has been regarded as a feature specific to *Methylocella tundrae*, acidophilic, psychrotolerant methanotrophs isolated from acidic wetlands of tundra (Dedysh *et al.*, 2004). Significant amounts of C_{18:1} ω 7c (11.1–15.2 %), C_{16:0} (4.6–21.3 %) and C_{18:0} 3-OH (5.6–10.7 %) were also detected in cells of the new isolates. Polar lipid profiles of Siberian strains TPB606^T and TPB621 were nearly identical and consisted of phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, an unknown aminolipid, an unknown polar lipid, and minor amounts of phosphatidylserine and several unknown polar lipids (Supplementary Fig. S3a in IJSEM Online; data are shown only for strain TPB606^T). Tundra strain WM1^T contained phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an

Table 2. Cellular fatty acid compositions (% of the total) of acidophilic strains WM1^T, TPB606^T and TPB621

–, Not detected.

Fatty acid	Strain WM1 ^T	Strain TPB606 ^T	Strain TPB621
C _{10:0}	0.1	–	–
C _{12:0}	0.2	–	–
C _{14:0}	0.2	0.7	0.5
Unknown 14.959	3.1	1.4	0.7
Summed feature 2*	1.9	2.0	2.2
Summed feature 3†	–	1.2	0.7
C _{16:0} N alcohol	0.4	–	–
C _{16:1} ω 5c	–	0.2	–
C _{16:0}	4.7	21.3	17.3
Cyclo C _{17:0}	–	1.2	0.7
C _{16:0} 2-OH	–	0.5	0.7
C _{16:0} 3-OH	0.2	1.2	1.4
C _{18:1} ω 9c	2.4	0.7	–
C _{18:1} ω 7c	11.1	13.8	15.2
C _{18:1} ω 5c	0.4	–	–
C _{18:0}	6.0	2.9	3.2
11-Methyl C _{18:1} ω 7c	1.2	0.8	0.9
C _{17:0} 3-OH	0.4	–	–
Cyclo C _{19:0} ω 8c	55.4	44.4	48.8
C _{18:1} 2-OH	–	0.8	0.6
C _{18:0} 3-OH	10.7	5.6	6.1
C _{20:2} ω 6,9c	1.4	1.1	1.0

*Summed feature 2 comprises iso-C_{16:1} I and/or C_{14:0} 3-OH.

†Summed feature 3 comprises C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH.

unknown aminolipid and an unknown polar lipid as the predominant polar lipids. Additionally, minor amounts of phosphatidylserine, phosphatidic acid and several unknown polar lipids were detected (Supplementary Fig. S3b).

Comparative sequence analysis of the 16S rRNA gene placed strains WM1^T, TPB606^T and TPB621 in the class *Alphaproteobacteria*. However, these strains were related only distantly to the type strains of acidophilic alphaproteobacteria *Acidisphaera rubrifaciens* (93.4–94.3% 16S rRNA gene sequence similarity), *Rhodopila globiformis* (92.2–93.3%), and members of the genera *Acidiphilium* (91.3–93%) and *Acidocella* (91.8–92.4%) (Fig. 2). The two strains isolated from Siberian wetland, TPB606^T and TPB621, possessed almost identical 16S rRNA gene sequences and shared 97.2% sequence similarity with tundra strain WM1^T. The DNA G+C content of strains WM1^T, TPB606^T and TPB621 was 61.9, 60.5 and 61.3 mol%, respectively. Levels of DNA–DNA relatedness between strains WM1^T and TPB606^T and between strains TPB606^T and TPB621 were 37 and 79%, respectively. Thus, the results of DNA–DNA hybridization experiments indicated that the two Siberian strains and the strain isolated from tundra represent two separate species. This conclusion was also supported by significant morphological and phenotypic differences between these strains (Table 1) as well as by clear differences in their cellular fatty acid compositions (Table 2) and polar lipid profiles (Supplementary Fig. S3). Compared with data for the

isolate from tundra, cells of the two Siberian strains were smaller, contained carotenoids, were less acidophilic and psychrotolerant, and were more resistant to NaCl.

In summary, the novel isolates from *Sphagnum*-dominated wetlands possessed a number of characteristics that clearly distinguished them from phylogenetically related members of the *Alphaproteobacteria* (Table 3). They could be differentiated from members of the genera *Acidisphaera*, *Acidiphilium*, *Rhodopila*, *Craurococcus*, *Paracraurococcus*, *Roseococcus* and *Roseomonas* based on the absence of bacteriochlorophyll *a*. Absence of motility distinguished them from members of the genera *Acidocella*, *Acidiphilium*, *Rhodopila* and *Roseococcus*. They could be differentiated from members of the genus *Acidocella* based on the presence of diphosphatidylglycerol in polar lipid profiles. Compared with the phylogenetically related bacteria listed in Table 3, strains WM1^T, TPB606^T and TPB621 had greater tolerance to low temperatures, were more sensitive to NaCl and possessed distinct fatty acid profiles. Thus, strain WM1^T and strains TPB606^T and TPB621 are considered to represent two novel species of a new genus, for which the names *Acidisoma tundrae* gen. nov., sp. nov. and *Acidisoma sibiricum* sp. nov. are proposed.

Description of *Acidisoma* gen. nov.

Acidisoma [A.ci.di.so' ma. M.L. n. *acidum* in acid; Gr. neut. n. *soma* body; M.L. neut. n. *Acidisoma* an acid (-requiring) body].

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strains WM1^T, TPB606^T and TPB621 in relation to some representatives of the family *Acetobacteraceae*. The tree was constructed based on 1280 nt positions. Bootstrap values (based on 1000 data resamplings) of >50% are shown at nodes. The 16S rRNA gene sequence of *Rhodospirillum rubrum* (GenBank no. D30778) was used as an outgroup (not shown). Bar, 0.05 substitutions per nucleotide position. The same tree topology was obtained with the maximum-likelihood and maximum-parsimony methods.

Table 3. Major characteristics that distinguish *Acidisoma* gen. nov. from phylogenetically related genera of the *Alphaproteobacteria*

Genera: 1, *Acidisoma* gen. nov.; 2, *Acidisphaera* (data from Hiraishi *et al.*, 2000; Hiraishi, 2005a); 3, *Rhodopila* (Madigan & Imhoff, 2005); 4, *Acidiphilium* (Hiraishi & Imhoff, 2005); 5, *Acidocella* (Hiraishi, 2005b); 6, *Craurococcus* (Saitoh *et al.*, 1998); 7, *Paracraurococcus* (Saitoh *et al.*, 1998); 8, *Roseococcus* (Yurkov, 2005); 9, *Roseomonas* (Weyant & Whitney, 2005); 10, *Stella* (Vasilyeva, 2005). NA, Data not available; v, variable; w, weakly positive.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell shape	Coccobacilli	Cocci, short rods	Cocci	Rods	Rods, coccobacilli	Cocci	Cocci	Cocci	Cocci, cocco-bacilli	Flat stars
Cell size (μm)	$0.7\text{--}1.5 \times 1.4\text{--}4.1$	$0.7\text{--}0.9 \times 0.9\text{--}1.6$	$1.6\text{--}1.8$	$0.3\text{--}1.2 \times 4.2$	$0.5\text{--}0.8 \times 1.0\text{--}2.0$	$0.8\text{--}2.0$	$0.8\text{--}1.5$	$0.9\text{--}1.6$	NA	$0.7\text{--}3.0$
Motility	—	—	+	+	+	—	—	+	v	—
Colony colour	White, cream	Salmon-pink	Purple-red	Pink, red	White, cream, light brown	Pink	Red	Pink	Pale pink	Greyish white
Bacteriochlorophyll <i>a</i>	—	+	+	+	—	+	+	+	+	—
Anaerobic phototrophy	—	—	+	—	—	—	—	—	NA	—
Optimal growth temperature ($^{\circ}\text{C}$)	18–22	30–35	30–35	27–30	31–41	28–32	30–34	25–30	35	28–30
Growth below 5 $^{\circ}\text{C}$	+	—	—	—	—	—	—	—	—	NA
pH range	3.7–7.5	3.5–6.0	4.2–6.5	2.0–5.9	3.0–6.0	>6.8	NA	6–9	NA	NA
Optimal growth pH	5.0–5.7	4.5–5.0	4.8–5.0	3.2–3.5	NA	7.5	6.6–6.8	7–8	NA	7–8
Growth at pH 7	+	—	—	—	—	+	+	+	NA	+
Tolerance of >2.5 % NaCl	—	+	+	+	+	+	+	NA	+	—
Major quinone(s)	Q-10	Q-10	Q-9 (10), MK-9 (10), RQ-9 (10)	Q-10	Q-10	Q-10	Q-10	NA	NA	NA
Major fatty acid	Cyclo $\text{C}_{19:0}\omega 8c$	NA	$\text{C}_{18:1}$	$\text{C}_{18:1}$	$\text{C}_{18:1}$	$\text{C}_{18:1}$	$\text{C}_{18:1}$	NA	$\text{C}_{18:1}$	NA
Diphosphatidylglycerol	+	NA	NA	+	—	NA	NA	NA	NA	NA
Oxidase	+	+	NA	—/w	—/w	+	+	+	v	+
DNA G+C content (mol%)	60.5–61.9	69.1–69.8	66.3	62.9–68.3	58.7–64.4	70.5	70.3–71.0	70.4	65–71	69.3–73.5

Non-spore-forming, non-motile coccobacilli that occur singly, in pairs or in chains and are covered by large capsules. Cells stain Gram-negative and have a Gram-negative cell-wall structure as revealed by electron microscopy. Colonies are circular, smooth, convex and slimy. Colony colour varies from white to cream and pinkish. Bacteriochlorophyll *a* is absent. Produce poly- β -hydroxybutyrate. Oxidase- and catalase-positive. Chemo-organotrophic strict aerobes. Sugars, polyalcohols and some organic acids are the preferred growth substrates. Hydrolyse starch, heparin, xylan and aesculin. Some strains can utilize laminarin, pectin and fucoidan. Dissimilatory nitrate reduction is negative. Moderately acidophilic and psychrotolerant. The major cellular fatty acid is cyclo C_{19:0} ω 8; the major quinone is Q-10. Phosphatidylcholine, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unknown aminolipid are present as major phospholipids. The G + C content of the DNA varies between 60.5 and 61.9 mol%. Member of the class *Alphaproteobacteria*. The type species is *Acidisoma tundrae*.

Description of *Acidisoma tundrae* sp. nov.

Acidisoma tundrae (tun'drae. N.L. gen. fem. n. *tundrae* from the tundra, of the northern zone of Eurasia and North America).

The description is as for the genus but with the following additional traits. Cells are 0.8–1.5 μ m wide and 1.9–4.1 μ m long. Colony colour is white or ivory. Able to utilize the following carbon sources (0.05 %, w/v): sorbose, *myo*-inositol, gluconate, lactate, pyruvate, galacturonic acid, yeast extract, Casamino acids, peptone, asparagine, butyrate, glycerol, arabinose, mannose, glucose, trehalose, dulcitol, ethanol, sorbitol, xylose, fructose, malate, galactose and mannitol. Unable to utilize acetate, propionate, capronate, formate, citrate, benzoate, succinate, methanol, butanol, glucuronic acid, alanine, leucine, maltose, raffinose, fumarate, lactose, oxalate, cellobiose, sucrose or aspartate. Capable of hydrolysing fucoidan and pectin, but not laminarin, pullulan, alginate, carboxymethylcellulose or chondroitin sulfate. The following enzyme activities are present: acid phosphatase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, valine arylamidase, phosphohydrolase and α -glucosidase (API ZYM test). Able to utilize the following nitrogen sources (0.05 %, w/v): ammonia, urea, Bacto yeast extract, histidine HCl, proline, alanine, methionine, glutamine and nitrate. Contains phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown aminolipid as predominant polar lipids. Phosphatidylserine, phosphatidic acid and several unknown polar lipids are present in minor amounts. Resistant to ampicillin and lincomycin, but susceptible to kanamycin, novobiocin, gentamicin, streptomycin, chloramphenicol and neomycin. Capable of growth at pH 3.0–7.5 (optimum, pH 4.5–5.7) and at 2–30 °C (optimum, 15–22 °C). NaCl inhibits growth at concentrations above 1.5 % (w/v).

The type strain, WM1^T (=DSM 19999^T=VKM B-2488^T), was isolated from an acidic oligotrophic *Sphagnum*-dominated wetland in the Vorkuta region of northern Russia.

Description of *Acidisoma sibiricum* sp. nov.

Acidisoma sibiricum (si.bi'ri.cum. N.L. neut. adj. *sibiricum* from Siberia, the region of Russia from where the type strain was isolated).

The description is as for the genus but with the following additional traits. Cells are 0.7–1.2 μ m wide and 1.4–3.1 μ m long. Spirilloxanthin is present in small amounts. Able to utilize the following carbon sources (0.05 %, w/v): sorbitol, arabinose, galactose, fructose, sorbose, *myo*-inositol, mannitol, dulcitol, glycerol, gluconate, pyruvate, galacturonic acid, yeast extract, Casamino acids, peptone, asparagine, xylose and glucose. Utilization of lactose, trehalose, mannose, cellobiose and sucrose is variable. Cannot utilize acetate, propionate, butyrate, capronate, formate, oxalate, citrate, benzoate, succinate, lactate, malate, ethanol, propanol, butanol, glucuronic acid, raffinose, maltose, fumarate, alanine, aspartate or leucine. Does not hydrolyse pullulan, pectin, alginate, carboxymethylcellulose or chondroitin sulfate. The ability to hydrolyse laminarin and fucoidan is variable. The following enzyme activities are present: alkaline phosphatase, acid phosphatase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, valine arylamidase, phosphohydrolase and urease (API ZYM test). Able to utilize the following nitrogen sources (0.05 %, w/v): ammonia, urea, Bacto yeast extract, glutamine, proline, histidine HCl, methionine and nitrate. Utilization of asparagine is variable. Contains phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unknown aminolipid and an unknown polar lipid as major lipids. Phosphatidylserine and several unknown polar lipids are present in minor amounts. Resistant to ampicillin, lincomycin, novobiocin, streptomycin, chloramphenicol and neomycin, but susceptible to kanamycin and gentamicin. Capable of growth at pH 3.7–7.6 (optimum, pH 5.0–6.5) and at 2–30 °C (optimum, 20–25 °C). NaCl inhibits growth at concentrations above 2.5 % (w/v).

The type strain, TPB606^T (=DSM 21000^T=VKM B-2487^T), was isolated from the *Sphagnum* peat bog Bakchar in the Tomsk region of western Siberia. TPB621, isolated from the same locality, is a second strain of the species.

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