Singulisphaera acidiphila gen. nov., sp. nov., a non-filamentous, *Isosphaera*-like planctomycete from acidic northern wetlands

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Four novel strains of budding bacteria, designated MOB10^T, PO2, MPL1015 and BG32, were isolated from acidic wetlands of northern Russia. Cells of these four strains were aerobic, non-motile spheres that occurred singly or in shapeless aggregates and attached to surfaces by means of a holdfast material. The isolates were moderately acidophilic, mesophilic organisms capable of growth between pH 4.2 and 7.5 (optimum growth at pH 5.0–6.2) and at temperatures between 4 and 33 °C (optimum growth at 20–26 °C). The strains possessed a complex intracellular membrane system that compartmentalized the cells. The major fatty acids were C_{16:0}, C_{18:1ω9c} and C_{18:2ω6c,12c}. The major quinone was menaquinone-6 (MK-6). The G+C content of the DNA was 57.8–59.9 mol%. 16S rRNA gene sequence analysis showed that strains MOB10^T, PO2, MPL1015 and BG32 were members of the order *Planctomycetales* and belonged to a phylogenetic lineage defined by the genus *Isosphaera*, exhibiting 90% sequence similarity to the type strain of the thermophilic planctomycete *Isosphaera pallida* and 95–95.5% sequence similarity to a taxonomically uncharacterized group of filamentous bacteria from activated sludge, '*Nostocoida limicola*' III. However, compared with '*Nostocoida limicola*' III and *Isosphaera pallida*, the new isolates from acidic wetlands were non-filamentous, unpigmented bacteria, which possessed highly distinctive phospholipid fatty acid profiles and were capable of growth and of degrading several biopolymers under acidic, microaerobic and cold conditions. The data suggest that the four isolates should be considered as representing a novel species of a new genus of the order *Planctomycetales*, for which the name *Singulisphaera acidiphila* gen. nov., sp. nov. is proposed. The type strain of *Singulisphaera acidiphila* is MOB10^T (=ATCC BAA-1392^T=VKM B-2454^T=DSM 18658^T).

Members of the order *Planctomycetales* are ubiquitous in a wide range of terrestrial and aquatic environments with diverse conditions (Staley *et al.*, 1992; Schlesner, 1994; Fuerst, 1995, 2004; Ward *et al.*, 2006). We recently showed

Abbreviations: FISH, fluorescence *in situ* hybridization; PLFA, phospholipid fatty acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MOB10^T, MPL1015, PO2 and BG32 are AM850678, AM902525, AM902526 and AM902527, respectively.

A graph showing the influence of medium pH on growth of strain MOB10^T is available as supplementary material with the online version of this paper.

that these micro-organisms are highly abundant in acidic *Sphagnum*-dominated northern wetlands (Dedys *et al.*, 2006; Kulichevskaya *et al.*, 2006; Ivanova & Dedys, 2006). Bacterial cells revealed in *Sphagnum* peat by means of fluorescence *in situ* hybridization (FISH) with two phylum-specific 16S rRNA-targeted oligonucleotide probes for planctomycetes, PLA46 and PLA886 (Neef *et al.*, 1998), had ellipsoid or spherical morphology. By using an effective combination of a traditional isolation technique with FISH-based monitoring of the enrichment procedure, we succeeded in isolating several peat-inhabiting, acidophilic planctomycetes in pure culture (Dedys *et al.*, 2006; Kulichevskaya *et al.*, 2006). Three of these isolates were

ellipsoid-shaped and rosette-forming bacteria, which belonged to a phylogenetic lineage defined by the genus *Planctomyces*. They were recently described as representing a novel species of a new genus, *Schlesneria paludicola* (Kulichevskaya *et al.*, 2007). Another morphologically distinct group of isolates from acidic peat was represented by large, non-motile, spherical cells that occurred singly, in pairs or in shapeless aggregates. Similar cell morphology was previously reported for a few *Isosphaera*-like planctomycetes isolated from diverse sources, i.e. from leakage water of a compost heap, a pitcher of *Nepenthes* sp. and a laboratory ampicillin solution (Schlesner, 1994; Ward *et al.*, 1995; Wang *et al.*, 2002). None of these strains has been described taxonomically. Thus, the goal of the present study was to characterize the newly isolated planctomycete strains from acidic wetlands and to determine their taxonomic position.

Strain MOB10^T was obtained from acidic peat (pH 4.2) collected from the upper (0–10 cm) oxic layer of the bog Obukhovskoe, in the Yaroslavl region of northern European Russia (58° 14' N 38° 12' E) (Kulichevskaya *et al.*, 2006). Two further strains, PO2 and MPL1015, were isolated from a peat soil (pH 4.0) sampled at a depth of 10–20 cm (with a water table at approximately 15 cm depth) of the *Sphagnum* peat bog Bakchar, Tomsk region, western Siberia (56° 51' N 82° 50' E). A fourth strain, BG32, was obtained from a peat sample (pH 4.2) collected from the upper (0–10 cm) layer of an unnamed ombrotrophic (rainwater-dependent) bog located within the catchment area of the acidic humic lake Dubrovskoe, Darwin State Preserve, Vologda region, northern European Russia (58° 30' N 37° 80' E). The isolation procedure and culture conditions used were as described by Dedysh *et al.* (2006) and Kulichevskaya *et al.* (2006). The isolates were maintained on agar medium M31 (modification of medium 31 described by Staley *et al.*, 1992) containing (per litre distilled water): 0.1 g KH₂PO₄, 20 ml Hutner's basal salts, 1.0 g *N*-acetylglucosamine, 0.2 g ampicillin (sodium salt), 0.1 g peptone, 0.1 g yeast extract and 15 g agar-agar (Difco), pH 5.8.

Morphological observations and cell size measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). For the preparation of ultrathin sections, cells from 10-day-old agar cultures were fixed in 2% (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 6.8) for 0.5 h and then post-fixed in 1% (w/v) osmium tetroxide in the same buffer for 4 h, dehydrated through a graded ethanol series and embedded in araldite. Thin sections were cut on an LKB-8800 ultramicrotome, stained with lead citrate (Reynolds, 1963) and examined with a transmission electron microscope (JEM-1011; JEOL). For scanning electron microscopy, bacteria were fixed in 2% (w/v) glutaraldehyde in liquid medium M31 without carbon sources for 0.5–1 h and in some cases post-fixed in 1% (w/v) osmium tetroxide in the same medium for 4 h. The samples were then dehydrated through an ethanol series, left in absolute

acetone overnight, critical-point dried (Dryer HCP-2), coated with Au–Pd alloy (IB-3 Ion Coater) and examined with a JEOL JSM-6380LA scanning electron microscope.

Physiological tests were performed in batch cultures grown in liquid medium M31 (in 100 ml flasks containing 20 ml medium). Growth of strains MOB10^T, MPL1015, PO2 and BG32 was monitored by nephelometry at 600 nm in an Eppendorf BioPhotometer for 7–14 days under a variety of conditions, including temperatures of 4–37 °C, pH 3.8–8.0 and NaCl concentrations of 0–3.0% (w/v). Except for temperature experiments, the flasks were incubated on a rotary shaker at 120 r.p.m. and at 24 °C. Variations in pH were achieved by mixing 0.1 M solutions of H₂SO₄ and KOH. At intervals of 1–2 days, the flasks were examined for cell growth by measuring the OD₆₀₀ and pH of the culture liquid. The specific growth rate calculated for each incubation period was correlated with the mean pH observed for a particular flask at the beginning and at the end of the incubation period. Carbon source utilization was determined by using liquid mineral medium (MM) supplemented with the respective carbon source (0.05% w/v). MM contained (per litre distilled water): 0.1 g KH₂PO₄, 0.1 g (NH₄)₂SO₄, 0.1 g MgSO₄·7H₂O and 1 ml of metal salt solution '44' (Staley *et al.*, 1992), with pH adjusted to 5.5. Cultivation was done in 100-ml flasks containing 10 ml medium. Cultures were incubated at 25 °C for 2–3 weeks on a shaker. The ability of the strains to degrade different biopolymers was examined by measuring the rate of CO₂ production in tightly closed 120-ml serum bottles containing 10 ml liquid MM 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, 1981) and by using an API 20 NE kit (bioMérieux). Enzyme activities were examined by using an API ZYM kit (bioMérieux). Nitrogen source utilization was tested by using liquid MM with 0.05% glucose in which (NH₄)₂SO₄ was replaced with one of the following compounds at a concentration of 0.05% (w/v): KNO₃, KNO₂, urea or one of the amino acids listed in Table 1. Cultures were tested for growth under anaerobic conditions in jars by using AnaeroGen anaerobic system envelopes (Oxoid). Susceptibility to antibiotics was determined on M31 agar plates by using discs containing the following: 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) (Oxoid).

For fatty acid, neutral lipid and quinone analyses, cells of the new isolates were grown on liquid medium M31 and then harvested at the late-exponential growth phase. Phospholipid fatty acid (PLFA) and isoprenoid quinone analyses were performed as described for *Schlesneria paludicola* (Kulichevskaya *et al.*, 2007). For neutral lipid analysis, the procedures described by Sinninghe Damsté

Table 1. Phenotypic characteristics of strains MOB10^T, MPL1015, PO2 and BG32

None of the strains showed pigmentation, and all strains grew at 4–33 °C. All strains utilized glucose, fructose, galactose, lactose, cellobiose, maltose, mannose, melibiose, rhamnose, ribose, trehalose, sucrose, xylose, *N*-acetylglucosamine and salicin. All strains were capable of hydrolysing laminarin, pectin, chondroitin sulfate, aesculin, pullulan, lichenan and xylan and were able to liquefy gelatin. None of the strains utilized raffinose, sorbose, glycerol, methanol, ethanol, starch, glucuronic acid, benzoate, caproate, citrate, formate, formaldehyde, fumarate, glutarate, malate, succinate, propionate, mannitol, tartrate, alanine, arginine, asparagine, aspartate, cysteine, cystine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. None of the strains was capable of hydrolysing starch or chitin. All strains utilized ammonia, alanine, aspartate, glutamine, threonine, arginine, tryptophan and glycine as nitrogen sources. None of the strains utilized nitrate, nitrite, urea, cysteine, cystine, histidine, leucine, methionine, norleucine, ornithine, serine or tyrosine as nitrogen sources. ND, Not determined.

Characteristic	MOB10 ^T	MPL1015	PO2	BG32
Cell diameter (µm)	1.6–2.5	1.8–2.3	1.8–2.6	1.7–2.5
Salinity tolerance (% NaCl)	<0.4	<0.3	<0.2	<0.5
pH growth range	4.2–7.2	4.2–7.5	4.2–7.5	4.2–7.5
pH optimum	5.1–6.2	5.0–6.2	5.0–6.1	5.0–6.2
Temperature optimum (°C)	20–28	18–26	15–26	15–26
Carbon source utilization				
Fucose	–	+	+	+
Leucrose	+	+	–	+
Acetate	–	–	–	+
Lactate	+	–	–	+
Pyruvate	+	–	–	+
Nitrogen source utilization				
Asparagine	+	+	–	–
Isoleucine	+	–	–	–
Lysine	–	–	–	+
Phenylalanine	–	–	–	+
Proline	+	–	+	–
Valine	–	–	–	+
DNA G+C content (mol%)	59.9	59.4	57.8	ND

et al. (2004) were used. Genomic DNA from the novel strains was extracted as described by Marmur (1961). The G+C content of the DNA was determined by means of thermal denaturation by using a Unicam SP1800 spectrophotometer at a heating rate of 0.5 °C min⁻¹ and was calculated according to Owen *et al.* (1969). PCR-mediated amplification of the 16S rRNA gene from positions 28 to 1491 (numbering according to the International Union of Biochemistry nomenclature for the *Escherichia coli* 16S rRNA gene) was performed by using primers 9f and 1492r and the reaction conditions described by Weisburg *et al.* (1991). The 16S rRNA gene amplicons were purified by using QIAquick spin columns (Qiagen) and were sequenced on an ABI Prism 377 DNA sequencer with BigDye terminator chemistry, as specified by the manufacturer

(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. 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) via PHYLIP (Felsenstein, 1989).

On agar medium M31, the isolates from *Sphagnum* peat formed raised, opaque, colourless, circular colonies with an entire edge and a smooth surface. At an early stage of isolation, 1-month-old colonies of these bacteria did not exceed 1 mm in diameter; after a series of further transfers on laboratory media at 24 °C, the isolates began to form significantly larger colonies, 2–4 mm in diameter. No gliding motility was observed in cultures of the new isolates. Mature cells of strains MOB10^T, MPL1015, PO2 and BG32 were spherical and typically varied in size from 1.6 to 2.6 µm, although some cells in old cultures were up to 3 µm in diameter (Fig. 1a, b). Cells were non-motile and encapsulated. They reproduced by budding and occurred singly, in pairs or were arranged in shapeless aggregates. No stable cell chains or filaments were observed in cultures of the novel strains. Examination of old cultures grown on agar plates revealed the presence of an amorphous holdfast substance excreted from the poles of many cells (Fig. 1b). Similar holdfast production, which enables the cells to attach to surfaces or to form aggregates by attaching to each other, has been reported for *Pirellula*-like bacteria (Schlesner, 1994).

Thin-sectioned cells of strain MOB10^T displayed the pattern of compartmentalization typical for *Isosphaera*-like planctomycetes (Fig. 1c) (Giovannoni *et al.*, 1987a, b; Lindsay *et al.*, 2001; Liu *et al.*, 2001; Wang *et al.*, 2002; Fuerst, 2005; Ward *et al.*, 2006). Two electron-dense layers separated by an electron-transparent layer were clearly visible in the ultrastructure of the cell wall. These may correspond to a thin glycocalyx-like layer, an outer membrane and the underlying proteinaceous layer. Total wall thickness was about 12 nm. Pit-like structures with a diameter of 25 nm were distributed uniformly over the cell surface. An enlarged view of the cross-section of one of these pit-like structures showed that it represents a cell-wall invagination, which is connected to underlying paryphoplasm (Fig. 1d). The intracytoplasmic membrane compartmentalized the cell to produce irregularly shaped membrane-bound regions ramifying throughout the cytoplasm. The paryphoplasm in cells of strain MOB10^T was invaginated such that this resulted in a large central region and numerous peripheral regions. These regions were filled with an electron-dense substance. Multiple nucleoid zones, possibly from a single folded nucleoid being sectioned more than once, and electron-dense ribosome-like particles were observed in the central part of the cell. Many ribosome-like particles were aligned regularly against the

Fig. 1. Phase-contrast micrographs of cells of strain MOB10^T grown for 10 days in liquid culture (a) and of cells of strain MPL1015 grown for 1 month on agar medium (b). White arrowheads in (b) indicate amorphous holdfast substance excreted by the cells. Bars, 10 μ m. (c) Electron micrograph of an ultrathin section of a cell of strain MOB10^T. CW, Cell wall; ICM, intracytoplasmic membrane; P, paryphoplasm; R, ribosome-like particles; N, nucleoid. Black arrowheads indicate pit-like invaginations of the cell wall [enlarged view in (d)]. Bar, 0.2 μ m. (e) Scanning electron micrograph of cells of strain MOB10^T grown on agar medium M31. B, Bud. Bar, 1 μ m.

intracytoplasmic membrane. Apart from the large central ribosome-containing region, the cells also possessed numerous peripheral electron-transparent areas with scattered ribosomes. Scanning electron microscopy did not reveal any additional structures on the cell surface of the novel strains (Fig. 1e).

Strains MOB10^T, MPL1015, PO2 and BG32 were obligately aerobic chemoheterotrophs. In contrast to other peat-inhabiting planctomycetes of the genus *Schlesneria*, they were not capable of fermenting carbohydrates; however, they grew well under microaerobic conditions.

A list of carbon substrates tested in our experiments and the results of the tests are given in the species description below and in Table 1. Most sugars and *N*-acetylglucosamine were the preferred growth substrates. Organic acids either were not utilized or were utilized poorly by some of the strains. All the new isolates were capable of hydrolysing laminarin, pectin, chondroitin sulfate, aesculin, gelatin, pullulan, lichenan and xylan, but not starch, chitin or cellulose. All the strains showed catalase, cytochrome oxidase and urease activities. Dissimilatory nitrate reduction was negative. Growth factors were not required.

Ammonia, *N*-acetylglucosamine, Bacto peptone, Bacto yeast extract, alanine, aspartate, arginine, glutamine, threonine, tryptophan and glycine were utilized as nitrogen sources. Some of the novel strains were also able to utilize asparagine, isoleucine, lysine, phenylalanine, proline and valine. Nitrate and nitrite were not utilized. The following enzyme activities (API ZYM) were detected in all of the novel strains: alkaline and acid phosphatases, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, valine arylamidase, phosphohydrolase, *N*-acetyl- β -glucosaminidase and β -galactosidase. The following enzyme activities were absent: arginine dihydrolase, lipase, trypsin, chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase and α -mannosidase. All the isolates were resistant to ampicillin, streptomycin, chloramphenicol, lincomycin, kanamycin and novobiocin, but sensitive to neomycin and gentamicin.

Strains MOB10^T, MPL1015, PO2 and BG32 grew in the range pH 4.2–7.5, with optimum growth at pH 5.0–6.2 (Table 1; see also Supplementary Fig. S1, available in IJSEM Online). The temperature range for growth was 4–33 °C, with optimum growth at 20–26 °C (Table 1). The isolates

from wetlands were highly sensitive to salt stress. Growth inhibition of 50–80 % was observed in the presence of NaCl in the medium at concentrations of 0.2–0.5 % (w/v), and NaCl at concentrations above 0.5 % (w/v) completely inhibited growth.

Similar to other described planctomycetes (Sittig & Schlesner, 1993; Ward *et al.*, 2006; Kulichevskaya *et al.*, 2007), the new isolates contained menaquinone-6 (MK-6) as the predominant isoprenoid quinone. The major components of the PLFA profiles of strains MOB10^T, MPL1015, PO2 and BG32 were C_{16:0}, C_{18:1ω9c} and C_{18:2ω6c,12c} (Table 2). One of the strains, BG32, also contained a significant amount (about 22 %) of C_{18:2ω7c,12c}. High levels of C_{16:0} and C_{18:1ω9c} fatty acids are characteristic of members of the family *Planctomycetaceae*, i.e. the genera *Planctomyces* and *Pirellula* (Kerger *et al.*, 1988). However, none of the taxonomically described planctomycetes, including *Isosphaera pallida* (Giovannoni *et al.*, 1987b) or representatives of the recently described genus *Schlesneria* (Kulichevskaya *et al.*, 2007), possess C_{18:2} fatty acids. The PLFA profiles of the novel strains are thus distinct and suggest differentiation at the genus level. The neutral lipids of strains MOB10^T, MPL1015 and PO2 were dominated by an n-C_{31:9} hydrocarbon previously detected in Antarctic bacteria (Nichols *et al.*, 1995) as well as in *Planctomyces limnophilus* and *Schlesneria* species (Kulichevskaya *et al.*, 2007) (Table 3). Squalene and, to a lesser extent, the hopanoids diplopterol and 3-methyl diplopterol were also detected. Upon base hydrolysis of the residue following extraction, in addition to C₁₆ and C₁₈ fatty acids, the

Table 2. Phospholipid fatty acid (PLFA) composition of the novel planctomycete strains from acidic peatlands

Values shown are percentages of total PLFAs. Major components are shown in bold. Components comprising <0.1 % of the total were not included.

Fatty acid	MOB10 ^T	MPL1015	PO2	BG32
C _{14:0}	1.06	1.07	1.50	0.73
C _{15:0}	0.23	0.75	0.26	0.61
iso-C _{16:0}		0.15	0.13	0.14
C _{16:0}	31.12	34.12	36.59	23.89
C _{16:1ω5t}			0.13	
C _{16:1ω9c}		0.13		
C _{16:1ω7c}	1.65	1.66	1.41	1.40
C _{17:0}	0.13	0.41	0.17	0.26
cyclo C _{17:0}	0.43	0.86	0.35	0.89
C _{18:0}	3.51	3.77	2.56	3.39
C _{18:1ω11t}	0.12	0.14		
C _{18:1ω9t}				0.58
C _{18:1ω9c}	46.73	40.84	30.58	23.48
C _{18:1ω7c}	0.79	0.81		0.51
C _{18:2ω7c,12c}				21.72
C _{18:2ω6c,12c}	13.79	15.19	25.58	21.89
C _{20:0}	0.11	0.29	0.22	0.15

Table 3. Major neutral lipids of strains MOB10^T, MPL1015 and PO2

Values shown are percentages of total neutral lipids quantified. Strain BG32 was not included in this analysis.

Compound	MOB10 ^T	MPL1015	PO2
Squalene	28	40	18
n-C _{31:9}	68	54	79
Diplopterol	3	4	2
3-Methyl diplopterol	1	2	1

uncommon 27-hydroxy-octacosanoic acid was released in considerable proportions in all the novel strains.

Comparative 16S rRNA gene sequence analysis showed that strains MOB10^T, PO2, MPL1015 and BG32 were members of the order *Planctomycetales* and belonged to a phylogenetic lineage defined by the genus *Isosphaera* (Fig. 2). However, levels of sequence similarity between the type strain of the only taxonomically described member of this lineage, *I. pallida* – a thermophilic gliding bacterium from hot springs (Giovannoni *et al.*, 1987a) – and the novel isolates from northern wetlands were quite low (about 90 %), suggesting their differentiation at the genus level. The four novel strains possessed nearly identical (99.8–99.9 % similarity) 16S rRNA gene sequences and were most closely related to the taxonomically uncharacterized *Isosphaera*-like isolate Schlesner 640 isolated from a compost leakage water (Ward *et al.*, 1995) (95.5 % sequence similarity), *Isosphaera*-like strain C2-3 from a laboratory ampicillin solution (Wang *et al.*, 2002) (95 % sequence identity) and a group of filamentous planctomycete strains from activated sludge, '*Nostocoida limicola*' III (Liu *et al.*, 2001) (94.5–95.5 % sequence identity). The DNA G + C content of the novel strains ranged from 57.8 to 59.9 mol%. The corresponding value reported for *I. pallida* IS1B^T is 62.2 mol% (Giovannoni *et al.*, 1987a).

Morphologically, the non-filamentous, unpigmented isolates from acidic wetlands were clearly distinct from both *I. pallida* and '*Nostocoida limicola*' III. As no information except a detailed morphological characterization is available for strains of '*Nostocoida limicola*' III, we compared the phenotypic and genotypic features of the novel strains with those of *I. pallida* (Table 4). In contrast to *I. pallida*, the new isolates were non-thermophilic bacteria capable of growth under microaerobic, acidic and cold conditions. They showed hydrolytic capabilities and possessed quite distinct PLFA profiles. We also failed to detect gliding motility and phototactic activity in the novel strains under the conditions described by Giovannoni *et al.* (1987a). Thus, based on significant morphological, phenotypic and genotypic differences between the newly isolated strains and *I. pallida*, we suggest that strains MOB10^T, MPL1015, PO2 and BG32 represent a novel species of a new genus within the order *Planctomycetales*, for which the name *Singulisphaera acidiphila* gen. nov., sp. nov. is proposed.

Fig. 2. Neighbour-joining tree (Jukes-Cantor correction) based on 16S rRNA gene sequences showing the phylogenetic position of strains MOB10^T, MPL1015, PO2 and BG32 in relation to *I. pallida* DSM 9630^T, strains assigned to '*Nostocoida limicola*' III and some other representatives of the phylum Planctomycetes. Bootstrap values (from 1000 data resamplings) >50% are shown. The 16S rRNA gene sequence of *Chlorobium limicola* UdG 6040 (GenBank accession no. Y10642) was used as an outgroup (not shown). Bar, 0.1 substitutions per nucleotide position. The same tree topology was obtained with the maximum-likelihood and maximum-parsimony methods.

Description of *Singulisphaera* gen. nov.

Singulisphaera (Sin.gu.li.sphae'ra. L. adj. *singuli* single, separate; L. fem. n. *sphaera* sphere; N.L. fem. n. *Singulisphaera* a single spherical cell).

Cells are spherical and occur singly, in pairs or in shapeless aggregates. Non-motile. Reproduce by budding. Stalk-like structures are absent. Attach to surfaces by means of amorphous holdfast material. Encapsulated. Colonies are opaque and colourless. Chemo-organotrophic aerobes. Capable of growth under microaerobic conditions. Catalase-, cytochrome oxidase- and urease-positive. Dissimilatory nitrate reduction is negative. Moderately acidophilic and mesophilic. Sensitive to NaCl. The major quinone is MK-6. The major fatty acids are C_{16:0}, C_{18:1ω9c} and C_{18:2ω6c,12c}, the last of these being characteristic for the genus. The genus is a member of the phylum Planctomycetes, order Planctomycetales, family

Planctomycetaceae. The type species is *Singulisphaera acidiphila*.

Description of *Singulisphaera acidiphila* sp. nov.

Singulisphaera acidiphila (a.ci.di'phi.la. N.L. n. *acidum* acid from L. adj. *acidus* sour; Gr. adj. *philos* loving; N.L. fem. adj. *acidiphila* acid-loving).

Has the following characteristics in addition to those given for the genus. Mature cells are 1.6–2.6 μm in diameter. Crateriform pits are scattered over the whole cell surface. Carbon sources (0.05 %, w/v) include glucose, fructose, galactose, lactose, cellobiose, maltose, mannose, melibiose, rhamnose, ribose, trehalose, sucrose, xylose, leucrose, *N*-acetylglucosamine and salicin. Ability to utilize fucose, lactate and pyruvate is variable. Unable to utilize raffinose, sorbose, melezitose, glycerol, methanol, ethanol, starch, glucuronic acid, benzoate, caproate, citrate, formate,

Table 4. Major differential characteristics between *Singulisphaera* gen. nov. and the genus *Isosphaera*

Data for *Isosphaera* were taken from Giovannoni *et al.* (1987a) and Ward *et al.* (2006).

Characteristic	<i>Singulisphaera</i>	<i>Isosphaera</i>
Arrangement of cells	Single or in pairs	Filaments
Colony pigmentation	None	Pink
Gliding motility	—*	+
Phototaxis	—*	+
Respiration	Aerobic or microaerophilic	Strictly aerobic
Hydrolytic capability	+	—
Growth at pH 4.5–5.5	+	—
Growth at 4 °C	+	—
Growth above 35 °C	—	+
Vitamin requirement	—	+
Presence of C _{18:2} fatty acids	+	—
DNA G + C content (mol%)	57–60	62

*Determined under the conditions given by Giovannoni *et al.* (1987a).

formaldehyde, fumarate, glutarate, malate, succinate, propionate, mannitol, tartrate, alanine, arginine, asparagine, aspartate, cysteine, cystine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. Capable of hydrolysing laminarin, pectin, chondroitin sulfate, aesculin, gelatin, pullulan, lichenan and xylan. Unable to hydrolyse starch, chitin or cellulose. Shows the following enzyme activities: alkaline and acid phosphatases, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, valine arylamidase, phosphohydrolase, *N*-acetyl- β -glucosaminidase and β -galactosidase (API ZYM test). Able to utilize the following nitrogen sources (0.05 %, w/v): ammonia, *N*-acetylglucosamine, Bacto peptone, Bacto yeast extract, alanine, aspartate, arginine, glutamine, threonine, tryptophan and glycine. Some strains can also utilize asparagine, isoleucine, lysine, phenylalanine, proline and valine. Nitrate and nitrite are not utilized. Vitamins are not required. Resistant to ampicillin, streptomycin, chloramphenicol, lincomycin, kanamycin and novobiocin, but sensitive to neomycin and gentamicin. Capable of growth between pH 4.2 and 7.5 (optimum at pH 5.0–6.2) and at temperatures between 4 and 33 °C (optimum at 20–26 °C). NaCl inhibits growth at concentrations above 0.5 % (w/v). The G+C content of the DNA varies between 57.8 and 59.9 mol% (59.9 mol% for the type strain).

The type strain, MOB10^T (=ATCC BAA-1392^T =VKM B-2454^T=DSM 18658^T), was isolated from the *Sphagnum* peat bog Obukhovskoe, Yaroslavl region, northern European Russia. Strains PO2, MPL1015 and BG32, isolated from similar acidic wetlands, are also included in the species.

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