

Telmatocola sphagniphila gen. nov., sp. nov., a novel dendriform planctomycete from northern wetlands

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Members of the phylum *Planctomycetes* are common inhabitants of northern wetlands. We used barcoded pyrosequencing to survey bacterial diversity in an acidic (pH 4.0) *Sphagnum* peat sampled from the peat bog Obukhovskoye, European North Russia. A total of 21189 bacterial 16S rRNA gene sequences were obtained, of which 1081 reads (5.1%) belonged to the *Planctomycetes*. Two-thirds of these sequences affiliated with planctomycete groups for which characterized representatives have not yet been available. Here, we describe two organisms from one of these previously uncultivated planctomycete groups. One isolate, strain OB3, was obtained from the peat sample used in our molecular study, while another strain, SP2^T (=DSM 23888^T=VKM B-2710^T), was isolated from the peat bog Staroselsky moss. Both isolates are represented by aerobic, budding, pink-pigmented, non-motile, spherical cells that are arranged in unusual, dendriform-like structures during growth on solid media. These bacteria are moderately acidophilic and mesophilic, capable of growth at pH 4.0–7.0 (optimum pH 5.0–5.5) and at 6–30°C (optimum 20–26°C). The preferred growth substrates are various heteropolysaccharides and sugars, the latter being utilized only if provided in low concentrations ($\leq 0.025\%$). In contrast to other described planctomycetes, strains SP2^T and OB3 possess weak cellulolytic potential. The major fatty acids are C16:1 ω 5c, C18:1 ω 5c, C16:0, and C18:0. Characteristic lipids are the *n*-C31 polyunsaturated alkene (9–10 double bonds) and C30:1/C32:1 (ω -1) hydroxy fatty acids. The G + C content of the DNA is 58.5–59.0 mol%. Strains SP2^T and OB3 share identical 16S rRNA gene sequences, which exhibit only 86 and 87% similarity to those of *Gemmata obscuriglobus* and *Zavarzinella formosa*. Based on the characteristics reported here, we propose to classify these novel planctomycetes as representatives of a novel genus and species, *Telmatocola sphagniphila* gen. nov., sp. nov.

Keywords: the phylum *Planctomycetes*, *Telmatocola sphagniphila* gen. nov., sp. nov., cellulolytic planctomycete, dendriform cell morphology, acidic northern wetlands

INTRODUCTION

The planctomycetes is a ubiquitously distributed and a largely unexplored bacterial phylum, which contains only a limited number of taxonomically characterized representatives (Fuerst, 1995; Ward et al., 2006; Fuerst and Sagulenko, 2011). At present, the list of validly described planctomycetes includes the genera *Planctomyces* (Gimesi, 1924), *Pirellula* (Schlesner and Hirsch, 1987), *Blastopirellula*, *Rhodopirellula* (Schlesner et al., 2004), *Isosphaera* (Giovannoni et al., 1987), *Gemmata* (Franzmann and Skerman, 1984), *Schlesneria* (Kulichevskaya et al., 2007b), *Singulisphaera* (Kulichevskaya et al., 2008), *Zavarzinella* (Kulichevskaya et al., 2009), and *Aquisphaera* (Bondoso et al., 2011). These 10 genera accommodate 16 species, which differ in cell morphology, eco-physiological adaptations, substrate preferences, and other phenotypic and genotypic characteristics. However, all members of these species display a number of common features including a budding mode of cell division, peptidoglycan-less cell walls, and a unique cell organization (Staley et al., 1992; Fuerst, 1995, 2005; Ward et al., 2006). All taxonomically characterized planctomycetes

are chemoheterotrophs; many of them are capable of degrading various heteropolysaccharides but not cellulose or chitin.

Highly distinctive cell morphology is typical for many planctomycetes. Rosettes of spherical cells joined together by non-cellular stalks represent one of the most fascinating planctomycete morphotypes, which is characteristic of *Planctomyces bekefii*. This species has never been isolated in pure culture and was described based solely on its specific morphology (Gimesi, 1924). The same is true for two other species of the genus *Planctomyces*, *P. guttaeformis*, and *P. stranskae* (Starr and Schmidt, 1984). Traditionally, these rosette-forming stalked morphotypes that are commonly observed in various aquatic environments have been classified as belonging to the genus *Planctomyces*. Recently, however, we reported that the presence of a stalk and a similar rosette-like cell arrangement is typical for the novel *Gemmata*-like planctomycete, *Zavarzinella formosa* (Kulichevskaya et al., 2009). The latter bacterium was isolated from acidic *Sphagnum* peat, which is densely colonized by members of the *Planctomycetes* (Dedysh et al., 2006; Kulichevskaya et al., 2006; Ivanova and Dedysh, 2012).

Our further cultivation efforts resulted in the isolation of two other peat-inhabiting, morphologically unique, *Gemmata*-like planctomycetes, strains SP2^T and OB3, from two North European *Sphagnum* peat bogs Staroselsky moss and Obukhovskoye. When grown on solid media, spherical cells of these bacteria were arranged in unusual, dendriform-like structures. The peat sample used for isolating strain OB3 was also used for pyrosequencing-based *Bacteria* diversity analysis. This analysis indicated that microorganisms related to strains SP2^T and OB3 made a significant proportion of peat-inhabiting planctomycetes. In order to characterize phenotypic properties of the newly isolated planctomycetes and to understand their functional role in *Sphagnum* peat, this work was initiated.

MATERIALS AND METHODS

SAMPLING SITES

The acidic (pH 3.8) peat soil used for isolation of strain SP2^T was sampled in August 2008 from the upper oxic layer (5–10 cm) of the *Sphagnum*-dominated ombrotrophic peat bog Staroselsky moss, Tver region, European North Russia (56°34'N, 32°46'E). The second peat sample (pH 4.0), which was used for isolation of strain OB3 and for the molecular diversity analysis, was collected in June 2010 at a depth of 0–10 cm of the *Sphagnum*-dominated, ombrotrophic peat bog Obukhovskoye, Yaroslavl region, European North Russia (58°14'N, 38°120'E). This wetland was described previously (Pankratov et al., 2011). During each sampling event, five subsamples collected from the particular wetland site were combined to form one composite sample. The samples were transported to the laboratory in boxes containing ice packs, homogenized by cutting the peat material into small fragments (about 0.5 cm) with sterile scissors, and used to inoculate enrichment cultures or frozen at –20°C for DNA extraction within 1 day after sampling.

DNA EXTRACTION, PCR AMPLIFICATION, AND PYROSEQUENCING

Three subsamples, each of 0.5 g wet weight, were taken from the peat sample collected from the ombrotrophic bog Obukhovskoye and processed separately. The extraction was performed using a FastDNA SPIN kit for Soil (Bio101, Carlsbad, USA) according to manufacturer's instruction. Polymerase chain reaction was carried out using the *Bacteria*-specific primers 907F (5'-AAA CTY AAA KGA ATT GAC GG-3') and 1392R (5'-ACG GGC GGT GTG TRC-3'; Lane, 1991). The 907F primer included a 454-A pyrosequencing adaptor and a 6-bp barcode sequence, while the 1392R primer incorporated a 454-B pyrosequencing adaptor. PCR amplifications were performed in a DNA thermal cycler (model 9700; PE Applied Biosystems) under the following conditions: initial denaturation (3 min at 95°C); 30 cycles consisting of denaturation (30 s at 95°C), primer annealing (45 s at 55°C), and elongation (90 s at 72°C), with a final elongation step for 8 min at 72°C. All samples were amplified in triplicate, pooled in equal amounts, and purified using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA). Quantification of the PCR products was performed using Quant-iT dsDNA BR assay kit and Qubit fluorometer (Invitrogen GmbH, Karlsruhe, Germany). The purified products were subjected to 454-pyrosequencing at the Max Planck Genome Centre Cologne, Germany.

DATA ANALYSIS

We used Mothur (Schloss et al., 2009) to trim the primer sequences and to remove low quality and short (<400-bp) sequences. The sequences were further refined by removing chimeras with the UCHIME (Edgar et al., 2011) algorithm implemented in Mothur. Taxonomy-based analysis of the resulting sequence pool was made by using Mothur and SILVA-based template database as a reference (Pruesse et al., 2007), at a confidence threshold of 80%. The 16S rRNA gene sequences of representatives of the planctomycetes were retrieved from the total sequence pool and clustered by using Mothur.

ISOLATION AND MAINTENANCE OF STRAINS SP2^T AND OB3

Two grams of wet peat were suspended in 10 ml of sterile water and treated in a laboratory stomacher at 240 rpm for 5 min. The resulting peat suspension was used to inoculate 500-ml serum bottles containing 90 ml of sterile dilute mineral medium M1 of the following composition (gram per liter of distilled water): KH₂PO₄, 0.1; (NH₄)₂SO₄, 0.1; MgSO₄ × 7H₂O, 0.1; CaCl₂ × 2H₂O, 0.02; 1 ml of trace element solution “44” and 1 ml Staley's vitamin solution (Staley et al., 1992); pH 4.8–5.5. The bottles were tightly closed and incubated in the dark at room temperature. After 4 weeks of incubation, 20-μl aliquots of the resulting enrichment cultures were spread plated onto medium M1 containing 0.2 g l⁻¹ of Na-ampicillin and solidified with 10 g Phytigel (Sigma – Aldrich) The plates were then incubated at 22°C for 4 weeks in gastight jars containing 5% CO₂ (v/v) in air, generated by GENbox CO₂ system envelopes (Biome'rieux). Colonies and microbial cell masses that developed on plates were screened microscopically for the presence of cells with planctomycete-like morphology. The selected cell material was re-streaked onto the same medium supplemented with 0.01% yeast extract and 0.025% glucose (medium M1N). This procedure was repeated until the target microorganism was obtained in a pure culture.

Isolates were maintained on medium M1N and were subcultured at 2 month intervals. *Z. formosa* A10^T (Kulichevskaya et al., 2009) and *Gemmata obscuriglobus* DSM 5831^T (Franzmann and Skerman, 1984) were used as the reference strains in our study. *Z. formosa* A10^T was cultivated on agar medium M31 (Kulichevskaya et al., 2009), while *G. obscuriglobus* DSM 5831^T was maintained on medium 629 recommended by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) for cultivation of this bacterium.

MORPHOLOGICAL OBSERVATIONS AND PHYSIOLOGICAL TESTS

Morphological observations and cell size measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss, Germany). Physiological tests were performed in liquid medium M1N. Growth of strains SP2^T and OB3 was monitored by nephelometry at 600 nm in an Eppendorf BioPhotometer for 14–21 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). Variations in the pH were achieved by mixing 0.1M solutions of H₂SO₄ and KOH. Carbon source utilization was determined using mineral medium M1 supplemented with 0.005% yeast extract and respective carbon sources (0.025%, w/v). Cultivation was done in 120-ml flasks containing 10 ml medium.

Cultures were incubated at 22°C for 2–3 weeks on a shaker. The capability to degrade different biopolymers was examined by measuring the rate of CO₂ production in tightly closed 160-ml serum bottles for 1.5 months at 22°C. The serum bottles contained 10 ml of liquid medium M1 with 0.005% yeast extract as a growth factor and 0.05% (w/v) of the corresponding polymer substrate. Control incubations were run in parallel under the same conditions but without a polymer substrate. Cellulolytic capabilities of strains SP2^T and OB3 were determined using sodium carboxymethyl cellulose (Fluka), microcrystalline cellulose (Aldrich), and fibrous cellulose (0.05%, w/v) as carbon sources. Fibrous cellulose was prepared from Whatman filter paper as described by Pankratov et al. (2011). Cellulose degradation was monitored by measuring CO₂ production rates during incubation. CO₂ concentration was measured with a non-dispersive infra-red gas-analyzer “Infralit” (Germany). The reducing sugar product was assayed by the dinitrosalicylic (DNS) method (Miller, 1959), using glucose as the sugar standard. All experiments were performed in triplicate. The purity of strains SP2^T and OB3 in the course of our experiments on cellulose degradation was controlled by means of whole-cell hybridization with Cy3-labeled 16S rRNA-targeted planctomycetes-specific oligonucleotide probes PLA46 and PLA886 (Neef et al., 1998).

Oxidative and fermentative utilization of carbohydrates was determined as described for the Hugh–Leifson test (Gerhardt, 1981). Nitrogen sources were tested using liquid medium M1N in which (NH₄)₂ SO₄ was replaced with one of the following compounds at a concentration of 0.01% (w/v): KNO₃, KNO₂, urea, or one of the amino acids listed in **Table 1**. Analyses of enzymatic profiles, gelatin, and urease hydrolysis, indole production and Hugh–Leifson test were made with API ZYM and API 20NE kits (Biome’rieux). Catalase and oxidase tests were carried out by standard methods (Gerhardt, 1981). Cultures were tested for growth under anaerobic conditions in anaerobic jars by using AnaeroGen anaerobic system envelopes (Oxoid). Susceptibility to antibiotics was determined on solid medium M1N using disks (Oxoid) containing the following antibiotics: ampicillin (10 µg), gentamycin (10 µg), kanamycin (30 µg), neomycin (10 µg), novobiocin (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), and lincomycin (10 µg; Oxoid).

LIPID ANALYSES

For lipid analyses, cells of strains SP2^T and OB3 were grown on liquid medium M1N and harvested in the late exponential growth phase. Lipids were analyzed using the procedures described by Goossens et al. (1989). Briefly, cells were extracted with dichloromethane/methanol (2;1, v/v) and the total extract was hydrolyzed by reflux in 1N methanolic KOH for 1 h. The residue of the extraction was also subjected to base hydrolysis. Fatty acids were methylated with diazomethane in diethyl ether and alcohols were silylated with BSTFA [N,O-Bis(trimethylsilyl)trifluoroacetamide] prior to analysis by gas chromatography and gas chromatography-mass spectrometry. To compare the results with the lipid compositions of *Z. formosa* A10^T and *G. obscuriglobus* DSM 5831^T, we used previously obtained extracts (Kulichevskaya et al., 2009) and performed direct hydrolysis on these extracts.

GENOTYPIC ANALYSES

Genomic DNA from strains SP2^T and OB3 was extracted as described by Marmur (1961). The G + C content of DNA was determined by means of thermal denaturation using a Unicam SP1800 spectrophotometer (UK) at a heating rate of 0.5°C min⁻¹ and calculated according to Owen et al. (1969). DNA of *Escherichia coli* K-12 (G + C value 51.7 mol%) was used as the standard. PCR-mediated amplification of the 16S rRNA gene from positions 28 to 1491 (numbering according to the International Union of Biochemistry nomenclature for *E. coli* 16S rRNA) was performed using primers 9f and 1492r and reaction conditions described by Weisburg et al. (1991). The 16S rRNA gene amplicons were purified using QIAquick spin columns (Qiagen) and sequenced on an ABI Prism 377 DNA sequencer using BigDye terminator chemistry, as specified by the manufacturer (PE Applied Biosystems). Phylogenetic analysis was carried out using the ARB program package (Ludwig et al., 2004).

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

The 16S rRNA gene sequence of *Telmatocola sphagniphila* SP2^T has been deposited in the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession number JN880417.

RESULTS AND DISCUSSION

PYROSEQUENCING-BASED ASSESSMENT OF PLANCTOMYCETE DIVERSITY IN ACIDIC PEAT

A total of 21189 partial (average length ~490 bp) bacterial 16S rRNA gene sequences were obtained from the *Sphagnum* peat sample examined in our study. The major groups of sequences affiliated with the *Acidobacteria* (32.5%), *Proteobacteria* (24.2%), *Actinobacteria* (8.3%), and *Verrucomicrobia* (7.5%; Serkebaeva et al., unpublished data). This pattern of bacterial diversity is typical for *Sphagnum*-dominated wetlands (reviewed in Dedysh, 2011).

The relative abundance of the *Planctomycetes* in the bacterial community according to the pyrosequencing data was 5.1% (1081 reads). The diversity of the planctomycete-related 16S rRNA gene sequences in the amplicon library is illustrated in **Figure 1A** by rarefaction curves showing the expected number of operational taxonomic units encountered with increasing sequencing depth. Rarefaction curves showed a progressive trend to cover diversity but did not reach the asymptote, indicating that we failed to survey the full extent of planctomycete diversity in the particular peat sample.

Taxonomy-based analysis revealed that only 29% of all the planctomycete-related 16S rRNA gene sequences affiliate with phylogenetic lineages defined by taxonomically described organisms, including members of the genera *Isosphaera*, *Pirellula*, *Planctomyces*, *Schlesneria*, and *Singulisphaera* (**Figure 1B**). The most frequently detected organisms were *Isosphaera*-like bacteria (16.7% of all planctomycete-related sequences), which is line with the recently reported data on the planctomycetes diversity in oxic peat layers (Ivanova and Dedysh, 2012). The majority (71%) of all planctomycete-related 16S rRNA gene sequences retrieved from acidic peat could not be assigned to taxonomically characterized organisms. One of the major sequence groups within this unclassified diversity, however, affiliated with strain SP2^T, one of the

Table 1 | Phenotypic characteristics of strains SP2^T and OB3 in comparison to those of *Zavarzinella formosa* A10^T and *Gemmata obscuriglobus* DSM 5831^T.

Characteristic	Strain SP2 ^T	Strain OB3	<i>Z. formosa</i>	<i>G. obscuriglobus</i>
Cell shape	Spherical	Spherical	Ellipsoidal	Spherical to ovoid
Cell size (μm)	1.2–2.0	1.4–2.2	2.5–3.2 × 2.0–2.5	1.4–3.0 × 1.4–3.0
Motile swarm cells	–	–	+	+
Flagellation	–	–	Monotrichous	Polytrichous
Rosette formation	+	+	+	–
Stalk formation	+	+	+	–
Colony color	Pink	Pink	Pink	Rose
Salinity tolerance	<0.1%	<0.1%	<0.6%	<0.6%
Glucose concentration	≤0.025%	≤0.025%	≤0.05%	≤0.1%
pH growth range	4.0–7.0	4.2–7.0	3.8–7.2	7.8–8.8
pH optimum	5.0–5.5	5.0–5.8	5.5–6.0	ND
Temperature range, °C	6–30	6–30	10–30	16–35
Temperature optimum, °C	20–26	20–26	20–25	ND
Oxidase	–	–	+	–
CARBON SOURCES				
Lactose	–	–	+	+
Mannose	+	+	–	+
Sorbose	–	–	+	–
Raffinose	+	–	+	–
<i>N</i> -acetylglucosamine	+	+	+	+
Pyruvate	–	–	+	–
Chondroitin sulfate	–	–	+	+
Pectin	–	–	+	+
Carboxymethyl cellulose	+	+	–	–
Cellulose	W	W	–	–
ENZYMATIC ACTIVITIES				
α-Galactosidase	–	–	–	+
β-Galactosidase	+	+	–	+
Cystine arylamidase	+	+	+	–
Valine arylamidase	+	+	+	–
<i>N</i> -acetyl-β-glucosaminidase	w	W	+	+
α-Mannosidase	–	–	–	+
DNA G + C content, mol%	58.5	59.0	62.5	64.4

All strains utilized *D*-glucose, *D*-galactose, cellobiose, maltose, melizitose, L-rhamnose, ribose, sucrose, trehalose, xylose, salicin, and *N*-acetylglucosamine (strains SP2^T and OB3 < 0.01%). All strains were capable of hydrolyzing laminarin, aesculin, starch, gelatin, lichenan, and xylan. Substrates tested but not utilized: fructose, fucose, glycerol, gluconate, methanol, ethanol, galacturonate, acetate, benzoate, caproate, citrate, formate, formaldehyde, fumarate, glutarate, lactate, malate, succinate, propionate, mannitol, tartrate, alanine, arginine, asparagine, aspartate, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine. None of the strains was capable of hydrolyzing casein, chitosan, and chitin. All strains utilized nitrate, ammonia, Bacto Yeast Extract, glutamine, asparagine as nitrogen sources. All strains were positive for catalase, did not reduce nitrate to nitrite, did not produce indole from tryptophane, and did not ferment glucose. ND, not determined; w, weak growth or weak activity.

as-yet-uncharacterized isolates in our collection of peat-inhabiting planctomycetes. This isolate was obtained in 2008 from the *Sphagnum* peat bog Staroselsky moss, European North Russia, and was phylogenetically identified as a planctomycete distantly related to *G. obscuriglobus* and *Z. formosa* (86 and 87% sequence similarity, respectively; **Figure 2**). Strain SP2^T-related 16S rRNA gene sequences comprised 10% of all the planctomycete-related 454 reads obtained from the peat bog Obukhovskoye, suggesting that strain SP2^T is a typical representative of the planctomycetes community in peat bogs. This finding prompted us address this bacterial group in more detail.

ISOLATION AND IDENTIFICATION

The enrichment approach that implies the use of dilute media and gives a selective advantage to planctomycetes was designed earlier (Hirsch and Müller, 1985; Schlesner, 1994). In our recent experiments on *Sphagnum* moss decomposition, we noticed that moss (or moss-derived peat) can be used as the growth substrate for planctomycetes, which usually develop at the final stage of decomposition process, after several weeks of incubation (Kulichevskaya et al., 2007a). Therefore, we used long-term incubation of a dilute mineral medium inoculated with peat suspension as a specific factor to enrich for peat-inhabiting planctomycetes. Using this

approach, we isolated strain SP2^T from the *Sphagnum* peat bog Staroselsky moss in 2008. Growth requirements of this isolate, however, were somewhat different from those in other characterized peat-inhabiting planctomycetes, and it took us nearly 2 years to optimize its laboratory growth conditions. By that time, we

obtained the second isolate, strain OB3, from another *Sphagnum*-dominated wetland, peat bog Obukhovskoye, using the same isolation approach. *Sphagnum* peat sample used for isolating strain OB3 was also used for the pyrosequencing-based *Bacteria* diversity analysis described above.

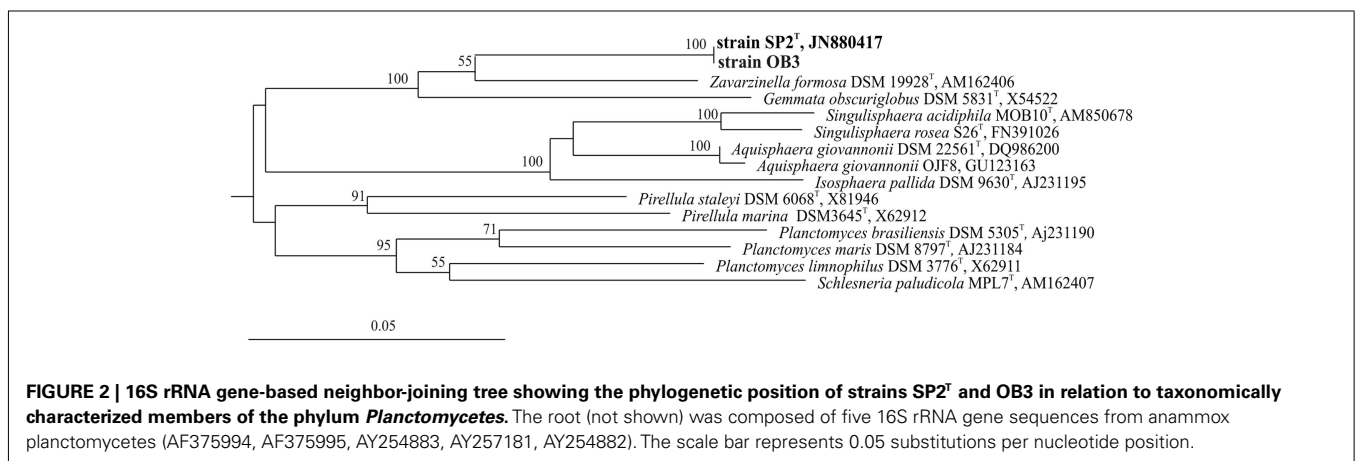
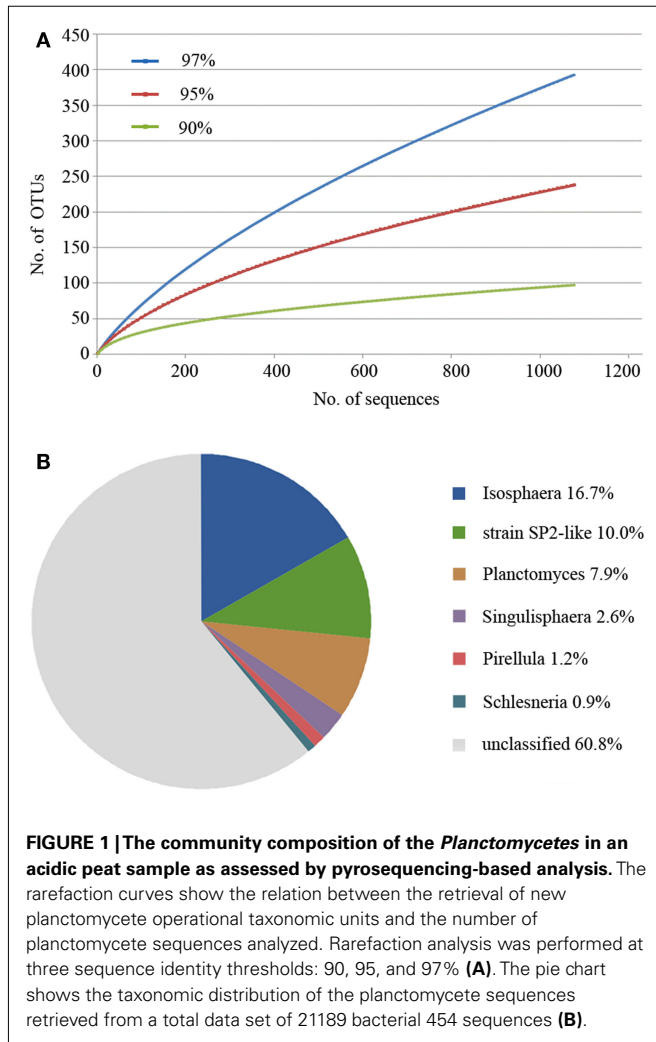
Strains SP2^T and OB3 shared identical 16S rRNA gene sequences (Figure 2) and possessed a highly distinctive morphology. On media solidified with Phytigel, the two novel isolates formed small (1–2 mm), circular, pink-pigmented colonies with an entire edge and a smooth surface. These colonies were composed of non-motile, spherical cells, which varied in size from 1.2 to 2.0 μm and reproduced by budding. In contrast to many other taxonomically characterized planctomycetes, buds of novel isolates were non-motile. On aging, cells formed rosettes or large, dendriform-like structures (Figure 3).

GROWTH SUBSTRATES AND PHENOTYPIC CHARACTERISTICS

At the stage of isolation, strains SP2^T and OB3 developed on a mineral medium solidified with Phytigel, an agar substitute produced from exopolysaccharide of *Sphingomonas elodea*. This polysaccharide is composed of glucuronic acid, rhamnose, and glucose. Presumably, Phytigel was used by strains SP2^T and OB3 as the carbon and energy source. Our attempts to maintain the isolates on agar medium M31 containing 0.1% (w/v) *N*-acetylglucosamine, which was used in our previous studies for cultivation of peat-inhabiting planctomycetes (Kulichevskaya et al., 2007b, 2008, 2009, 2012), were unsuccessful. Long-term growth optimization procedures showed that strains SP2^T and OB3 do not develop on agar media. This is typical for many bacteria from northern wetlands (Dedysh, 2011).

Major phenotypic characteristics of strains SP2^T and OB3 are listed in Table 1. These bacteria were moderately acidophilic, mesophilic organisms capable of growth at pH values between 4.0 and 7.0 with an optimum at pH 5.0–5.5 (Figure 4) and at temperatures between 6 and 30°C (with an optimum at 20–26°C). Similar to phylogenetically related *Z. formosa* and *G. obscuriglobus*, the two novel isolates were obligately aerobic chemoheterotrophs. They were unable to grow in micro-oxic or anoxic conditions. NaCl inhibited growth at concentrations above 0.1% (w/v).

The preferred growth substrates of strains SP2^T and OB3 were various heteropolysaccharides and sugars (Table 1), the latter



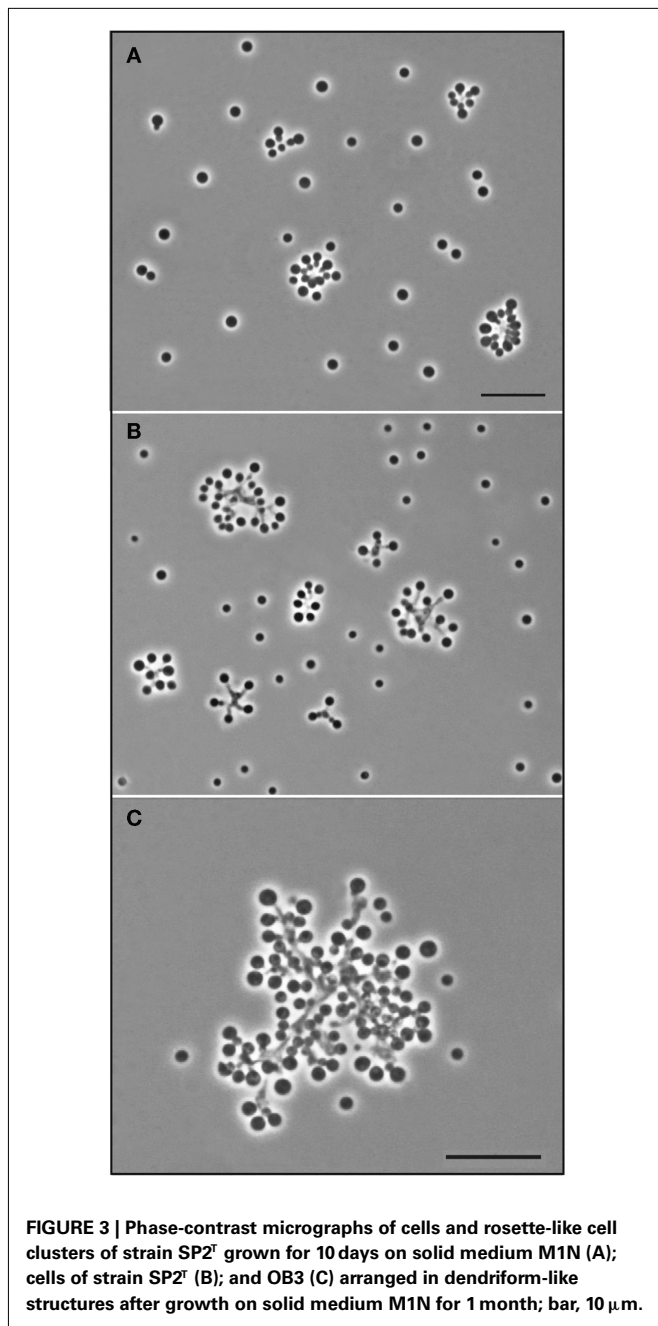


FIGURE 3 | Phase-contrast micrographs of cells and rosette-like cell clusters of strain SP2^T grown for 10 days on solid medium M1N (A); cells of strain SP2^T (B); and OB3 (C) arranged in dendriform-like structures after growth on solid medium M1N for 1 month; bar, 10 μm .

being utilized only if provided in low concentrations [below 0.025% (w/v)]. Growth inhibition of 60–70% was observed in the presence of substrates in the medium at a concentration of 0.05% (w/v), and substrate concentrations of 0.1% (w/v) completely inhibited growth. A similar phenomenon was earlier reported for the filamentous planctomycete *Isosphaera pallida* (Giovannoni et al., 1987). Notably, *N*-acetylglucosamine was also utilized by strains SP2^T and OB3 only if provided in concentrations below 0.01% (w/v). It appears that high concentrations of available substrates, which are provided in most of the routinely employed media, is one of the reasons for low planctomycete recovery in cultivation-based studies.

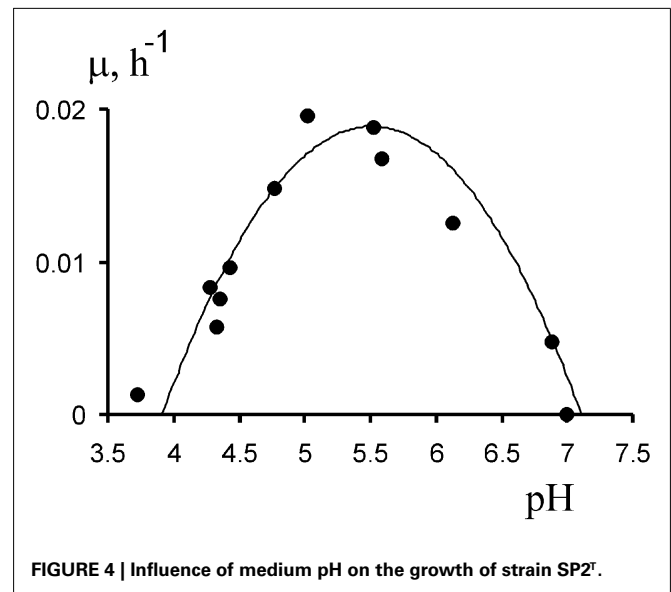
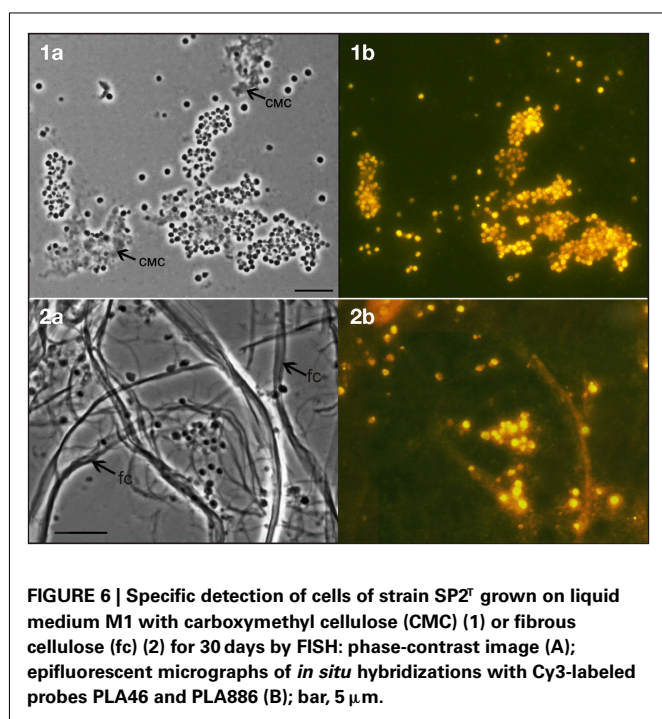
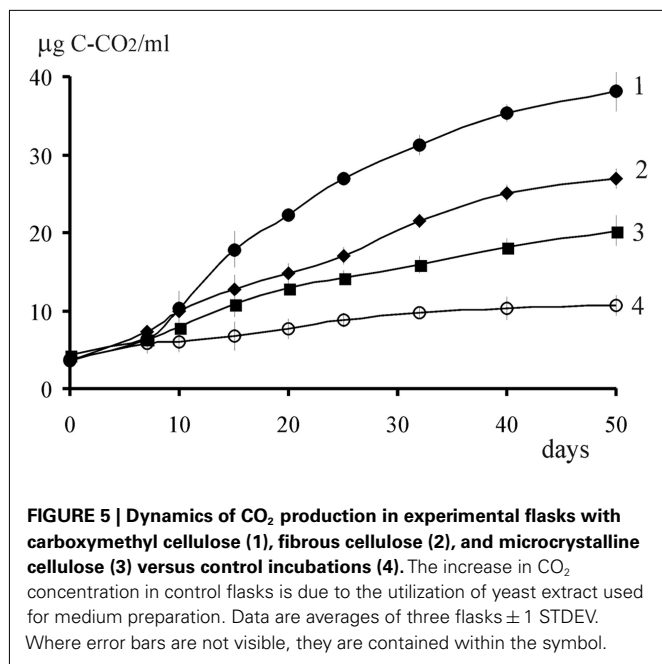


FIGURE 4 | Influence of medium pH on the growth of strain SP2^T.

In contrast to other taxonomically characterized planctomycetes, strains SP2^T and OB3 displayed weak cellulolytic potential. Continuous CO_2 production was detected during incubation of these bacteria with carboxymethyl cellulose, fibrous cellulose, and microcrystalline cellulose (Figure 5). Microscopic examination of the respective culture liquids revealed numerous cells of planctomycetes being attached to micro-particles of carboxymethyl cellulose (Figure 6, panel 1A,B) or micro-fibers of cellulose (Figure 6, panel 2A,B). The concentrations of reducing sugars detected in the medium after 30 days of cultivation comprised 0.071 ± 0.012 , 0.063 ± 0.007 , and 0.069 ± 0.016 mg per ml for carboxymethyl cellulose, fibrous and microcrystalline cellulose, respectively (compared to 0.010 ± 0.005 mg per ml at the beginning of the experiment and after 30 days of incubation in control). These values are one order of magnitude lower than those commonly measured for known fast-growing cellulose degraders. Other methods routinely employed for testing cellulose degrading capabilities in microorganisms, including cellulose disappearance from culture medium, could not be used for strains SP2^T and OB3 due to their very slow growth on this substrate. Obviously, cellulolytic potential of these planctomycetes cannot be compared to that in well-known cellulose degrading bacteria, such as clostridia, bacilli, or *Cytophaga* species. However, it is quite comparable to that in some slow-growing cellulolytic acidobacteria (Pankratov et al., 2011, 2012). It appears that strains SP2^T and OB3 can potentially play a role of slow-acting cellulose decomposers being, nonetheless, incapable of competing with traditionally known cellulose degraders. This hypothesis is supported by the fact that planctomycetes were identified as a numerically abundant component of a bacterial community that developed in the course of *Sphagnum* moss decomposition (Kulichevskaya et al., 2007a). The finding of weak cellulose degrading capabilities in strains SP2^T and OB3 strongly extends our understanding of the biogeochemical role of planctomycetes in peatlands and shows that they can function as slow-acting primary degraders in these ecosystems.



LIPID COMPOSITION

Both free and residually bound lipids of strains SP2^T and OB3 were analyzed and compared with those of *Z. formosa* A10^T and *G. obscuriglobus* DSM 5831^T. The major fatty acids of strain SP2^T and OB3 were C16:1ω5c, C18:0, and C18:1ω5c in both the extract and the residue after extraction (Table 2). This fatty acid profile is clearly distinct from that of *G. obscuriglobus* DSM 5831^T, which does not show C18:1ω5c in high amounts. However, it is not that much different to the profile of *Z. formosa* A10^T, although the

latter contains substantially more of this fatty acid (Table 2). Saturated and monounsaturated C26–C32 (ω-1) hydroxy fatty acids form a characteristic group of lipids of strains SP2^T and OB3 (Table 2). They occur only residually bound. The same series of (ω-1) hydroxy fatty acids was detected in *Z. formosa* A10^T, but not in *G. obscuriglobus* DSM 5831^T (Table 2).

The neutral lipids of strain A10^T are dominated by a polyunsaturated C31 hydrocarbon (Table 2) that has been detected in many other planctomycetes (Kulichevskaya et al., 2007b, 2008, 2009). Parkeol, a characteristic steroid of *G. obscuriglobus* DSM 5831^T (Pearson et al., 2003), was not detected in strains SP2^T and OB3. Overall, the lipid profiles indicate that, based on chemotaxonomic criteria, strains SP2^T and OB3 are closer related to *Z. formosa* A10^T than to *G. obscuriglobus* DSM 5831^T.

In summary, strains SP2^T and OB3 were clearly distinct from *Z. formosa* A10^T and *G. obscuriglobus* DSM 5831^T. As compared to *Gemmata* and *Zavarzinella*, cells of the novel isolates were smaller in size and did not show motility; they displayed cellulolytic activity and utilized sugars and *N*-acetylglucosamine only when these substrates were provided in low concentrations [$<0.025\%$ (w/v) for sugars and $<0.01\%$ (w/v) for *N*-acetylglucosamine]. In addition, the presence of a stalk, formation of rosettes or dendriform-like structures, and ability to grow in acidic conditions differentiated strains SP2^T and OB3 from *G. obscuriglobus*. Unique cell morphology, differences in substrate utilization patterns, and a number of chemotaxonomic and genotypic characteristics suggest that strains SP2^T and OB3 should be considered to represent a novel genus and species of planctomycetes, *Telmatocola sphagniphila* gen. nov., sp. nov. Strain SP2^T is the type strain (=DSM 23888^T = VKM B-2710^T).

DESCRIPTION OF TELMATOCOLA gen. nov.

Telmatocola (Tel.ma.to'co.la. Gr. n. *telma* – atos, swamp, bog; L. masc. or fem. suff. – *cola* (from L. n. *incola*) inhabitant, dweller; N.L.fem. n. *Telmatocola* a bog-dweller).

Spherical cells occur singly, in pairs or are assembled in large rosette-like clusters and dendriform-like structures during growth on solid media. Reproduce by budding. Both daughter and adult cells are non-motile. Produce stalk-like structures. Chemo-heterotrophic aerobe. Obligately oligotrophic. Moderately acidophilic and mesophilic. Major fatty acids are C18:0, C18:1ω5c, and C16:1ω5c. Member of the phylum *Planctomycetes*, order *Planctomycetales*, family *Planctomycetaceae*. The type species is *T. sphagniphila*.

DESCRIPTION OF TELMATOCOLA SPHAGNIPHILA sp. nov.

Telmatocola sphagniphila (sphag.ni'phi.la. N.L. n. *Sphagnum* generic name of sphagnum moss; Gr. adj. *philos* loving; N.L. fem. adj. *sphagniphila* *Sphagnum*-loving).

Exhibit the following properties in addition to those given in the genus description. Colonies are pink. Mature spherical cells are non-motile 1.2–2.0 µm in size. Catalase – positive and cytochrome – oxidase and urease – negative. Dissimilatory nitrate reduction and glucose fermentation are negative. Do not produce indole from tryptophane. Carbon sources (0.025%, w/v) include glucose, galactose, cellobiose, maltose, melizitose, manose, rhamnose, ribose, sucrose, trehalose, xylose, salicin, and

Table 2 | PLFA and neutral lipid composition of strains SP2^T, OB3, Zavarzinella formosa A10^T, and Gemmata obscuriglobus DSM 5831^T (major PLFAs are shown in bold).

% Of total	Strain SP2 ^T		Strain OB3		<i>Z. formosa</i>		<i>G. obscuriglobus</i>	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
FATTY ACIDS								
nC14:0					2.1	1.5		
iC15:0					2.9	2.3		
nC16:1 ω 5c	37.9	18.5	33.9	18.3	18.0	12.2	18.3	17.6
nC16:0	8.0	3.7	12.3	3.7	8.0	5.4	4.8	2.7
iC17:0	1.4	0.6	3.7	1.0	0.8			
aiC17:0			1.0					
nC17:0	0.8	1.4	2.2	2.8				
nC18:1 ω 9c	1.3	0.8	0.7	0.7			0.7	0.7
nC18:1 ω 5c	36.8	15.0	24.8	9.4	48.7	21.2	1.7	1.3
nC18:0	9.6	25.7	16.4	33.2	11.6	15.8	61.1	59.1
nC20:0							1.8	1.7
ω OH-C24:0								16.9
(ω -1)OH-C26:0						1.0		
(ω -1)OH-C28:1		0.6		0.9		9.0		
(ω -1)OH-C28:0				1.6		8.4		
(ω -1)OH-C30:1		28.7		25.2		18.7		
(ω -1)OH-C30:0						3.0		
(ω -1)OH-C32:1		4.8		3.2		1.5		
NEUTRAL LIPIDS								
n-C31:9	4.2		5.0		7.9		8.9	
Parkeol							2.8	

(1) Base hydrolyzed extract.

(2) Base hydrolyzed residue after extraction.

N-acetylglucosamine (<0.01%). Hydrolyze laminarin, aesculin, starch, gelatin, lichenan, gellan-gum, phytigel, xanthan, and xylan. Possess weak cellulolytic potential and capable of slow growth on carboxymethyl cellulose, fibrous, and microcrystalline cellulose. Cannot utilize lactose, fructose, sorbose, fucose, glycerol, gluconate, methanol, ethanol, galacturonate, acetate, benzoate, caproate, citrate, formate, formaldehyde, fumarate, glutarate, lactate, 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, valine. Cannot hydrolyze casein, chondroitin sulfate, pectin, chitosan, and chitin. Shows the following enzyme activities: alkaline and acid phosphatase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase, valine arylamidase, phosphohydrolase, *N*-acetyl- β -glucosaminidase, β -galactosidase (API ZYM test). Utilize nitrate, ammonia, Bacto

Yeast Extract, glutamine, asparagine as nitrogen sources. Sensitive to novobiocin, kanamycin, and gentamicin, but resistant to ampicillin, chloramphenicol, streptomycin, cycloserine, and neomycin. Growth occurs at pH 4.0–7.0 (optimum, pH 5.0–5.5) and at temperatures between 6 and 30°C (optimum, 20–26°C). NaCl inhibits growth at concentrations above 0.1% (w/v). The G + C content of the DNA varies between 58.5 and 59.0 mol% (58.5 mol% for the type strain). *Sphagnum* peat bogs are the main habitat. The type strain is strain SP2^T (=DSM 23888^T = VKM B-2710^T), which was isolated from the *Sphagnum* peat bog Staroselsky moss, Tver region, European North Russia.

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