International Journal of Systematic and Evolutionary Microbiology (2012), **62**, 654–664 **DOI** 10.1099/ijs.0.031898-0

Bryocella elongata gen. nov., sp. nov., a member of subdivision 1 of the *Acidobacteria* isolated from a methanotrophic enrichment culture, and emended description of *Edaphobacter aggregans* Koch *et al.* 2008

Svetlana N. Dedysh,[1] Irina S. Kulichevskaya,[1] Yulia M. Serkebaeva,[1] Maria A. Mityaeva,[1] Vladimir V. Sorokin,[1] Natalia E. Suzina,[2] W. Irene C. Rijpstra[3] and Jaap S. Sinninghe Damsté[3]

Correspondence
Svetlana N. Dedysh
dedysh@mail.ru

[1]S. N. Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow 117312, Russia

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

[3]NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, PO Box 59, 1790 AB Den Burg, The Netherlands

An aerobic, pink-pigmented, chemo-organotrophic bacterium, designated strain SN10^T, was isolated from a methanotrophic enrichment culture obtained from an acidic *Sphagnum* peat. This isolate was represented by Gram-negative, non-motile rods that multiply by normal cell division and form rosettes. Strain SN10^T is an obligately acidophilic, mesophilic bacterium capable of growth at pH 3.2–6.6 (with an optimum at pH 4.7–5.2) and at 6–32 °C (with an optimum at 20–24 °C). The preferred growth substrates are sugars and several heteropolysaccharides of plant and microbial origin, such as pectin, lichenan, fucoidan and gellan gum. While not being capable of growth on C_1 compounds, strain SN10^T can develop in co-culture with exopolysaccharide-producing methanotrophs by utilization of their capsular material. The major fatty acids determined in strain SN10^T using the conventional lipid extraction procedure are iso-$C_{15:0}$ and $C_{16:1}\omega7c$. Upon hydrolysis of total cell material, substantial amounts of the uncommon membrane-spanning lipid 13,16-dimethyl octacosanedioic acid (isodiabolic acid) were also detected. The polar lipids are two phosphohexoses, phosphatidylethanolamine, phosphatidylglycerol and several phospholipids of unknown structure. The major quinone is MK-8. Pigments are carotenoids. The G+C content of the DNA is 60.7 mol%. Strain SN10^T forms a separate lineage within subdivision 1 of the phylum *Acidobacteria* and displays 94.0–95.4 % 16S rRNA gene sequence similarity to members of the genera *Edaphobacter* and *Granulicella*, 93.0–93.7 % similarity to members of the genus *Terriglobus* and 92.2–92.3 % similarity to the type strains of *Telmatobacter bradus* and *Acidobacterium capsulatum*. Therefore, strain SN10^T is classified within a novel genus and species, for which the name *Bryocella elongata* gen. nov., sp. nov. is proposed. Strain SN10^T (=LMG 25276^T =DSM 22489^T) is the type strain of *Bryocella elongata*. An emended description of *Edaphobacter aggregans* Koch *et al.* 2008 is also given.

In contrast to the rapidly growing pool of environmental 16S rRNA gene sequences affiliated with the phylum *Acidobacteria*, the taxonomically described diversity within this group remains very limited. Half of the currently characterized acidobacteria belong to subdivision 1, which at present encompasses the genera *Acidobacterium* (Kishimoto *et al.*, 1991), *Terriglobus* (Eichorst *et al.*, 2007; Männistö *et al.*, 2011), *Edaphobacter* (Koch *et al.*, 2008), *Granulicella* (Pankratov & Dedysh, 2010) and *Telmatobacter* (Pankratov *et al.*, 2012). All cultivated representatives of this subdivision

Abbreviation: IPL, intact polar lipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Bryocella elongata* SN10^T is FR666706.

Three supplementary figures are available with the online version of this paper.

 031898 © 2012 IUMS *Printed in Great Britain*

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

are Gram-negative, mesophilic, acidophilic or acid-tolerant chemo-organotrophs. *Terriglobus*, *Edaphobacter* and *Granulicella* species are strict aerobes, *Acidobacterium capsulatum* is an aerobe capable of poor growth under anoxic conditions and *Telmatobacter bradus* is a facultatively anaerobic bacterium (Pankratov *et al.*, 2012). Members of subdivision 1 of the *Acidobacteria* have been isolated from diverse soils (Janssen *et al.*, 2002; Sait *et al.*, 2002; Joseph *et al.*, 2003; Eichorst *et al.*, 2007; Koch *et al.*, 2008; Stott *et al.*, 2008), acidic mineral environments (Kishimoto *et al.*, 1991), the termite hindgut (Eichorst *et al.*, 2007), *Sphagnum*-dominated wetlands (Pankratov *et al.*, 2008, 2012; Pankratov & Dedysh, 2010) and decaying wood (Folman *et al.*, 2008; Valášková *et al.*, 2009). In one particular case, a member of subdivision 1 of the *Acidobacteria* was recovered from a co-culture with the slime-producing methanotrophic bacterium *Methylocella silvestris*. This acidobacterium, strain Wbg-1^T, was further described as the type strain of *Edaphobacter aggregans* (Koch *et al.*, 2008). Here, we report the characterization of another subdivision 1 acidobacterium, which was also isolated from a methanotrophic enrichment culture and showed an ability to develop in a co-culture with the exopolysaccharide-producing methanotroph *Methylocapsa acidiphila*.

Strain SN10^T was isolated from a methanotrophic enrichment culture that was established from an acidic peat soil (pH 3.8) sampled at a depth of 10 cm of the oligo-mesotrophic fen Torfjanoye, Archangelsk region, European North Russia (65° 01′ N 35° 44′ E), in June 2006. A methanotrophic enrichment culture was obtained from this peat sample using liquid mineral medium M2, pH 5.0 (Dedysh *et al.*, 2000), and incubation with 30 % (v/v) methane. The resulting enrichment was analysed by means of whole-cell hybridization with a set of 16S rRNA-targeted oligonucleotide probes developed for differential detection of type II methanotrophs (Dedysh *et al.*, 2003). According to this analysis, most cells in the enrichment belonged to the species *Methylocapsa acidiphila*. Aliquots of cell suspension from this enrichment were spread-plated onto solid medium M2 prepared with gellan gum (Gel-Gro; ICN Biomedicals) and incubated in closed glass jars with 30 % (v/v) methane. Colonies that developed on these plates after 1 month of incubation were picked and the bacteria were identified by means of partial (~500 bp) sequencing of the 16S rRNA gene. Two isolates obtained from this enrichment, strains V1 and N2, were identified as *Methylocapsa acidiphila* based on 99.9–100 % 16S rRNA gene sequence identity to *Methylocapsa acidiphila* B2^T. Besides these acidophilic methanotrophs, an isolate of pink-pigmented and rod-shaped bacteria was recovered from these plates and designated strain SN10^T. Partial sequencing of the 16S rRNA gene from this isolate showed that it belongs to subdivision 1 of the phylum *Acidobacteria*. Though none of the currently characterized acidobacteria is able to grow on C_1 compounds, a response of these bacteria to amendments with methane and methanol was noticed in several cultivation-independent studies. In a study of Radajewski *et al.* (2002), several 16S rRNA gene sequences affiliated with subdivision 1 of the *Acidobacteria* were retrieved from a ^{13}C-labelled DNA fraction obtained after amendment of acidic soil with ^{13}C-methanol. Another study reported activation of uncultured acidobacteria in Lake Washington sediment in response to methane (Kalyuzhnaya *et al.*, 2008). Finally, an amendment of peat soil with methanol showed an increase in cell numbers of acidobacteria (Pankratov *et al.*, 2008). These results suggested that members of the *Acidobacteria* might be involved in the turnover of C_1 compounds in natural environments. Therefore, the present study was initiated in order to characterize this novel acidobacterium from a methanotrophic enrichment culture and to examine its ability to utilize C_1 substrates.

Once obtained as a pure culture, strain SN10^T was maintained on agar medium MM and was subcultured at 3-week intervals. Medium MM contained (g per litre distilled water): KH_2PO_4, 0.1; $(NH_4)_2SO_4$, 0.1; $MgSO_4 . 7H_2O$, 0.05; $CaCl_2 . 2H_2O$, 0.02; glucose, 0.5; yeast extract, 0.05; with the addition of 0.1 % (v/v) of a trace element stock solution containing (g l^{-1}) EDTA, 5; $FeSO_4 . 7H_2O$, 2; $ZnSO_4 . 7H_2O$, 0.1; $MnCl_2 . 4H_2O$, 0.03; $CoCl_2 . 6H_2O$, 0.2; $CuCl_2 . 5H_2O$, 0.1; $NiCl_2 . 6H_2O$, 0.02; and Na_2MoO_4, 0.03. The pH of the medium was 5.5.

Morphological observations and cell size measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). For preparation of ultrathin sections, cells harvested in the exponential growth stage were pre-fixed with 1.5 % (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 6.5) for 1 h at 4 °C and then fixed in 1 % (w/v) OsO_4 in the same buffer for 4 h at 20 °C. Capsule substances were contrasted by glutaraldehyde/osmium fixation in the presence of ruthenium red (Luft, 1964). After dehydration in an ethanol series, the samples were embedded in a Spurr epoxy resin. Thin sections were cut on an LKB-4800 microtome, mounted on copper grids covered with Formvar film, contrasted with uranyl acetate (3 % solution in 70 % ethanol) for 30 min and then stained with lead citrate (Reynolds, 1963) at 20 °C for 4–5 min. Specimen samples were examined with a JEM-100C electron microscope (JEOL) at an accelerating voltage of 80 kV. X-ray microanalysis of unstained ultrathin sections was performed using a JEM-100CX2 electron microscope (JEOL) equipped with an EM-ASID4D scanning device and a LINK-860 X-ray analyser with an E5423 detector (Link-System). Pigments were extracted from cells of strain SN10^T with acetone/methanol (7 : 2, v/v) and the absorption spectrum of this extract was determined between 250 and 1000 nm using a Cary-50 spectrophotometer (Varian).

Physiological tests were performed in batch cultures grown in liquid medium MM in tightly closed 120 ml serum bottles containing 10 ml medium. With the exception of growth temperature experiments, the flasks were incubated on a rotary shaker at 120 r.p.m. at 24 °C. Variations in pH were achieved by mixing 0.1 M solutions of H_2SO_4 and KOH. Carbon source utilization and the ability of isolates

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

to degrade different biopolymers were determined using liquid medium MM in which glucose was replaced with one of the carbon sources or polymer substrates (0.05 %, w/v). Control incubations were run in parallel under the same conditions but without substrate. Oxidative and fermentative utilization of carbohydrates was determined by using the API 20 NE kit (bioMérieux). Enzyme activities were examined by using the API ZYM kit (bioMérieux). Nitrogen sources were tested using liquid MM medium with 0.05 % glucose in which $(NH_4)_2SO_4$ was replaced with one of the following compounds at a concentration of 0.05 % (w/v): KNO_3, KNO_2, urea or one of the amino acids listed in the species description. Cultures were tested for growth under anaerobic conditions in anaerobic jars by using AnaeroGen anaerobic system envelopes (Oxoid) and also in liquid cultures using MM medium prepared under a nitrogen atmosphere. Nitrate respiration was examined in the same medium by replacing $(NH_4)_2SO_4$ with KNO_3. Susceptibility to antibiotics was determined on MM agar plates using discs 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) (all from Oxoid).

The ability of strain SN10^T to develop in a co-culture with *Methylocapsa acidiphila* N2 was tested in 120 ml serum vials containing 20 ml liquid mineral medium M2. For this experiment, cells of strain SN10^T were grown in liquid medium MM with glucose, while *Methylocapsa acidiphila* N2 was grown in mineral medium M2 with methane. One-week-old cells from both cultures were collected by centrifugation, washed with sterile mineral medium M2 and used to inoculate experimental vials. The first series of vials was inoculated with both cultures, while a second series of vials contained only acidobacterial cells. One additional series of vials contained the spent growth medium from *Methylocapsa acidiphila* N2 instead of medium M2, and was inoculated with cells of strain SN10^T. All incubations were performed in triplicate. Vials were sealed with rubber septa and methane (20 %, v/v) was added aseptically using syringes equipped with disposable filters (0.22 μm). Vials were incubated under static conditions at 20 °C. Once in 2–4 days, the cultures were shaken vigorously and 5 μl cell suspension was spread on an eight-well Teflon-coated slide, air-dried, mounted with a drop of glycerol, covered with a coverslip and viewed with a Zeiss Axioplan 2 microscope. Cells of strain SN10^T and *Methylocapsa acidiphila* N2 could be differentiated easily from each other because of differences in cell morphology. Cell counting was performed on 100 randomly chosen fields of view for each test sample. HPLC analysis of the spent growth medium from *Methylocapsa acidiphila* N2 was made using a Stayer analyser (Aquilon). Culture aliquots of *Methylocapsa acidiphila* N2 were collected in the early- and late-exponential phases as well as in the stationary growth phase (days 6, 14 and 20 of cultivation), cells were removed by centrifugation and the resulting liquid was purified by filtration through 0.22 μm pore-size filters and used for the analysis.

Cell biomass for analyses of cellular fatty acids, intact polar lipids and quinones was obtained from batch cultures of strain SN10^T grown in liquid medium MM as described for *Granulicella* species (Pankratov & Dedysh, 2010). For comparison, *Terriglobus roseus* DSM 18391^T, *Edaphobacter aggregans* DSM 19364^T, *Acidobacterium capsulatum* DSM 11244^T and *Telmatobacter bradus* DSM 23630^T were grown in the same medium under identical growth conditions for 1 week, with the exception of the slowly growing *Telmatobacter bradus* DSM 23630^T, which was collected after 2 weeks of incubation. 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) using an Agilent 6890N gas chromatograph, Sherlock MIS version 4.5 and the library TSBA50 5.00. Intact polar lipids (IPLs) were analysed as described elsewhere (Sinninghe Damsté *et al.*, 2011). Briefly, an Agilent 1200 series LC equipped with thermostatted auto-injector and column oven was used, coupled to a Thermo LTQ XL linear ion trap with Ion Max source with electrospray ionization (ESI) probe (Thermo Scientific). Isoprenoid quinones were extracted according to Collins (1985) and analysed using tandem-type mass spectrometer LCQ ADVANTAGE MAX and ionization mass spectrometer Finnigan Mat 8430 with atmospheric pressure chemical ionization (APCI). Mass spectra were first recorded in MS mode and then analysed using MS/MS mode.

The DNA base composition of strain SN10^T was determined by thermal denaturation using a Unicam SP1800 spectrophotometer at a heating rate of 0.5 °C min^{-1}. The G+C content was calculated according to Owen *et al.* (1969). A nearly full-length (1416 bp) 16S rRNA gene sequence was determined for strain SN10^T as described by Dedysh *et al.* (2000). Phylogenetic analysis was carried out using the ARB program package (Ludwig *et al.*, 2004). Trees were reconstructed using distance-based (neighbour-joining), maximum-likelihood (DNAML) and maximum-parsimony methods.

On agar media, strain SN10^T formed smooth, light-pink, semi-transparent, circular colonies, 2–5 mm in diameter. On media solidified with gellan gum, growth of the isolate was accompanied by the formation of depressions around colonies (Fig. 1a), which is indicative of gellanase production (Kennedy & Sutherland, 1994; Mikolajczak *et al.*, 1994; Sutherland & Kennedy, 1996; Hashimoto *et al.*, 1998). Cells of strain SN10^T were Gram-negative, non-motile, elongated rods, 0.7–1.0 μm wide and 1.7–4.0 μm long. They reproduced by normal cell division and occurred singly or in pairs or were arranged in rosettes (Fig. 1b). The formation of rosettes was most pronounced in old (2 weeks and older) cultures. No resting cell forms were observed.

The main absorption maxima detected in methanol extracts of our novel isolate were at 475, 499 and

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

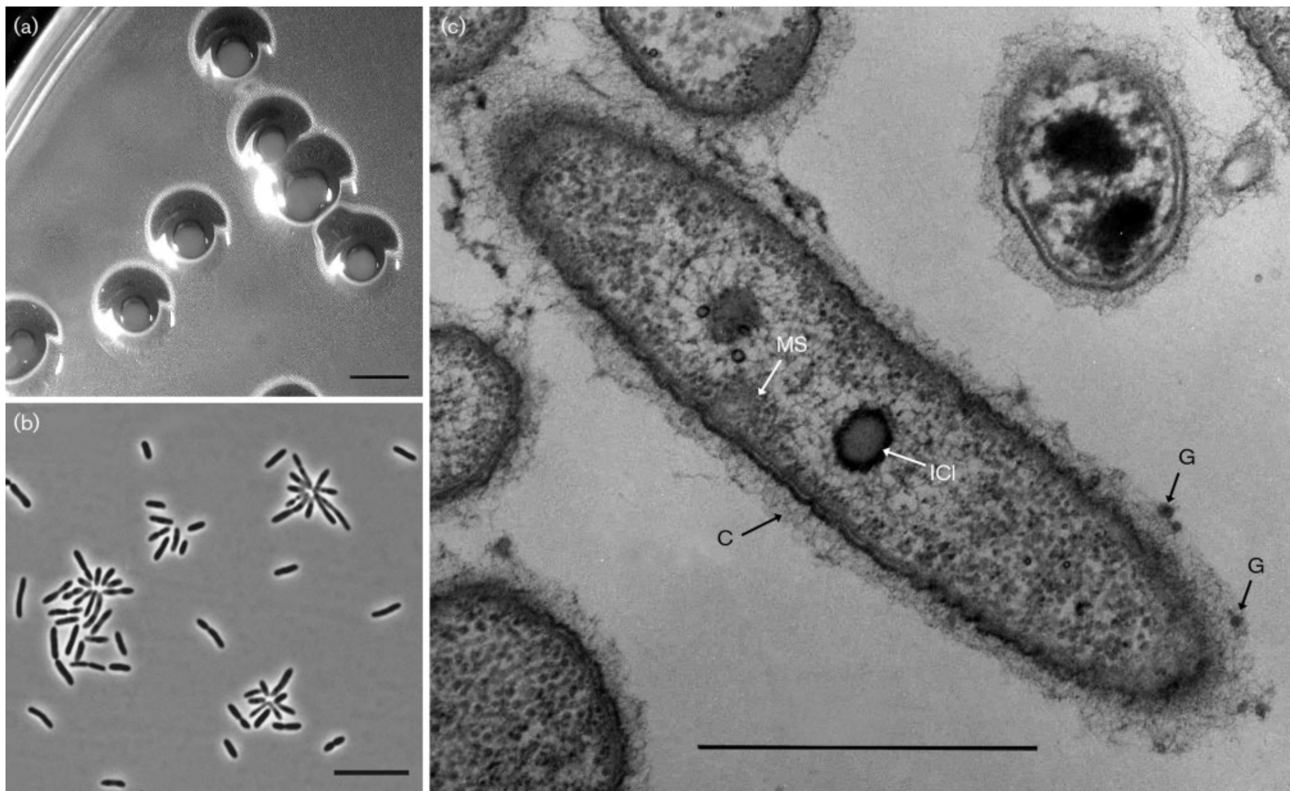

Fig. 1. (a) Development of depressions in gellan-solidified medium during colony growth of strain SN10^T. Bar, 5 mm. (b, c) Phase-contrast (b) and electron micrograph (c) images of an ultrathin section of cells of strain SN10^T in 14-day-old culture. Bars, 10 μm (b) and 1.0 μm (c). ICl, Intracytoplasmic inclusion; C, capsule; G, electron-dense granule; MS, mesosome-like structure.

540 nm, typical of carotenoids and highly similar to the peaks detected in *Terriglobus roseus* (Eichorst *et al.*, 2007) and *Granulicella* species (Pankratov & Dedysh, 2010).

Electron microscopy revealed a cell-wall structure typical of Gram-negative bacteria (Fig. 1c). As revealed by contrasting the acid mucopolysaccharides with ruthenium red, the cells possessed 0.1–0.2 μm thick capsules. The outer surfaces of these capsules were covered with electron-dense granules. A specific feature of cells of strain SN10^T was the presence of intracytoplasmic inclusions. The size and number of these inclusions varied in different cells. Most cells contained one or two large (150–200 nm in diameter) and several small (30–50 nm) inclusions. We attempted to determine the nature of these inclusions by means of X-ray microanalysis. X-ray spectra of the inclusions showed strong signals for P, Ca, Cl and Fe (Supplementary Fig. S1a, available in IJSEM Online). Therefore, the composition of these inclusions in strain SN10^T was somewhat different from that in commonly known polyphosphate granules in other bacteria. X-ray spectra of the cytoplasm as well as of the exopolysaccharide matrix around the cells also showed a detectable signal for Fe (Supplementary Fig. S1b, c). These data suggest that cells of our novel acidobacterium were able to accumulate iron, though the rationale for this behaviour remains unclear. Interestingly, analysis of three recently sequenced genomes of acidobacteria revealed the presence of genes that should enable these organisms to take up iron from the environment, including the genes *feoAB*, which encode a high-affinity ferrous iron transporter, as well as other iron-transporter genes (Ward *et al.*, 2009).

Strain SN10^T was a strictly aerobic chemo-organotroph. Fermentation and anaerobic growth with nitrate were not detected. The isolate grew best on media with various sugars, such as glucose, cellobiose, fructose, galactose, lactose, leucrose, maltose, mannose, melibiose, melezitose, raffinose, rhamnose, sucrose, xylose and *N*-acetylglucosamine. Poor growth was observed on ethanol, fumarate and lactate. Other alcohols and organic acids were not utilized. Strain SN10^T was also capable of growth on several heteropolysaccharides of plant and microbial origin, such as pectin, lichenan, fucoidan and gellan gum, but not on starch, xylan, cellulose, CM-cellulose or chitin. The preferred nitrogen sources were nitrate, ammonia and yeast extract. Strain SN10^T was unable to fix dinitrogen. The complete list of carbon and nitrogen sources tested in our experiments and the results of those tests are given in the species description. Growth factors (50 mg yeast extract l^{-1}) were required.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

Despite its isolation from a methanotrophic enrichment culture, strain SN10^T was unable to utilize any of the C_1 compounds tested in our experiments: methane, methanol, formaldehyde and formate. However, good development of this acidobacterium was observed in co-culture with *Methylocapsa acidiphila* N2, which was isolated from the same methanotrophic enrichment. Since these two bacteria could easily be distinguished from each other by differences in cell morphology (Supplementary Fig. S2), we were able to determine the growth dynamics of strain SN10^T in the presence and absence of *Methylocapsa acidiphila* N2 by means of direct cell counting (Fig. 2). In liquid mineral medium M2 with methane as the only available carbon source, the number of cells of strain SN10^T increased by two orders of magnitude for 2 weeks of growth in a co-culture with *Methylocapsa acidiphila* N2, while no growth of the acidobacterium was observed either in the same medium without this methanotroph or in the spent growth medium from *Methylocapsa acidiphila* N2 collected after 12 days of cultivation (Fig. 2). We also subjected aliquots of the spent growth medium from this methanotroph collected in the early- and late-exponential phases as well as in the stationary growth phase (days 6, 14 and 20 of cultivation) to HPLC analysis, but did not detect the presence of any potential growth substrates for strain SN10^T. These data suggest that development of strain SN10^T probably occurred by utilization of the capsular material produced by the methanotrophic partner in this association. Production of a thick fibrous polysaccharide capsule is one of the characteristic features of the species *Methylocapsa acidiphila* (Dedysh *et al.*, 2002). The structure of this polysaccharide was not identified and, at present, we cannot offer an insight into the mechanism(s) of its utilization by strain SN10^T. The conclusion that development of the acidobacterium resulted from feeding on this capsular material was confirmed indirectly by our failure to establish a co-culture of strain SN10^T with the

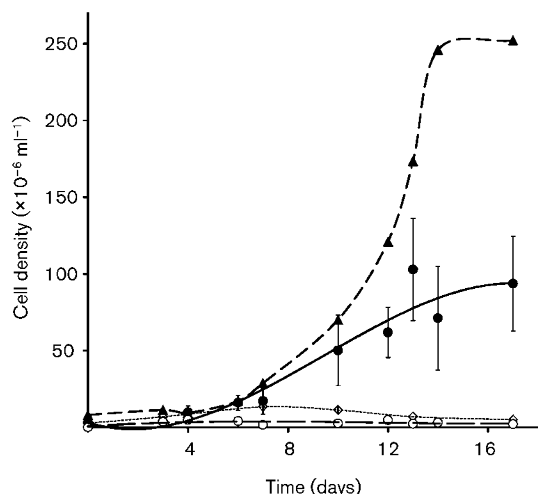

Fig. 2. Dynamics of cell numbers of strain SN10^T in mineral liquid medium M2 with methane as the sole carbon and energy source in pure culture (open circles), in co-culture with *Methylocapsa acidiphila* N2 (filled circles) and in spent growth medium from *Methylocapsa acidiphila* N2 collected after 12 days of methanotroph cultivation (diamonds). Data are means ± SEM from three flasks. Where error bars are not visible, they are contained within the symbol. Growth dynamics of *Methylocapsa acidiphila* N2 are shown by triangles.

Table 1. Whole-cell fatty acid compositions of strain SN10^T and other described representatives of subdivision 1 of the *Acidobacteria*

Strains: 1, SN10^T; 2, *Granulicella* strains (all strains described by Pankratov & Dedysh, 2010); 3, *Terriglobus roseus* DSM 18391^T; 4, *Edaphobacter aggregans* DSM 19364^T; 5, *Acidobacterium capsulatum* DSM 11244^T; 6, *Telmatobacter bradus* DSM 23630^T. Data were obtained in this study and are percentages of total fatty acids. All strains were grown in medium MM (pH 5.5) at 24 °C. Major fatty acids are shown in bold.

Fatty acid	1	2	3	4	5	6
Saturated						
$C_{12:0}$	0.2	–	–	–	–	–
$C_{14:0}$	0.8	1.2–5.2	3.3	1.9	2.3	–
$C_{15:0}$	0.1	0–1.6	–	0.1	–	–
$C_{16:0}$	**7.1**	**6.0–12.5**	**10.6**	**6.0**	**16.3**	1.7
$C_{17:0}$	0.1	0.3–0.9	0.6	–	3.2	–
$C_{18:0}$	1.2	0.3–1.1	0.5	0.2	6.6	0.6
$C_{20:0}$	0.4	0.3–1.7	0.5	0.9	–	–
Unsaturated						
$C_{14:1}\omega 5c$	0.6	0.1–3.4	0.5	2.0	–	–
$C_{15:1}\omega 6c$	0.1	0–1.5	0.6	0.3	–	1.8
$C_{16:1}\omega 5c$	–	0–0.1	0.3	–	–	–
$C_{16:1}\omega 7c$*	**34.2**	**12.7–42.4**	**25.6**	**38.9**	3.1	–
$C_{16:1}\omega 9c$	0.8	–	–	–	–	–
$C_{17:1}\omega 8c$	–	0–0.1	0.4	–	1.9	–
$C_{18:1}\omega 7c$	0.6	0–0.2	0.5	–	–	–
$C_{18:1}\omega 9c$	1.7	–	–	–	**17.3**	–
Methyl-branched						
iso-$C_{11:0}$	–	0–0.5	–	–	–	–
iso-$C_{13:0}$	0.1	0.1–9.7	3.4	–	–	–
iso-$C_{15:0}$	**47.8**	**35.5–59.9**	**46.8**	**46.1**	**42.6**	**69.8**
iso-$C_{17:1}\omega 9c$	1.1	0–1.1	–	1.8	–	–
iso-$C_{17:1}\omega 8c$	–	–	–	–	–	**20.1**
iso-$C_{17:0}$	2.0	0.6–3.6	1.9	1.2	3.1	6.8
Hydroxy						
$C_{12:0}$ 3-OH	0.1	0–0.2	–	–	–	–
iso-$C_{15:0}$ 3-OH	–	0–1.2	1.0	–	–	–
Summed feature						
iso-$C_{15:1}$ I/H and/or $C_{13:0}$ 3-OH	0.3	–	–	0.3	3.7	–

*Determined as summed feature 3 ($C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH) by the DSMZ and identified as $C_{16:1}\omega 7c$ by Sinninghe Damsté *et al.* (2011) using GC/MS.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

capsule-free methanotroph *Methyloferula stellata* AR4[T] (data not shown), which was isolated from the same enrichment culture (Vorobev *et al.*, 2011).

Additional evidence for feeding on methanotroph capsular material is provided by the ability of strain SN10[T] to grow on another polysaccharide of microbial origin, gellan gum. Gellan is the exopolysaccharide produced by *Sphingomonas elodea* and consists of a linear repeating tetrasaccharide [→3)-β-D-Glc-(1→4)–β-D-GlcA-(1→4)–β-D-Glc-(1→4)–α-L-Rha-(1→] composed of D-glucose (Glc), D-glucuronic acid (GlcA) and L-rhamnose (Rha) at a ratio of 2 : 1 : 1 (Jansson *et al.*, 1983). Gellan degradation is catalysed by extracellular, eliminase-type enzymes (gellan lyases or gellanases), which cleave the sequence ...-β-D-Glc-(1→4)–β-D-GlcA-... in the tetrasaccharide repeat unit of the substrate polysaccharides (Kennedy & Sutherland, 1994; Sutherland & Kennedy, 1996). Gellanases occur in various micro-organisms and display different properties and specificity. Formation of depressions around colonies on gellan-solidified media and the ability to grow on gellan as a sole carbon and energy source are indicative of gellanase production by a micro-organism (Kennedy & Sutherland, 1994; Hashimoto *et al.*, 1998). In our study, we examined the ability of different subdivision 1 acidobacteria to form depressions in gellan-solidified media. Only weak gellan-degrading ability was noticed in *Acidobacterium capsulatum* DSM 11244[T] after 6 weeks of incubation, while no depressions appeared around colonies of *Granulicella* type strains, *Terriglobus roseus* DSM 18391[T] or *Edaphobacter aggregans* DSM 19364[T]. Therefore, the gellanase activity was much higher in strain SN10[T] than in other described subdivision 1 acidobacteria. Since gellan-degrading enzymes are inducible in the presence of gellan and β-glucosidase is expressed at higher levels in order to utilize gellan depolymerization products efficiently (Hashimoto *et al.*, 1996), we compared the results of two API ZYM tests with glucose- and gellan-grown cells of strain SN10[T]. Glucose-grown cells displayed high activities of leucyl and valyl arylamidases, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase and relatively low activities of esterases (C4 and C8), β-galactosidase and α- and β-glucosidases. Gellan-grown cells differed from glucose-grown cells in the presence of much higher activities of α- and β-glucosidases, β-galactosidase and β-glucuronidase. These results confirm that the cells grown with gellan utilized depolymerization products of this polysaccharide.

Strain SN10[T] was obligately acidophilic and grew at pH 3.2–6.6 (Supplementary Fig. S3). The highest growth rates, μ=0.02–0.025 h^{-1}, corresponding to doubling times of 28–34 h, were observed at pH 4.7–5.2. The temperature range for growth was 6–32 °C (optimum at 20–24 °C). No growth occurred at 4 or 37 °C. Growth of strain SN10[T] was inhibited in the presence of NaCl in the medium at concentrations above 3.0 % (w/v). This bacterium was resistant to ampicillin, gentamicin, streptomycin, neomycin and chloramphenicol, but sensitive to kanamycin, lincomycin and novobiocin.

Table 2. Relative abundances and fatty acid compositions of IPLs of strain SN10[T] and other described representatives of subdivision 1 of the *Acidobacteria*

Strains: 1, SN10[T]; 2, *Terriglobus roseus* DSM 18391[T]; 3, *Granulicella aggregans* LMG 25274[T]; 4, *Acidobacterium capsulatum* DSM 11244[T]; 5, *Telmatobacter bradus* DSM 23630[T]. Data in columns 2 and 3 were taken from Sinninghe Damsté *et al.* (2011). Abundance is given relative to the major peak in the LC/MS base peak chromatogram: + + +, base peak; + +, 50–100 % of base peak; +, 10–50 % of base peak; −, not detected. Note that the mass spectral response factors for different IPL groups can be quite different. IPLs are listed in order of elution: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; P-hex, phosphohexose. The predominant fatty acid composition is reported, where possible, as the total number of carbon atoms of the acyl moieties and the number of double bonds.

IPL	1	2	3	4	5
Unknown[a]*	−	−	−	−	+ + +
Unknown†	+	+	+	+	+
PE	+ + (30 : 0, 31 : 1, 32 : 2)	+ + + (28 : 0, 30 : 0, 32 : 2)	+ + + (31 : 1, 30 : 0, 32 : 2)	+ + + (30 : 0, 33 : 1)	+ (30 : 0, 32 : 1)
PG	+ (30 : 0, 31 : 1)	−	+ (30 : 0)	+ (33 : 1, 30 : 0)	+ (30 : 0, 34 : 1)
Unknown‡	+	+	+	+	+
PC	−	−	−	+	−
Unknown[b]	+	−	+	+	+
Unknown[c]	−	+ +	−	−	−
P-hex-1	+ + + (30 : 0)	−	+ (30 : 0)	+ (30 : 0)	+ (30 : 0)
P-hex-2	+ + (30 : 0)	+ + (30 : 0)	+ (30 : 0)	−	−
Unknown[d]	−	+	−	−	−

*Characterized by the following molecular ions: *a*, *m*/*z* 522 and 550; *b*, *m*/*z* 708 and 730 (two peaks); *c*, *m*/*z* 1338 (two peaks); *d*, *m*/*z* 1336 (two peaks).

†Loses an *m*/*z* 198 fragment from the molecular ion.

‡Unknown IPL containing four acyl moieties.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

Similarly to other taxonomically described subdivision 1 acidobacteria, strain SN10^T contained menaquinone-8 (MK-8) as the predominant isoprenoid quinone. The major fatty acids determined in strain SN10^T using the conventional lipid extraction procedure were iso-$C_{15:0}$ and $C_{16:1}\omega7c$ (Table 1). High contents of these two fatty acids are also characteristic of the genera *Granulicella*, *Terriglobus* and *Edaphobacter* (Table 1). The fatty acid profile of *Edaphobacter aggregans* DSM 19364^T determined in our study was different from that reported by Koch *et al.* (2008), but Sinninghe Damsté *et al.* (2011) recently reported a fatty acid distribution for this bacterium grown at the DSMZ similar to that reported here. In summary, according to the currently available dataset (Table 1), strain SN10^T and members of the genera *Granulicella*, *Terriglobus* and *Edaphobacter* seem to be nearly indistinguishable based on their cellular fatty acid profiles.

Direct acid hydrolysis of cell material of strain SN10^T released a relatively large amount (26 % of total fatty acids) of the uncommon membrane-spanning lipid 13,16-dimethyl octacosanedioic acid (isodiabolic acid). This characteristic lipid was recently detected in all studied subdivision 1 and 3 acidobacteria (Sinninghe Damsté *et al.*, 2011) and cannot therefore be used to distinguish strain SN10^T from members of *Granulicella*, *Terriglobus* and *Edaphobacter*.

Analysis of IPLs in strain SN10^T by LC/MS revealed the presence of two phosphohexoses as abundant IPLs, phosphatidylglycerol and phosphatidylethanolamine and several unknown IPL groups (Table 2). The IPL profile of strain SN10^T is quite distinct from those of related acidobacterial species (Table 2). The fatty acid composition of the two abundant phosphohexoses is relatively simple ($C_{30:0}$, probably two iso-$C_{15:0}$ moieties) for both strain SN10^T and the other acidobacteria, whilst the phosphatidylglycerol and phosphatidylethanolamine IPLs contain a more complex suite of fatty acids (Table 2).

Strain SN10^T formed a separate lineage within subdivision 1 of the phylum *Acidobacteria* (Fig. 3) and displayed 94.4–95.4 % 16S rRNA gene sequence similarity to members of the genus *Edaphobacter*, 94.0–94.8 % to members of the genus *Granulicella*, 93.0–93.7 % to members of the genus *Terriglobus*, 92.3 % to *Acidobacterium capsulatum* JCM 7670^T and 92.2 % to *Telmatobacter bradus* TPB6017^T. The

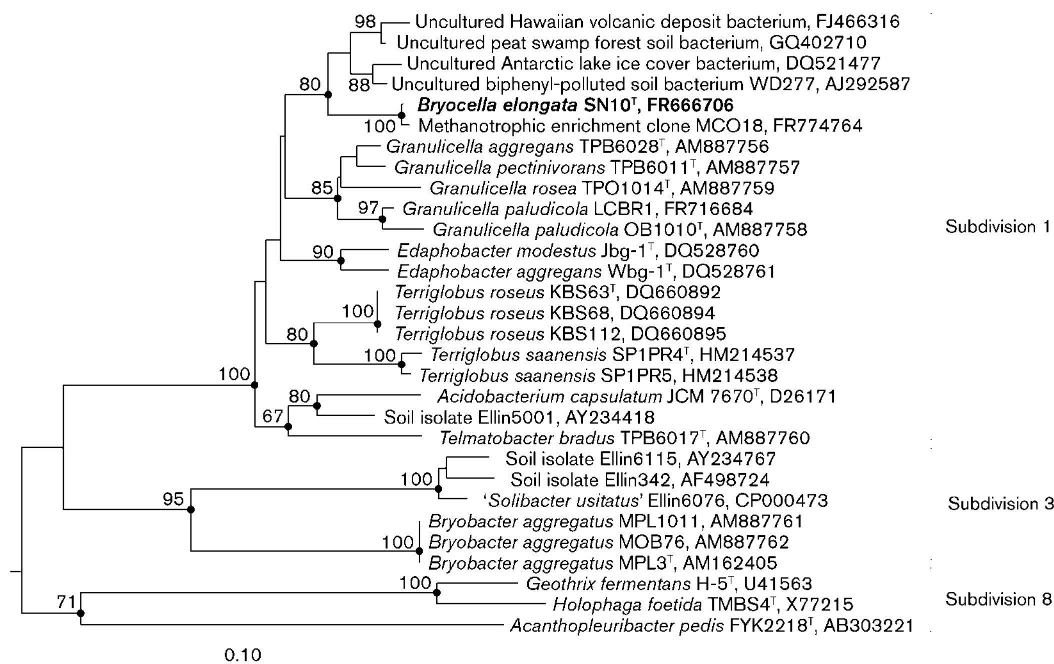

Fig. 3. 16S rRNA gene sequence-based neighbour-joining tree (Jukes–Cantor correction) showing phylogenetic relationships of strain SN10^T to taxonomically characterized representatives and some non-described members of the phylum *Acidobacteria*. The tree was calculated based on 1329 nt positions using the filter implemented in ARB for the phylum *Acidobacteria*. Significance levels of interior branch points obtained in neighbour-joining analysis were determined by bootstrap analysis (1000 data resamplings) using PHYLIP (Felsenstein, 1989). Bootstrap values (1000 data resamplings) >50 % are shown. Filled circles indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. Six members of the *Planctomycetes* were used as an outgroup (not shown): *Isosphaera pallida* DSM 9630^T (GenBank accession no. AJ231193), *Gemmata obscuriglobus* UQM 2246^T (X54522), *Planctomyces brasiliensis* DSM 5305^T (AJ231190), *Planctomyces maris* DSM 8797^T (AJ231184), *Schlesneria paludicola* MPL7^T (AM162407) and *Singulisphaera acidiphila* ATCC BAA-1392^T (AM850678). Bar, 0.1 substitutions per nucleotide position.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

Table 3. Major characteristics that distinguish strain SN10^T (*Bryocella* gen. nov.) from related genera

Unless indicated, data for reference genera were taken from Pankratov & Dedysh (2010) (*Granulicella*), Eichorst *et al.* (2007) (*Terriglobus*), Koch *et al.* (2008) (*Edaphobacter*), Kishimoto *et al.* (1991) and Pankratov *et al.* (2012) (*Acidobacterium*) and Pankratov *et al.* (2012) (*Telmatobacter*). ND, No data available.

Characteristic	*Bryocella*	*Granulicella*	*Terriglobus*	*Edaphobacter*	*Acidobacterium*	*Telmatobacter*
Source(s) of isolation	*Sphagnum* peat	*Sphagnum* peat and *Cladonia* sp.	Soil and termite hindgut	Alpine and forest soil	Acidic mineral environment	*Sphagnum* peat
Cell length (μm)	1.7–4.0	1.5–15	1.0–1.2	1.0–2.1	1.1–2.3	2–10
Motility	−	−	−	+*	+	+
Formation of rosettes	+	−	−	−	−	−
Copious EPS production	+	+	+	−	−	−
Structured capsule	+	−	−	−	ND	−
Pigment	Light pink	Pink to red	Pink or none	None	Orange	None
pH range	3.2–6.6	3.0–7.5	5.0–7.0	4.0–7.0	3.0–6.0	3.0–7.5
pH optimum	4.7–5.2	3.8–4.5	5.0–6.0	5.5	ND	4.5–5.0
Growth at below pH 4	+	+	−	−	+	+
Growth at 3.5 % NaCl	−	+	−	−	−	−
Anaerobic growth	−	−	−	−	+	+
Cellulose degradation	−	−	−	−	−	+
Gellan gum hydrolysis	+	−	−	−	Weak†	−
Phosphohexoses as major polar lipids	+	−	−	−	−	−
DNA G+C content (mol%)	60.7	57.3–59.3	58.1–59.8	55.8–56.9	59.9–60.8	57.6

*Positive for *E. modestus* Jbg-1^T only (Koch *et al.*, 2008).
†Determined in this study.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

phylogenetic lineage defined by strain SN10^T included several 16S rRNA gene sequences detected by cultivation-independent methods in various habitats, including soils (GenBank accession numbers AJ292587 and GQ402710), volcanic deposits (FJ466316) and Antarctic lake ice (DQ521477). Interestingly, the most closely related environmental 16S rRNA gene sequence in this cluster (FR774764; 99 % 16S rRNA gene similarity to strain SN10^T) was retrieved from another methanotrophic consortium enriched from the ombrotrophic peat bog Obukhovskoye, Yaroslavl region, European North Russia (58° 14′ N 38° 12′ E) (Y. M. Serkebaeva and S. N. Dedysh, unpublished). The phylogenetic cluster containing strain SN10^T and related clones remained stable independently of the algorithm used for the tree construction. This suggests a wide distribution of strain SN10^T-like organisms in diverse environments and highlights methane-oxidizing microbial communities as a specific ecological niche for development of these bacteria. The DNA G+C content of strain SN10^T was 60.7 mol%.

In summary, our novel isolate from a methanotrophic enrichment culture formed a phylogenetic lineage that was separate from the lineages defined by the genera *Granulicella*, *Terriglobus*, *Edaphobacter*, *Acidobacterium* and *Telmatobacter* (Fig. 3) and displayed a number of features that clearly distinguished it from all previously described subdivision 1 acidobacteria (Table 3). Formation of rosettes, a strongly pronounced ability to degrade gellan gum and the possession of phosphohexoses as major polar lipids made strain SN10^T different from all other currently described members of this subdivision. Cells of strain SN10^T were longer than those of members of *Terriglobus*, *Edaphobacter* and *Acidobacterium*, and the strain did not produce globular starvation forms typical of the genus *Granulicella*. Pink pigmentation distinguished our strain from members of *Edaphobacter*, *Telmatobacter* and *Acidobacterium*. Absence of motility and the inability to develop under anoxic conditions distinguished the novel isolate from members of *Acidobacterium* and *Telmatobacter*. Its ability to grow at below pH 4 distinguished strain SN10^T from members of *Terriglobus* and *Edaphobacter*, while its inability to develop at above pH 7 distinguished it from the genus *Granulicella*. We therefore propose that strain SN10^T should be classified in a novel genus and species, *Bryocella elongata* gen. nov., sp. nov.

Description of *Bryocella* gen. nov.

Bryocella [Bry.o.cel′la. Gr. neut. n. *bryon* moss; L. fem. n. *cella* a storeroom, chamber and, in biology, a cell; N.L. fem. n. *Bryocella* moss(-associated) cell].

Cells are non-motile, Gram-negative rods that occur singly, in pairs or in rosettes. Cells are covered with capsules. Reproduce by normal cell division. The colony colour is light pink. Pigments are carotenoids. Oxidase-negative and catalase-positive. Strictly aerobic chemo-organotrophs. Sugars and heteropolysaccharides are the preferred growth substrates. Acidophilic and mesophilic. Major fatty acids are iso-$C_{15:0}$ and $C_{16:1}\omega7c$. Major polar lipids are phosphohexoses, phosphatidylglycerol and phosphatidylethanolamine. The quinone is MK-8. Member of subdivision 1 of the phylum *Acidobacteria*. Contains one species, *Bryocella elongata*, which is the type species of the genus.

Description of *Bryocella elongata* sp. nov.

Bryocella elongata (e.lon.ga′ta. L. fem. part. adj. *elongata* elongated, stretched out, pertaining to the elongated cell shape).

Description is as for the genus with the following additional information. Cells are 0.7–1.0 μm wide and 1.7–4.0 μm long. Formation of rosettes occurs in old cultures. Carbon sources utilized (0.05 %, w/v) include glucose, cellobiose, fructose, galactose, lactose, leucrose, maltose, mannose, melibiose, melezitose, raffinose, rhamnose, sucrose, xylose, *N*-acetylglucosamine, fumarate, lactate, and ethanol. Unable to utilize trehalose, sorbose, salicin, dulcitol, glycerol, glucuronic acid, acetate, benzoate, citrate, malate, succinate, propionate, methane, methanol, formaldehyde and formate. Capable of hydrolysing pectin, lichenan, fucoidan, chondroitin sulphate and gellan gum. Unable to hydrolyse starch, xylan, cellulose, CM-cellulose or chitin. Cells show the following enzyme activities (API ZYM test): β-galactosidase, acid phosphatase, esterases (C4 and C8), leucyl and valyl arylamidases, naphthol-AS-BI-phosphohydrolase, α- and β-glucosidases, *N*-acetyl-β-glucosaminidase and β-glucuronidase. Able to utilize the following nitrogen sources: ammonia, nitrate, Bacto peptone, Bacto yeast extract, glutamine, threonine and tyrosine. Nitrite, urea, dinitrogen gas, methionine, proline, tryptophan and valine are not utilized. Resistant to ampicillin, gentamicin, streptomycin, neomycin and chloramphenicol, but sensitive to kanamycin, lincomycin and novobiocin. Growth factors are required. Growth occurs at pH 3.2–6.6 (optimum at pH 4.7–5.2) and at 6–32 °C (optimum at 20–24 °C). NaCl inhibits growth at concentrations above 3 % (w/v). The DNA G+C content of the type strain is 60.7 mol%.

The type strain, SN10^T (=LMG 25276^T =DSM 22489^T), was isolated from a methanotrophic enrichment culture obtained from a *Sphagnum* peat bog in Archangelsk region, northern Russia.

Emended description of *Edaphobacter aggregans* Koch *et al.* 2008

The description of *Edaphobacter aggregans* is as given by Koch *et al.* (2008) with the following modifications. Major fatty acids are iso-$C_{15:0}$ and $C_{16:1}\omega7c$.

Acknowledgements

This research was supported by the Programs 'Molecular and Cell Biology' of Russian Academy of Sciences and the Russian Fund of

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

Basic Research (grants no. 09-04-00004 and 11-04-91333). The authors thank E. N. Detkova for DNA G+C content analysis.

References

Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.

Dedysh, S. N., Liesack, W., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. A., Semrau, J. D., Bares, A. M., Panikov, N. S. & Tiedje, J. M. (2000). *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. *Int J Syst Evol Microbiol* **50**, 955–969.

Dedysh, S. N., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. A., Semrau, J. D., Liesack, W. & Tiedje, J. M. (2002). *Methylocapsa acidiphila* gen. nov., sp. nov., a novel methane-oxidizing and dinitrogen-fixing acidophilic bacterium from *Sphagnum* bog. *Int J Syst Evol Microbiol* **52**, 251–261.

Dedysh, S. N., Dunfield, P. F., Derakshani, M., Stubner, S., Heyer, J. & Liesack, W. (2003). Differential detection of type II methanotrophic bacteria in acidic peatlands using newly developed 16S rRNA-targeted fluorescent oligonucleotide probes. *FEMS Microbiol Ecol* **43**, 299–308.

Eichorst, S. A., Breznak, J. A. & Schmidt, T. M. (2007). Isolation and characterization of soil bacteria that define *Terriglobus* gen. nov., in the phylum *Acidobacteria. Appl Environ Microbiol* **73**, 2708–2717.

Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.

Folman, L. B., Klein Gunnewiek, P. J. A., Boddy, L. & de Boer, W. (2008). Impact of white-rot fungi on numbers and community composition of bacteria colonizing beech wood from forest soil. *FEMS Microbiol Ecol* **63**, 181–191.

Hashimoto, W., Inose, T., Nakajima, H., Sato, N., Kimura, S. & Murata, K. (1996). Purification and characterization of microbial gellan lyase. *Appl Environ Microbiol* **62**, 1475–1477.

Hashimoto, W., Sato, N., Kimura, S. & Murata, K. (1998). Polysaccharide lyase: molecular cloning of gellan lyase gene and formation of the lyase from a huge precursor protein in *Bacillus* sp. GL1. *Arch Biochem Biophys* **354**, 31–39.

Janssen, P. H., Yates, P. S., Grinton, B. E., Taylor, P. M. & Sait, M. (2002). Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. *Appl Environ Microbiol* **68**, 2391–2396.

Jansson, P.-E., Lindberg, B. & Sandford, P. A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*. *Carbohydr Res* **124**, 135–139.

Joseph, S. J., Hugenholtz, P., Sangwan, P., Osborne, C. A. & Janssen, P. H. (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. *Appl Environ Microbiol* **69**, 7210–7215.

Kalyuzhnaya, M. G., Lidstrom, M. E. & Chistoserdova, L. (2008). Real-time detection of actively metabolizing microbes by redox sensing as applied to methylotroph populations in Lake Washington. *ISME J* **2**, 696–706.

Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of the coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.

Kennedy, L. & Sutherland, I. W. (1994). Gellan lyases – novel polysaccharide lyases. *Microbiology* **140**, 3007–3013.

Kishimoto, N., Kosako, Y. & Tano, T. (1991). *Acidobacterium capsulatum* gen. nov., sp. nov.: an acidophilic chemoorganotrophic bacterium containing menaquinone from acidic mineral environment. *Curr Microbiol* **22**, 1–7.

Koch, I. H., Gich, F., Dunfield, P. F. & Overmann, J. (2008). *Edaphobacter modestus* gen. nov., sp. nov., and *Edaphobacter aggregans* sp. nov., acidobacteria isolated from alpine and forest soils. *Int J Syst Evol Microbiol* **58**, 1114–1122.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Luft, J. H. (1964). Electron microscopy of cell extraneous coats as revealed by ruthenium red staining. *J Cell Biol* **23**, 54A–55A.

Männistö, M. K., Rawat, S., Starovoytov, V. & Häggblom, M. M. (2011). *Terriglobus saanensis* sp. nov., an acidobacterium isolated from tundra soil. *Int J Syst Evol Microbiol* **61**, 1823–1828.

Mikolajczak, M. J., Thorne, L., Pollock, T. J. & Armentrout, R. W. (1994). Sphinganase, a new endoglycanase that cleaves specific members of the gellan family of polysaccharides. *Appl Environ Microbiol* **60**, 402–407.

Owen, R. J., Hill, L. R. & Lapage, S. P. (1969). Determination of DNA base compositions from melting profiles in dilute buffers. *Biopolymers* **7**, 503–516.

Pankratov, T. A. & Dedysh, S. N. (2010). *Granulicella paludicola* gen. nov., sp. nov., *Granulicella pectinivorans* sp. nov., *Granulicella aggregans* sp. nov. and *Granulicella rosea* sp. nov., acidophilic, polymer-degrading acidobacteria from *Sphagnum* peat bogs. *Int J Syst Evol Microbiol* **60**, 2951–2959.

Pankratov, T. A., Serkebaeva, Y. M., Kulichevskaya, I. S., Liesack, W. & Dedysh, S. N. (2008). Substrate-induced growth and isolation of *Acidobacteria* from acidic *Sphagnum* peat. *ISME J* **2**, 551–560.

Pankratov, T. A., Kirsanova, L. A., Kaparullina, E. N., Kevbrin, V. V. & Dedysh, S. N. (2012). *Telmatobacter bradus* gen. nov., sp. nov., a cellulolytic facultative anaerobe from subdivision 1 of the *Acidobacteria*, and emended description of *Acidobacterium capsulatum* Kishimoto *et al.* 1991. *Int J Syst Evol Microbiol* **62**, 430–437.

Radajewski, S., Webster, G., Reay, D. S., Morris, S. A., Ineson, P., Nedwell, D. B., Prosser, J. I. & Murrell, J. C. (2002). Identification of active methylotroph populations in an acidic forest soil by stable-isotope probing. *Microbiology* **148**, 2331–2342.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* **17**, 208–212.

Sait, M., Hugenholtz, P. & Janssen, P. H. (2002). Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. *Environ Microbiol* **4**, 654–666.

Sinninghe Damsté, J. S., Rijpstra, W. I. C., Hopmans, E. C., Weijers, J. W. H., Foesel, B. U. F., Overmann, J. & Dedysh, S. N. (2011). 13,16-Dimethyl octacosanedioic acid (*iso*-diabolic acid), a common membrane-spanning lipid of *Acidobacteria* subdivisions 1 and 3. *Appl Environ Microbiol* **77**, 4147–4154.

Stott, M. B., Crowe, M. A., Mountain, B. W., Smirnova, A. V., Hou, S., Alam, M. & Dunfield, P. F. (2008). Isolation of novel bacteria, including a candidate division, from geothermal soils in New Zealand. *Environ Microbiol* **10**, 2030–2041.

Sutherland, I. W. & Kennedy, L. (1996). Polysaccharide lyases from gellan-producing *Sphingomonas* spp. *Microbiology* **142**, 867–872.

Valášková, V., de Boer, W., Klein Gunnewiek, P. J. A., Pospíšek, M. & Baldrian, P. (2009). Phylogenetic composition and properties of bacteria coexisting with the fungus *Hypholoma fasciculare* in decaying wood. *ISME J* **3**, 1218–1221.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15

Vorobev, A. V., Baani, M., Doronina, N. V., Brady, A. L., Liesack, W., Dunfield, P. F. & Dedysh, S. N. (2011). *Methyloferula stellata* gen. nov., sp. nov., an acidophilic, obligately methanotrophic bacterium that possesses only a soluble methane monooxygenase. *Int J Syst Evol Microbiol* **61**, 2456–2463.

Ward, N. L., Challacombe, J. F., Janssen, P. H., Henrissat, B., Coutinho, P. M., Wu, M., Xie, G., Haft, D. H., Sait, M. & other authors (2009). Three genomes from the phylum *Acidobacteria* provide insight into the lifestyles of these microorganisms in soils. *Appl Environ Microbiol* **75**, 2046–2056.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 18:27:15