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

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Five strains of strictly aerobic, heterotrophic bacteria that form pink–red colonies and are capable of hydrolysing pectin, xylan, laminarin, lichenan and starch were isolated from acidic *Sphagnum* peat bogs and were designated OB1010^T, LCBR1, TPB6011^T, TPB6028^T and TPO1014^T. Cells of these isolates were Gram-negative, non-motile rods that produced an amorphous extracellular polysaccharide-like substance. Old cultures contained spherical bodies of varying sizes, which represent starvation forms. Cells of all five strains were acidophilic and psychrotolerant, capable of growth at pH 3.0–7.5 (optimum pH 3.8–4.5) and at 2–33 °C (optimum 15–22 °C). The major fatty acids were iso-C_{15:0}, C_{16:0} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). The major menaquinone detected was MK-8. The pigments were carotenoids. The genomic DNA G + C contents were 57.3–59.3 mol%. The five isolates were found to be members of subdivision 1 of the phylum *Acidobacteria* and displayed 95.3–98.9% 16S rRNA gene sequence similarity to each other. The closest described relatives to strains OB1010^T, LCBR1, TPB6011^T, TPB6028^T, and TPO1014^T were members of the genera *Terriglobus* (94.6–95.8% 16S rRNA gene sequence similarity) and *Edaphobacter* (94.2–95.4%). Based on differences in cell morphology, phenotypic characteristics and hydrolytic capabilities, we propose a novel genus, *Granulicella* gen. nov., containing four novel species, *Granulicella paludicola* sp. nov. with type strain OB1010^T (=DSM 22464^T =LMG 25275^T) and strain LCBR1, *Granulicella pectinivorans* sp. nov. with type strain TPB6011^T (=VKM B-2509^T =DSM 21001^T), *Granulicella rosea* sp. nov. with type strain TPO1014^T (=DSM 18704^T =ATCC BAA-1396^T) and *Granulicella aggregans* sp. nov. with type strain TPB6028^T (=LMG 25274^T =VKM B-2571^T).

The phylum *Acidobacteria* is highly diverse and, at the time of publishing, comprises 26 distinct phylogenetic subdivisions (Barns *et al.*, 2007). Only three of them, subdivisions 1, 3 and 8, have taxonomically characterized representatives. The taxa with validly published names from subdivision 1 include the genera *Acidobacterium* (Kishimoto *et al.*, 1991),

Terriglobus (Eichorst *et al.*, 2007) and *Edaphobacter* (Koch *et al.*, 2008). The only described genus in subdivision 3 is *Bryobacter* (Kulichevskaya *et al.*, 2010), while subdivision 8 includes the genera *Holophaga* (Liesack *et al.*, 1994), *Geothrix* (Coates *et al.*, 1999) and *Acanthopleuribacter* (Fukunaga *et al.*, 2008).

Abbreviation: EPS, extracellular polymeric substance.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of *Granulicella aggregans* TPB6028^T, *G. pectinivorans* TPT6011^T, *G. paludicola* OB1010^T and *G. rosea* TPO1014^T are AM887756–AM887759, respectively.

Micrographs of negatively stained thin sections of vegetative TPO1014^T cells, and cells of strain TPB6011^T in 2-week-old culture, a chart showing the inhibitory effect of phosphate ions on the growth of acidobacteria and a table showing whole-cell fatty acid compositions of novel species of *Granulicella* gen. nov. are available with the online version of this paper.

Members of the phylum *Acidobacteria* inhabit a wide range of natural environments of diverse temperature, salinity and pH. They have been detected in soils and sediments (Barns *et al.*, 1999, 2007; Janssen, 2006; Lauber *et al.*, 2009), hot springs (Hugenholtz *et al.*, 1998; Barns *et al.*, 1999; Bryant *et al.*, 2007), acidic mining lakes (Kleinstuber *et al.*, 2008), water distribution systems (Martiny *et al.*, 2005) and caves (Zimmermann *et al.*, 2005; Meisinger *et al.*, 2007). Recently, we reported that one-third of all bacterial 16S rRNA gene sequences retrieved from acidic *Sphagnum* peat were grouped with the *Acidobacteria* (Dedysh *et al.*, 2006).

Our further cultivation efforts resulted in the isolation of several strains of peat-inhabiting acidobacteria in pure cultures (Pankratov *et al.*, 2008). Most of these isolates belonged to subdivision 1 of the *Acidobacteria* and represented strictly aerobic, acidophilic organisms that were able to hydrolyse a number of polysaccharides. In this paper, we provide a detailed characterization of five of these isolates, strains TPB6011^T, TPO1014^T, TPB6028^T, OB1010^T and LCBR1, and propose a novel genus, containing four novel species of these bacteria.

Strains TPO1014^T and OB1010^T were isolated from a peat sample (pH 4.2) collected from the upper oxic layer, 0–10 cm below the surface, of the bog Obukhovskoe, Yaroslavl region, European North Russia (58° 14' N 38° 12' E). Strains TPB6011^T and TPB6028^T were obtained from peat soil (pH 4.0) sampled at a depth of 10–20 cm of the *Sphagnum* peat bog Bakchar, Tomsk region, West Siberia (56° 51' N 82° 50' E). Strain LCBR1 was isolated from the thalli of lichen species of the genus *Cladonia* collected from the *Sphagnum* peat bog Bakchar. Isolation procedures and culture conditions were used as described by Pankratov *et al.* (2008). The isolation was performed using basal MM1 medium containing (per litre distilled water): MgSO₄·7H₂O (0.04 g); CaCl₂·2H₂O (0.02 g); and yeast extract (0.05 g). Dialysed humic acid (prepared as described by Pansu & Gautheyrou, 2006) (1 ml); galacturonic acid sodium salt, 30 mg; glucuronic acid, 30 mg, and homoserine, 10 mg were included in the MM1 medium as additional components. Xylan or pectin (0.05 %, w/v) were used as carbon sources. The solidifying agent used for the medium preparation was Gel-Gro (ICN Biomedicals). To avoid increasing ionic strength, pH was adjusted to 4.0–5.0 with 20–50 mg alginic acid l⁻¹. Once established as pure cultures, the isolates were maintained on MM1 agar medium (pH 4.6–5.0) with fructose and were subcultured at 4-week intervals. *Terrioglobus roseus* DSM 18391^T and *Edaphobacter aggregans* DSM 19364^T were used as reference strains in our study and were maintained on media 830 and 1135 as recommended by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

Cell morphology was examined using cultures grown both in liquid and on agar MM1 media. For the 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) osmium tetroxide 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). The samples were dehydrated in an ethanol series and embedded in Spurr's epoxy resin. Thin sections were cut on an LKB-4800 microtome, mounted on copper grids covered with Formvar film, contrasted with uranyl acetate (3 %, in 70 % ethanol) for 30 min and then stained with lead citrate (Reynolds, 1963) at 20 °C for 4–5 min. The specimens were examined with a JEM-100C (JEOL) electron microscope at 80 kV accelerating voltage. Absorption spectra of methanol extracts of cells of novel

isolates were determined between 250 and 1000 nm using a SPh-56 spectrophotometer (Lomo-Spectrum).

Growth of the novel strains was examined using batch cultures grown in liquid MM1 medium with fructose at 2–37 °C, pH 2–9 and in NaCl, KCl and K₂HPO₄ concentrations of 0.01–7.0 % (w/v). OD₆₀₀ was measured in an Eppendorf BioPhotometer at 2-day intervals for 3 weeks. To account for cases of non-homogeneous growth, measurement of CO₂ production rates by infrared gas spectroscopy with a thermal conductivity detector was used in place of OD₆₀₀. The range of potential growth substrates of the novel strains was examined by replacing fructose in MM1 medium with alternative carbon sources. The ability to degrade different biopolymers was examined by measuring the rate of CO₂ production of cultures grown in tightly closed 500 ml serum bottles containing 100 ml liquid MM1 medium with 0.05 % (w/v) of the corresponding polymer substrate for 3 weeks at 20 °C. Control incubations were run in parallel under the same conditions but without substrate. Isolates were also tested for their ability to grow anaerobically, determining the fermentation of glucose or fructose, or by anaerobic respiration with nitrate (1 mM) as the electron acceptor and glucose or fructose (0.05 %, w/v) as the sole carbon and energy source. These tests were performed in tightly closed 160 ml serum flasks containing 30 ml liquid MM1 medium. Before autoclaving, the medium and the headspace of the flasks were flushed for 10 min with a mixture of CO₂ (7%) and N₂ (93%) to replace the air.

Enzyme activity profiles, urease hydrolysis, indole production and the Hugh–Leifson test were analysed with API 20NE and API ZYM kits (bioMérieux). Catalase and oxidase tests were carried out by standard methods (Gerhardt *et al.*, 1981). Antibiotic susceptibilities were determined on MM1 agar plates using discs (Oxoid) containing the following antibiotics (µg): ampicillin (10), gentamicin (10), kanamycin (30), neomycin (10), novobiocin (30), streptomycin (10), chloramphenicol (30) and lincomycin (10).

Cell biomass for cellular fatty acid and isoprenoid quinone analyses and for DNA extraction was obtained from batch cultures grown in liquid MM1 medium at 24 °C for 1 week. Fatty acid profiles were analysed by the DSMZ Identification Service as described by Kämpfer & Kroppenstedt (1996). Identification of isoprenoid quinones was performed as reported by Pankratov *et al.* (2007). DNA base composition of each strain was determined by thermal denaturation using a Cary-100 UV-Vis spectrophotometer (Varian) at a heating rate of 0.5 °C min⁻¹. The DNA G+C contents were calculated according to Owen *et al.* (1969) using *Escherichia coli* K-12 (G+C 51.7 mol%) as a standard. DNA–DNA hybridization experiments were performed as described by De Ley *et al.* (1970). The 16S rRNA gene sequences of strains TPO1014^T, TPB6011^T, OB1010^T and TPB6028^T were determined previously (Pankratov *et al.*, 2008), whereas the corresponding gene sequence of strain LCBR1 was analysed in this study. Phylogenetic analyses were carried out using the ARB program package (Ludwig *et al.*, 2004). Trees were

constructed using distance-based (neighbour-joining), maximum-likelihood (DNAML) and maximum-parsimony methods. The significance levels of interior branch points obtained in neighbour-joining analyses were determined by bootstrap analysis (1000 data resamplings) using the PHYLIP program (Felsenstein, 1989).

All five strains formed convex, irregularly circular, semi-transparent or opaque colonies when grown on solid media made with Gel-Gro. Strains TPB6011^T and TPB6028^T produced slimy colonies, colonies of strains TPO1010^T and LCBR1 had a creamy consistency, while strain TPO1014^T produced solid, rubbery colonies that could be peeled from the agar surface. At the stage of isolation, colonies of strains TPO1014^T, OB1010^T, LCBR1 and TPB6028^T did not exceed 0.5 mm in diameter; after a series of further transfers on laboratory media under optimal conditions, these isolates began to form significantly larger colonies of up to 3 mm in diameter. The colonies of strain TPB6011^T were larger, reaching 3–5 mm in diameter. Colonies were pale pink–red in strains TPB6011^T, OB1010^T and LCBR1 and light pink to pink in strains TPO1014^T and TPB6028^T. The main absorption maxima detected in methanol extracts of the novel isolates were at 475, 509 and 540 nm, which are values typical of carotenoids and are most similar to those of spirilloxanthin. These absorption peaks were highly similar to the peaks of the carotenoids in *Terriglobus roseus* (Eichorst *et al.*, 2007). In liquid media, strains TPB6011^T, OB1010^T and LCBR1 grew homogeneously, while strains TPO1014^T and TPB6028^T displayed flocculent growth.

Cells of the novel isolates were Gram-negative, non-spore-forming, non-motile rods that divided by binary fission and occurred singly or in pairs (Supplementary Fig. S1a–d, available in IJSEM Online). Cultures of strains TPB6011^T and TPB6028^T also contained short chains of three to five cells. Cells of the novel isolates varied with regard to their length and width (Table 1). Cells of strains OB1010^T and LCBR1 were shorter than those of strains TPO1014^T, TPB6011^T and TPB6028^T. However, in general, cells of the novel isolates were significantly longer than cells of *Terriglobus roseus* DSM 18391^T and *Edaphobacter aggregans* DSM 19364^T when grown under the same conditions (Supplementary Fig. S1e–f). Production of an amorphous extracellular polymeric substance (EPS) was characteristic of all five novel isolates but was more strongly pronounced in strains TPB6011^T, TPO1014^T and TPB6028^T. Staining of this EPS with Alcian blue (Scott *et al.*, 1964) and ruthenium red (Luft, 1964) indicated that it was a polysaccharide-like substance (Supplementary Fig. S2).

Electron microscopy revealed a cell-wall structure typical of Gram-negative bacteria. The cytoplasmic membrane, peptidoglycan layer and outer membrane were evident in ultrathin sections (Fig. 1a). We also revealed the presence of numerous outer-membrane vesicles, ranging in size from 40 to 100 nm (Fig. 1b). Formation of these vesicles was most evident in strain TPO1014^T. Interestingly, the

analysis of three recently sequenced genomes of acidobacteria suggests they are able to synthesize a large number of high-molecular-mass excreted proteins (Ward *et al.*, 2009).

Old cultures of the novel isolates contained numerous elongated cells (up to 50 µm) as well as spherical bodies of varying sizes (0.5–3 µm) (Supplementary Figs S1a, b and S3a–c). These spherical cells did not show a peptidoglycan layer and had often lost most of their cytoplasm (Supplementary Fig. S3d–e). However, after transfer to fresh medium, some of these cells were able to grow out again into rods. Morphologically and structurally similar cells have been observed in several representatives of the order *Cytophagales* when starved during growth (Reichardt & Morita, 1982; Reichenbach, 2006), which suggests that the morphologies observed are examples of survival forms in nature when low temperature and nutrient deprivation conditions exist (Reichardt & Morita, 1982).

All five novel isolates were strictly aerobic and had a requirement for the presence of growth factors (50 mg yeast extract or Casamino acids l⁻¹) in the cultivation medium. Thus, utilization of a given carbon compound was assumed to have occurred when growth was distinctly improved by its presence, compared with basal MM1 medium alone. The carbon compounds tested and their effects on growth are given in the four species descriptions and are shown in Table 1. Most sugars were preferred as growth substrates. Strain TPO1014^T differed from the other strains by being able to utilize only a limited number of growth substrates. All five isolates possessed hydrolytic capabilities and were able to degrade pectin, laminarin, xylan, lichenan and starch, but not cellulose, CM-cellulose, chitin, chitosan, fucoidan, sodium alginate, chondroitin sulphate or pullulan. Hydrolytic capability was most strongly pronounced in strain TPB6011^T, and pectin was the preferred polymeric substrate. For comparison, *Terriglobus roseus* DSM 18391^T and *Edaphobacter aggregans* DSM 19364^T were tested along with our isolates. *Terriglobus roseus* DSM 18391^T was able to degrade pectin and starch but showed only poor growth on xylan and laminarin, and was unable to degrade lichenan. *Edaphobacter aggregans* DSM 19364^T showed good growth on laminarin and lichenan but grew poorly on xylan and starch and was unable to degrade pectin.

API ZYM tests revealed the strain with the largest number of enzyme activities was strain TPB6011^T, which also displayed the strongest hydrolytic capabilities. This isolate differed from the other four by the presence of trypsin and urease (Table 1). The smallest number of enzyme activities was detected in strain TPO1014^T, which utilized only a limited number of growth substrates. All five isolates were sensitive to novobiocin and were resistant to chloramphenicol, gentamicin, streptomycin and neomycin. Strains TPB6011^T, OB1010^T and LCBR1 differed from strains TPO1014^T and TPB6028^T by being susceptible to lincomycin (Table 1).

The novel isolates grew at pH 3.0–7.5, with optima between pH 3.8 and 4.5. The temperature range for growth

Table 1. Phenotypic characteristics of five novel strains of acidobacteria isolated from *Sphagnum* peat bogs

1, *Granulicella paludicola* OB1010^T; 2, *Granulicella paludicola* LCBR1; 3, *Granulicella pectinivorans* TPB6011^T; 4, *Granulicella rosea* TPO1014^T; 5, *Granulicella aggregans* TPB6028^T. All strains utilized D-glucose, D-fructose, sodium galacturonate, xylan, pectin, laminarin, lichenan and starch. Substrates tested but not utilized by all of the strains: D-arabinose, D-sorbose, D-fucose, butyrate, oxalate, propionate, formate, fumarate, sodium hexanoate, citrate, valerate, adonitol, ethanol, methanol, pullulan, fucoidan, CM-cellulose, cellulose, chitin and chitosan. All strains were positive for catalase, did not reduce nitrates to nitrites, did not produce indole from tryptophan, and did not ferment glucose. All strains were sensitive to novobiocin and were resistant to chloramphenicol, gentamicin, streptomycin and neomycin. w, Weak activity.

Characteristic	1	2	3	4	5
Cell length (µm)	1.5–3.5	1.5–3.0	1.5–15	1.5–9.0	1.5–10
Cell width (µm)	0.4–0.6	0.4–0.6	0.8–1.0	0.5–1.0	0.8–1.5
Homogeneous growth	+	+	+	–	–
Colony colour	Red	Red	Red	Pink	Pink
Utilization of sugars					
D-Galactose	+	+	+	–	+
Lactose	+	+	–	–	–
Lactulose	+	+	–	–	+
Leucrose	+	+	–	–	+
Maltose	+	+	–	+	+
D-Mannose	+	+	+	–	+
Melezitose	+	+	–	–	–
Melibiose	+	+	+	–	–
Raffinose	+	+	–	–	–
D-Rhamnose	+	+	–	–	+
D-Ribose	–	–	–	–	+
Salicin	+	+	–	+	+
Sucrose	+	+	+	–	+
Trehalose	–	–	+	–	–
Cellobiose	+	+	+	–	–
D-Xylose	+	–	+	+	+
N-Acetyl-D-glucosamine	+	+	+	–	+
Utilization of acids					
Acetate	–	–	+	–	–
Gluconate	w	+	+	–	+
Glucuronate	–	+	–	+	+
Lactate	–	–	+	–	–
Malate	–	–	–	+	+
Pyruvate	–	–	–	+	+
Succinate	–	–	+	–	–
Utilization of polyalcohols					
Arbutin	–	+	–	–	+
Inulin	+	–	+	–	–
Mannitol	–	–	+	–	+
myo-Inositol	–	+	+	–	+
Sorbitol	–	–	–	–	+
Dulcitol	–	–	–	–	+
Susceptibility to:					
Ampicillin	–	–	–	+	–

Table 1. cont.

Characteristic	1	2	3	4	5
Kanamycin	–	–	+	+	–
Lincomycin	+	+	+	–	–
Oxidase	+	+	+	–	–
Enzyme activities					
Esterase (C4)	+	–	w	–	+
Esterase lipase (C8)	–	–	+	+	+
Valine arylamidase	+	+	+	–	+
α-Chymotrypsin	–	+	+	–	+
α-Galactosidase	+	+	+	–	–
α-Glucosidase	+	+	+	–	+
N-Acetyl-β-glucosaminidase	+	+	+	–	+
Trypsin	–	–	+	–	–
Urease	–	–	+	–	–
DNA G + C content (mol%)	57.4	57.3	59.1	58.3	59.3

was 2–33 °C, with optima between 15 and 22 °C. Under optimal conditions, the growth rate of these bacteria was in the range 0.05–0.10 h^{–1}. Strains OB1010^T, LCBR1, TPB6011^T, TPO1014^T and TPB6028^T were relatively resistant to NaCl. They grew well in media at NaCl concentrations up to 3.5 % (w/v); complete inhibition of growth was observed at 5 % (w/v) NaCl and above. Growth

Fig. 1. Electron micrographs of ultrathin sections of vegetative cells of strain TPB6011^T (a) and TPO1014^T (b). OM, Outer membrane; CM, cytoplasmic membrane; P, peptidoglycan layer; V, outer membrane vesicles. Bars, 1.0 µm.

inhibition of 20 and 80 % was observed in the presence of KH_2PO_4 at concentrations of 0.7 and 6.7 mM, respectively, whereas no inhibition was detected in the presence of KCl within the same range of concentrations (Supplementary Fig. S4). Our results indicate that phosphate ions can inhibit growth of some acidobacteria.

The five novel isolates contained MK-8 as the predominant menaquinone. The major fatty acids of strains OB1010^T, LCBR1, TPB6011^T, TPO1014^T and TPB6028^T were iso- $\text{C}_{15:0}$, $\text{C}_{16:0}$ and summed feature 3 ($\text{C}_{16:1}\omega 7\text{c}/\text{iso-}\text{C}_{15:0}$ 2-OH) (Table 2). Strains OB1010^T and LCBR1 also contained iso- $\text{C}_{13:0}$ in substantial amounts (8.6–9.7 %). These cellular fatty acid profiles had significant similarity to those of the subdivision 1 acidobacteria *Terriglobus roseus* and *Edaphobacter modestus* (Eichorst *et al.*, 2007; Koch *et al.*, 2008) (Supplementary Table S1).

Comparative 16S rRNA gene sequence analysis showed that strains OB1010^T, LCBR1, TPB6011^T, TPO1014^T and TPB6028^T show between 95.3 and 98.9 % similarity to each other and belong to subdivision 1 of the phylum

Acidobacteria (Fig. 2). The closest described relatives of these novel isolates are members of the genera *Terriglobus* (94.6–95.8 % 16S rRNA gene sequence similarity) and *Edaphobacter* (94.2–95.4 %). Among taxonomically uncharacterized organisms, the highest sequence similarities (97.9–98.2 %) were to the soil isolate KBS89, which was described by Eichorst *et al.* (2007) but not included in the genus *Terriglobus*. Independently of the algorithms used for the phylogenetic tree construction, the five novel isolates from *Sphagnum* peat and the soil isolate KBS89 formed a common cluster, which was separate from the clusters defined by members of the genera *Terriglobus* and *Edaphobacter* (Fig. 2).

Despite the clear preference of isolate KBS89 for growth conditions of low pH (≤ 5.0), and its close phylogenetic relationship to strains OB1010^T, LCBR1, TPB6011^T, TPO1014^T and TPB6028^T, this soil isolate differed from the peat-inhabiting isolates by the absence of pigment and catalase activity, as well as by the inability to grow at 4 °C.

The DNA G+C content of strains OB1010^T, LCBR1, TPB6011^T, TPO1014^T and TPB6028^T ranged from 57.3 to 59.3 mol%. Only two of these isolates, strains OB1010^T and LCBR1, were highly similar to each other with regard to their cell size and morphology, phenotypic characteristics and DNA G+C content (Table 1). In contrast to other strains, they also contained substantial amounts (8.6–9.7 %) of iso- $\text{C}_{13:0}$ (Table 2). In comparative 16S rRNA gene sequence analyses, these strains always clustered together, independently of the algorithm used for phylogenetic tree construction (Fig. 2). This evidence allows us to conclude that strains OB1010^T and LCBR1 represent the same species. Other isolates displayed significant differences from each other with respect to cell morphology and phenotypic characteristics (Supplementary Fig. S1 and Table 1), which suggested that they may represent different species. This was further confirmed by the results of DNA–DNA hybridization studies, which revealed relatively low (9–20 %) DNA–DNA hybridization values among strains OB1010^T, TPB6011^T, TPO1014^T and TPB6028^T.

In summary, our novel isolates from *Sphagnum* peat bogs formed a coherent phylogenetic cluster, which was separate from the clusters defined by the genera *Terriglobus*, *Edaphobacter* and *Acidobacterium* (Fig. 2) and possessed a number of characteristics that clearly distinguished them from all earlier described members of subdivision 1 of the phylum *Acidobacteria* (Table 3). Cells of the novel isolates were longer than those of members of the genera *Terriglobus*, *Edaphobacter* and *Acidobacterium*, were more tolerant to low temperatures and less sensitive to NaCl. A pink colony pigmentation differentiated strains OB1010^T, LCBR1, TPB6011^T, TPB6028^T and TPO1014^T from members of the genera *Edaphobacter* and *Acidobacterium*, absence of motility distinguished them from members of the genus *Acidobacterium* and the ability to grow at below pH 4 distinguished them from members of the genera *Terriglobus* and *Edaphobacter*. The hydrolytic capabilities of

Table 2. Whole-cell fatty acid compositions (%) of five novel strains of acidobacteria from *Sphagnum* peat bogs

1, *Granulicella paludicola* OB1010^T; 2, *Granulicella paludicola* LCBR1; 3, *Granulicella pectinivorans* TPB6011^T; 4, *Granulicella rosea* TPO1014^T; 5, *Granulicella aggregans* TPB6028^T. Major fatty acids are shown in bold. –, Not detected.

Fatty acid	1	2	3	4	5
Saturated					
$\text{C}_{14:0}$	1.3	1.2	2.5	5.2	1.7
$\text{C}_{15:0}$	0.6	1.6	0.4	–	–
$\text{C}_{16:0}$	12.5	10.6	6.0	7.3	10.7
$\text{C}_{17:0}$	0.9	0.6	0.5	0.3	0.7
$\text{C}_{18:0}$	0.5	0.4	0.3	0.3	1.1
$\text{C}_{20:0}$	0.4	0.9	0.3	0.9	1.7
Unsaturated					
$\text{C}_{14:1}\omega 5\text{c}$	0.3	0.1	3.4	2.9	0.6
$\text{C}_{15:1}\omega 6\text{c}$	0.5	–	1.5	1.2	0.8
$\text{C}_{16:1}\omega 5\text{c}$	–	–	0.1	–	–
$\text{C}_{17:1}\omega 8\text{c}$	0.1	–	0.1	–	–
$\text{C}_{18:1}\omega 7\text{c}$	0.2	0.2	0.2	–	–
Methyl-branched					
iso- $\text{C}_{11:0}$	0.2	0.5	0.1	–	0.2
iso- $\text{C}_{13:0}$	9.7	8.6	0.4	0.1	0.4
iso- $\text{C}_{15:0}$	40.8	46.8	52.8	59.9	35.5
anteiso- $\text{C}_{15:0}$	1.3	0.9	0.2	0.2	–
iso- $\text{C}_{17:1}\omega 9\text{c}$	0.4	0.4	0.2	–	1.1
iso- $\text{C}_{17:0}$	2.3	3.6	1.5	0.6	2.8
anteiso- $\text{C}_{17:0}$	0.6	0.6	1.0	0.3	0.5
Hydroxy					
$\text{C}_{12:0}$ 3-OH	0.1	–	0.2	–	–
iso- $\text{C}_{15:0}$ 3-OH	–	1.2	0.1	–	–
Summed feature					
$\text{C}_{16:1}\omega 7\text{c}/\text{iso-}\text{C}_{15:0}$ 2-OH	26.8	12.7	27.7	20.7	42.4

Fig. 2. 16S rRNA gene-based neighbour-joining tree (Jukes–Cantor correction) showing the phylogenetic relationship of strains TPB6011^T, TPO1014^T, TPB6028^T, OB1010^T and LCBR1 to taxonomically characterized representatives and some non-described members of the phylum *Acidobacteria*. Bootstrap values (1000 data resamplings) >50% are shown. Filled circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. Six members of the phylum *Planctomycetes*, *Isosphaera pallida* strain 563 (AJ231193), *Gemmata obscuriglobus* strain UQM 2246^T (X54522), *Planctomyces brasiliensis* strain DSM 5305^T (AJ231190), *Planctomyces maris* strain DSM 8797^T (AJ231184), *Schlesneria paludicola* strain MPL7^T (AM162407) and *Singulisphaera acidiphila* ATCC BAA-1392^T (AM850678), were used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

the peat-inhabiting bacteria were much more pronounced than in members of the genera *Terriglobus* and *Edaphobacter*. Therefore, we propose a novel genus of acidophilic, polymer-degrading bacteria, *Granulicella* gen. nov., containing four novel species, *Granulicella paludicola* sp. nov. (strains OB1010^T and LCBR1), *Granulicella rosea* sp. nov. (TPO1014^T), *Granulicella pectinivorans* sp. nov. (TPB6011^T) and *Granulicella aggregans* sp. nov. (TPB6028^T).

Description of *Granulicella* gen. nov.

Granulicella (Gra.nu.li.cel'la L. neut. n. *granulum* a small grain; L. fem. n. *cella* a storeroom, chamber and, in biology, a cell; N.L. fem. n. *Granulicella* a grain-like cell).

Gram-negative, non-spore-forming, non-motile rods that occur singly, in pairs or in short chains. Reproduce by binary fission. Produce amorphous extracellular polysaccharide-like substances. Old cultures contain globular starvation forms. Colony colour varies from pale-pink to red. Pigments are carotenoids. Oxidase-variable and catalase-positive. Strictly aerobic chemo-organotrophs. Sugars are the preferred growth substrates. Capable of hydrolysing several polysaccharides but not cellulose or

chitin. Acidophilic and mesophilic. Do not produce H₂S from thiosulfate or indole from tryptophan. NaCl inhibits growth at concentrations above 3.5% (w/v). Major fatty acids are iso-C_{15:0}, C_{16:0} and summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH). The predominant menaquinone is MK-8. The DNA G+C content is 57.3–59.3 mol%. Strains have been isolated from acidic wetlands, specifically *Sphagnum* peat bogs. The type species is *Granulicella paludicola*.

Description of *Granulicella paludicola* sp. nov.

Granulicella paludicola (pa.lu.di'co.la. L. n. *palus*, -udis swamp, bog; L. suff. -cola derived from *incola* inhabitant, dweller; N.L. n. *paludicola* an inhabitant of bogs).

The description is as for the genus with the following additional traits. Cells are 0.4–0.6 × 1.5–3.5 μm. Colony colour varies from pink to red. Growth is homogeneous in liquid medium. Able to utilize the following carbon sources (0.05%, w/v): D-glucose, D-fructose, cellobiose, D-galactose, lactose, lactulose, leucrose, maltose, D-mannose, melibiose, melezitose, D-rhamnose, raffinose, sucrose, salicin, N-acetyl-D-glucosamine, sodium galacturonate, sodium D-gluconate.

Table 3. Major characteristics that distinguish *Granulicella* gen. nov. and related genera

Data for the related genera were taken from the following sources: Eichorst *et al.* (2007) (*Terriglobus*), Koch *et al.* (2008) (*Edaphobacter*) and Kishimoto *et al.* (1991) (*Acidobacterium*). ND, Not determined.

Characteristic	<i>Granulicella</i>	<i>Terriglobus</i>	<i>Edaphobacter</i>	<i>Acidobacterium</i>
Source of isolation	Species of <i>Sphagnum</i> peat and <i>Cladonia</i>	Soil and termite hindgut	Alpine and forest soil	Acidic mineral environment
Cell length (µm)	1.5–15	1.0–1.2	1.0–2.1	1.1–2.3
Motility	–	–	+	+
Copious EPS production	+	+	–	–
Pigment	Pink to red	Pink or none	None	Orange
pH range	3.0–7.5	5.0–7.0	4.0–7.0	3.0–6.0
pH optimum	3.8–4.5	5.0–6.0	5.5	ND
Growth at/in:				
pH <4	+	–	–	+
3.5 % NaCl	+	–	–	–
37 °C	–	–	+	+
4 °C	+	–	–	ND
Degradation of:				
Pectin	+	+	–	ND
Lichenan	+	–	+	ND
Presence of protease	–	ND	+	–
DNA G + C content (mol%)	57.3–59.3	58.1–59.8	55.8–56.9	59.9–60.8

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

Unable to utilize D-arabinose, D-sorbose, D-fucose, D-ribose, trehalose, mannitol, sorbitol, dulcitol, acetate, pyruvate, malate, lactate, succinate, butyrate, oxalate, propionate, formate, fumarate, capronate, citrate, valerate, adonitol, ethanol or methanol. Variable utilization of D-xylose, glucuronate, *myo*-inositol, arbutin and inulin. Hydrolyses aesculin, laminarin, pectin, lichenan, starch and xylan but not sodium alginate, CM-cellulose, cellulose, chitin, chitosan, fucoidan or pullulan. The following enzyme activities are present: valine arylamidase, α -glucosidase, *N*-acetyl- β -glucosaminidase. Activities of urease, trypsin and esterase (C8) are absent. Esterase (C4) and α -chymotrypsin activities are variable (API ZYM test). Oxidase- and catalase-positive. Capable of growth at pH 3.0–7.5 (optimum pH 4.2) and at 2–33 °C (optimum 18–22 °C). Resistant to ampicillin, kanamycin, chloramphenicol, gentamicin, streptomycin and neomycin but susceptible to lincomycin and novobiocin.

The type strain OB1010^T (=DSM 22464^T =LMG 25275^T) was isolated from the *Sphagnum* peat bog Obukhovskoe, Yaroslavl region, European North Russia.

Description of *Granulicella rosea* sp. nov.

Granulicella rosea (ro'se.a. L. fem. adj. *rosea* rose-coloured).

The description is as for the genus but with the following additional traits. Cells are 0.5–1.0 × 1.5–9.0 µm. Colony colour varies from pale pink to pink. Produces copious amounts of EPS. Growth is non-homogeneous in liquid medium. Able to utilize the following carbon sources

(0.05 %, w/v): D-glucose, D-fructose, maltose, D-xylose, sodium D-galacturonate, glucuronate, pyruvate, malate and salicin. Unable to utilize D-arabinose, sucrose, D-galactose, lactose, lactulose, leucrose, D-mannose, melibiose, melezitose, raffinose, D-rhamnose, D-ribose, D-sorbose, trehalose, D-fucose, cellobiose, *N*-acetylglucosamine, adonitol, mannitol, *myo*-inositol, sorbitol, dulcitol, arbutin, inulin, acetate, gluconate, lactate, succinate, butyrate, oxalate, propionate, formate, fumarate, capronate, citrate, valerate, ethanol or methanol. Hydrolyses aesculin, laminarin, pectin, lichenan, starch and xylan but not sodium alginate, CM-cellulose, cellulose, chitin, chitosan, fucoidan or pullulan. Esterase (C8) is present. The following enzyme activities are absent: valine arylamidase, α -glucosidase, *N*-acetyl- β -glucosaminidase, esterase (C4), urease and α -chymotrypsin (API ZYM test). Oxidase-negative. Catalase-positive. Capable of growth at pH 3.0–7.5 (optimum pH 4.5) and at 2–33 °C (optimum 18–22 °C). Resistant to chloramphenicol, gentamicin, streptomycin, neomycin, lincomycin and novobiocin but susceptible to ampicillin and kanamycin.

The type strain TPO1014^T (=DSM 18704^T =ATCC BAA-1396^T) was isolated from the *Sphagnum* peat bog Obukhovskoe, Yaroslavl region, European North Russia.

Description of *Granulicella pectinivorans* sp. nov.

Granulicella pectinivorans (pec.ti.ni.vor'ans. N.L. n. *pectinum* pectin; L. part. adj. *vorans* devouring; N.L. part. adj. *pectinivorans* pectin-devouring, referring to the ability to use pectin as a growth substrate).

The description is as for the genus but with the following additional traits. Cells are $0.8\text{--}1.0 \times 1.5\text{--}15\ \mu\text{m}$. Colony colour varies from pale pink to red. Produces copious amounts of EPS. Able to utilize the following carbon sources (0.05 %, w/v): D-glucose, D-fructose, D-galactose, D-mannose, melibiose, D-xylose, cellobiose, sucrose, trehalose, *N*-acetyl-D-glucosamine, acetate, gluconate, lactate, sodium D-galacturonate, succinate, mannitol, inulin and *myo*-inositol. Unable to utilize D-arabinose, lactose, lactulose, leucrose, maltose, melezitose, raffinose, D-rhamnose, D-ribose, D-sorbose, D-fucose, salicin, arbutin, adonitol, sorbitol, dulcitol, glucuronate, malate, pyruvate, butyrate, oxalate, propionate, formate, fumarate, capronate, citrate, valerate, ethanol or methanol. Hydrolyses aesculin, laminarin, pectin, lichenan, starch and xylan but not sodium alginate, CM-cellulose, cellulose, chitin, chitosan, fucoidan or pullulan. The following enzyme activities are present: valine arylamidase, α -glucosidase, *N*-acetyl- β -glucosaminidase, esterase, urease and α -chymotrypsin (API ZYM test). Oxidase-positive. Capable of growth at pH 3.0–7.5 (optimum pH 3.8–4.5) and at 2–33 °C (optimum 18–22 °C). Resistant to ampicillin, chloramphenicol, gentamicin, streptomycin and neomycin but susceptible to lincomycin, novobiocin and kanamycin.

The type strain TPB6011^T (=VKM B-2509^T=DSM 21001^T) was isolated from the *Sphagnum* peat bog Bakchar, Tomsk region, West Siberia.

Description of *Granulicella aggregans* sp. nov.

Granulicella aggregans (ag'gre.gans. L. part. adj. *aggregans* from L. v. *aggrego* attaching one's self to, adhering to, referring to the ability of the cells to form aggregates).

The description is as for the genus but with the following additional traits. Cells are $0.8\text{--}1.5 \times 1.5\text{--}10\ \mu\text{m}$. Colony colour varies from pale pink to reddish pink. Produces copious amounts of EPS. Growth is non-homogeneous in liquid medium. Able to utilize the following carbon sources (0.05 %, w/v): D-glucose, D-fructose, D-galactose, lactulose, leucrose, maltose, D-mannose, D-rhamnose, D-ribose, D-xylose, sucrose, salicin, *N*-acetyl-D-glucosamine, sodium D-galacturonate, gluconate, glucuronate, malate, pyruvate, arabinol, arbutin, dulcitol, mannitol, *myo*-inositol and sorbitol. Unable to utilize D-arabinose, lactose, melibiose, melezitose, raffinose, D-sorbose, trehalose, D-fucose, cellobiose, acetate, butyrate, capronate, citrate, valerate, lactate, oxalate, propionate, succinate, formate, fumarate, adonitol, inulin, methanol or ethanol. Hydrolyses aesculin, laminarin, pectin, lichenan, starch and xylan but not sodium alginate, CM-cellulose, cellulose, chitin, chitosan, fucoidan or pullulan. The following enzyme activities are present: esterase, valine arylaminidase, α -chymotrypsin, α -glucosidase, *N*-acetyl- β -glucosaminidase. The following enzyme activities are absent: α -galactosidase, trypsin and urease (API ZYM test). Oxidase-negative. Catalase-positive. Capable of growth at pH 3.0–7.5 (optimum pH 4.5) and at 2–33 °C (optimum 18–22 °C). Resistant to chloramphenicol, gentamicin,

streptomycin, neomycin, lincomycin, ampicillin and kanamycin but susceptible to novobiocin.

The type strain TPB6028^T (=LMG 25274^T =VKM B-2571^T) was isolated from the *Sphagnum* peat bog Bakchar, Tomsk region, West Siberia.

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References

- Barns, S. M., Takala, S. L. & Kuske, C. R. (1999). Wide distribution and diversity of members of the bacterial kingdom *Acidobacterium* in the environment. *Appl Environ Microbiol* **65**, 1731–1737.
- Barns, S. M., Cain, E. C., Sommerville, L. & Kuske, C. R. (2007). *Acidobacteria* phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum. *Appl Environ Microbiol* **73**, 3113–3116.
- Bryant, D. A., Costas, A. M., Maresca, J. A., Chew, A. G., Klatt, C. G., Bateson, M. M., Tallon, L. J., Hostetler, J., Nelson, W. C. & other authors (2007). *Candidatus Chloracidobacterium thermophilum*: an aerobic phototrophic acidobacterium. *Science* **317**, 523–526.
- Coates, J. D., Ellis, D. J., Gaw, C. V. & Lovley, D. R. (1999). *Geothrix fermentans* gen. nov., sp. nov., a novel Fe(III)-reducing bacterium from a hydrocarbon-contaminated aquifer. *Int J Syst Bacteriol* **49**, 1615–1622.
- De Ley, J., Cattoir, K. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Dedysh, S. N., Pankratov, T. A., Kulichevskaya, I. S., Belova, S. E. & Liesack, W. (2006). Phylogenetic analysis and *in situ* identification of bacteria community composition in an acidic *Sphagnum* peat bog. *Appl Environ Microbiol* **72**, 2110–2117.
- 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.
- Fukunaga, Y., Kurahashi, M., Yanagi, K., Yokota, A. & Harayama, Sh. (2008). *Acanthopleuribacter pedis* gen. nov., sp. nov., a marine bacterium isolated from a chiton, and description of *Acanthopleuribacteraceae* fam. nov., *Acanthopleuribacterales* ord. nov., *Holophagaceae* fam. nov., *Holophagales* ord. nov. and *Holophagae* classis nov. in the phylum 'Acidobacteria'. *Int J Syst Evol Microbiol* **58**, 2597–2601.
- Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. & Phillips, G. B. (editors) (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.
- Hugenholtz, P., Pitulle, C., Hershberger, K. L. & Pace, N. R. (1998). Novel division level bacterial diversity in a Yellowstone hot spring. *J Bacteriol* **180**, 366–376.
- Janssen, P. H. (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl Environ Microbiol* **72**, 1719–1728.

- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- 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.
- Kleinteuber, S., Müller, F.-D., Chatzinotas, A., Wendt-Potthoff, K. & Harms, H. (2008). Diversity and *in situ* quantification of *Acidobacteria* subdivision 1 in an acidic mining lake. *FEMS Microbiol Ecol* **63**, 107–117.
- 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.
- Kulichevskaya, I. S., Suzina, N. E., Liesack, W. & Dedysch, S. N. (2010). *Bryobacter aggregatus* gen. nov., sp. nov., a peat-inhabiting, aerobic chemo-organotroph from subdivision 3 of the *Acidobacteria*. *Int J Syst Evol Microbiol* **60**, 301–306.
- Lauber, C. L., Hamady, M., Knight, R. & Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* **75**, 5111–5120.
- Liesack, W., Bak, F., Kreft, J. U. & Stackebrandt, E. (1994). *Holophaga foetida* gen. nov., sp. nov., a new homoacetogenic bacterium degrading methoxylated aromatic compounds. *Arch Microbiol* **162**, 85–90.
- 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.
- Martiny, A. C., Albrechtsen, H.-J., Arvin, E. & Molin, S. (2005). Identification of bacteria in biofilm and bulk water samples from a nonchlorinated model drinking water distribution system: detection of a large nitriteoxidizing population associated with *Nitrospira* spp. *Appl Environ Microbiol* **71**, 8611–8617.
- Meisinger, D. B., Zimmermann, J., Ludwig, W., Schleifer, K.-H., Wanner, G., Schmid, M., Bennett, P. C., Engel, A. S. & Lee, N. M. (2007). *In situ* detection of novel *Acidobacteria* in microbial mats from a chemolithoautotrophically based cave ecosystem (Lower Kane Cave, WY, USA). *Environ Microbiol* **9**, 1523–1534.
- Owen, R. J., Lapage, S. P. & Hill, L. R. (1969). Determination of base composition from melting profiles in dilute buffers. *Biopolymers* **7**, 503–516.
- Pankratov, T. A., Tindall, B. J., Liesack, W. & Dedysch, S. N. (2007). *Mucilaginibacter paludis* gen. nov., sp. nov. and *Mucilaginibacter gracilis* sp. nov., pectin-, xylan- and laminarin-degrading members of the family *Sphingobacteriaceae* from acidic *Sphagnum* peat bog. *Int J Syst Evol Microbiol* **57**, 2349–2354.
- Pankratov, T. A., Serkebaeva, Y. M., Kulichevskaya, I. S., Liesack, W. & Dedysch, S. N. (2008). Substrate-induced growth and isolation of *Acidobacteria* from acidic *Sphagnum* peat. *ISME J* **2**, 551–560.
- Pansu, M. & Gautheyrou, J. (2006). *Handbook of Soil Analysis*. Berlin, Heidelberg: Springer.
- Reichardt, W. & Morita, R. Y. (1982). Survival stages of a psychrotrophic *Cytophaga johnsonae* strain. *Can J Microbiol* **28**, 841–850.
- Reichenbach, H. (2006). The order *Cytophagales*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 7, pp. 549–590. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- 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.
- Scott, J. E., Quintaretti, G. & Dellovo, M. C. (1964). The chemical and histochemical properties of alcian blue. I. The mechanisms of alcian blue staining. *Histochemie* **4**, 73–85.
- 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 their lifestyles in soils. *Appl Environ Microbiol* **75**, 2046–2056.
- Zimmermann, J., Gonzalez, J. M., Saiz-Jimenez, C. & Ludwig, W. (2005). Detection and phylogenetic relationships of highly diverse uncultured acidobacterial communities in Altamira Cave using 23S rRNA sequence analysis. *Geomicrobiol J* **22**, 379–388.