Terriglobus saanensis sp. nov., an acidobacterium isolated from tundra soil

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Two aerobic bacterial strains, designated SP1PR4^T and SP1PR5, were isolated from tundra soil samples collected from Saana field, North-western Finland (69° 03' N 20° 50' E). Cells of both strains were Gram-negative, non-motile rods. Phylogenetic analysis indicated that the strains belong to the genus *Terriglobus* in subdivision 1 of the phylum *Acidobacteria*. Strains SP1PR4^T and SP1PR5 shared identical BOX and ERIC fingerprints and 99.7% 16S rRNA gene similarity indicating that, together with their identical physiological features, these strains are members of the same species. The 16S rRNA gene sequence similarity of SP1PR4^T and SP1PR5 with *Terriglobus roseus* DSM 18391^T was 97.1%. A low DNA–DNA hybridization value (<20%) and *rpoB* gene sequence similarity (83.6%) with *T. roseus* DSM 18391^T indicated that the tundra soil isolates represent novel members of the genus *Terriglobus*. Strains SP1PR4^T and SP1PR5 grew at pH 4.5–7.5 and 4–30 °C. Sugars were the preferred growth substrates. The major cellular fatty acids were iso-C_{15:0}, C_{16:1ω7c}, iso-C_{13:0} and C_{16:0}. The DNA G+C content of strain SP1PR4^T was 57.3 mol%. Based on phylogenetic, chemotaxonomic and physiological analyses, the name *Terriglobus saanensis* sp. nov. is proposed to accommodate the two strains; the type strain is SP1PR4^T (=DSM 23119^T =ATCC BAA-1853^T).

Members of the phylum *Acidobacteria* are among the most abundant micro-organisms found in soil ecosystems (Janssen, 2006) and are also widely distributed in high-latitude terrestrial ecosystems (Männistö *et al.*, 2007; Pankratov *et al.*, 2008; Campbell *et al.*, 2010). Although ubiquitous in the environment, the ecological role of acidobacteria is poorly understood. Members of the phylum *Acidobacteria* are relatively difficult to cultivate and thus only a few strains have been isolated and characterized. To date, eight genera of this phylum have been described: *Acidobacterium*, *Edaphobacter*, *Granulicella* and *Terriglobus*, representing *Acidobacteria* subdivision 1; *Bryobacter* in *Acidobacteria* subdivision 3; and *Holophaga*, *Geothrix* and *Acanthopleuribacter* in *Acidobacteria* subdivision 8 (Kishimoto *et al.*, 1991; Liesack *et al.*, 1994; Coates *et al.*, 1999; Eichorst *et al.*, 2007; Fukunaga *et al.*, 2008; Koch *et al.*, 2008; Pankratov & Dedysh, 2010; Kulichevskaya *et al.*, 2010). ‘*Candidatus* Koribacter versatilis’ and ‘*Candidatus*

Solibacter usitatus’, from subdivisions 1 and 3, respectively, have been described recently (Ward *et al.*, 2009).

The phylum *Acidobacteria* is divided into 26 subdivisions (Barns *et al.*, 2007) and members of subdivisions 1, 3, 4 and 6 are most often detected in soil environments (Janssen, 2006; Jones *et al.*, 2009). The abundance of acidobacteria correlates with soil pH, members of subdivision 1 being most abundant in slightly acidic soils (Jones *et al.*, 2009). Bacterial communities in Arctic alpine tundra soils of northern Finland have been studied previously (Männistö *et al.*, 2007, 2009). Terminal restriction fragment length polymorphism and clone analysis indicated that acidobacteria dominate especially in acidic tundra heaths (Männistö *et al.*, 2007) and are highly active at low temperatures and after multiple freeze–thaw cycles (Männistö *et al.*, 2009). Various techniques have been used to isolate slow-growing and fastidious bacterial species from tundra environments including acidobacteria. One of the pre-treatment methods included freezing of soil samples in order to restrain the growth of, for example, some fast-growing proteobacteria. Tundra soil acidobacteria are cold-tolerant (Männistö *et al.*, 2009) and freezing of the sample was intended to select for species that tolerate harsh temperatures during

Abbreviations: BOX, BOX-repeat-based; ERIC, enterobacterial repetitive intergenic consensus.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SP1PR4^T is HM214537.

A supplementary figure is available with the online version of this paper.

the Arctic winter. Strains SP1PR4^T and SP1PR5 were isolated from soil samples collected from a wind-exposed ridge of Mt. Saana, north-western Finland (69° 03' N 20° 50' E). The soil samples were pre-treated by freezing at -20 °C for 7 days, after which they were thawed at +5 °C for 24 h and serially diluted and plated on ¼ strength R2A medium (pH 7; Difco). Cultivation of bacteria from these soil samples resulted in the isolation of two light-pink-pigmented strains that were members of *Acidobacteria* subdivision 1. Once obtained as pure cultures, the isolates were maintained on full strength R2A (pH 6 or 7).

Genomic DNA was isolated using the UltraClean microbial DNA kit (Mo Bio Laboratories). The nearly complete 16S rRNA gene was amplified using the universal primer set 27F and 1525R (Lane, 1991). Amplified genes were cloned and sequencing of both strands was performed using internal 16S rRNA specific primers and a Big Dye terminator cycle sequencing kit version 2.1 and a model 3100 sequencer (PE Applied Biosystems). Sequencing reactions were compiled using the software package Vector NTI (Invitrogen) and sequences were compared with those available in GenBank using the BLAST program (Altschul *et al.*, 1997). Sequences were aligned using CLUSTAL_X (Thompson *et al.*, 1997). Neighbour-joining, maximum-parsimony and maximum-likelihood phylogenetic trees with 1000 bootstrap replications were constructed using PHYLO_WIN (Galtier *et al.*, 1996) and MEGA4 with the maximum composite likelihood model for nucleotide substitutions (Tamura *et al.*, 2007).

Comparative 16S rRNA gene sequence analysis placed strains SP1PR4^T and SP1PR5 within *Acidobacteria* subdivision 1 on a separate branch, with the type strain of *Terriglobus roseus* as their closest relative (Fig. 1). This was supported by high bootstrap values in the neighbour-joining, maximum-parsimony and maximum-likelihood analyses. Sequences from SP1PR4^T and SP1PR5 were 99.7% similar to each other and both strains shared

97.1% 16S rRNA gene sequence similarity with their closest relative *T. roseus* DSM 18391^T.

Strains SP1PR4^T and SP1PR5 were further compared to *T. roseus* DSM 18391^T using BOX-repeat-based (BOX)-PCR, enterobacterial repetitive intergenic consensus (ERIC)-PCR, *rpoB* gene sequencing and DNA-DNA hybridization. BOX- and ERIC-PCR were performed as described by Rademaker & De Bruijn (1997). Partial *rpoB* genes (1007 bp) were amplified from SP1PR4^T, SP1PR5 and *T. roseus* DSM 18391^T using forward primer Acido_rpoB-F1 (5'-TGGGCCGCTCAAGTTCAA-3') and reverse primer Acido_rpoB-R1 (5'-GGACTGGCGTTGCATGTT-3') and the same PCR conditions as for the amplification of 16S rRNA genes. The *rpoB* primers were designed based on a conserved region identified by sequence alignments of *rpoB* genes from the genomes of *Acidobacterium capsulatum* JCM 7670^T, '*Candidatus* K. versatilis' Ellin345 and '*Candidatus* S. usitatus' Ellin6076. For DNA-DNA hybridization, cells were disrupted by using a French pressure cell (Thermo Spectronic) and DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA-DNA hybridization was carried out by the DSMZ as described by Huß *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multi-cell changer and a temperature controller with *in situ* temperature probe (Varian). Strains SP1PR4^T and SP1PR5 showed identical BOX- and ERIC-PCR patterns (Supplementary Fig. S1, available in IJSEM Online) and shared 99.8% *rpoB* gene sequence similarity, indicating that they represent the same species. BOX-PCR, ERIC-PCR and *rpoB* gene sequence similarities have been shown to correlate well with the results of DNA-DNA hybridization (Rademaker *et al.*, 2000; Adékambi *et al.*, 2008) and therefore DNA-DNA hybridization was not performed between these two strains. The genomic fingerprints of strains SP1PR4^T and SP1PR5 were distinctly different from that of *T. roseus* DSM 18391^T (Supplementary Fig. S1). DNA-DNA hybridization between strain SP1PR4^T and *T. roseus* DSM 18391^T revealed a low DNA relatedness value of 9.3% (mean of four replicate hybridizations). The low DNA relatedness of strain SP1PR4^T and *T. roseus* DSM 18391^T was further supported by low *rpoB* gene sequence similarity of 83.6%. A cut-off value of 97.7% *rpoB* gene sequence similarity has been suggested for the delineation of bacterial species (Adékambi *et al.*, 2008), below which strains can be classed as separate species.

Cell morphology of strains SP1PR4^T and SP1PR5 was studied by phase-contrast microscopy after 3 and 5 days cultivation on R2A at 20 °C using a Leitz Laborlux S light microscope and with a Nikon DXM1200 digital camera fitted on a Nikon E200 phase-contrast microscope. Gram-staining was performed using the Hucker staining method (Murray *et al.*, 1994). For transmission electron microscopy, cells were grown in R2 broth for 7–14 days and a cell pellet was processed for 3 h in Trump's fixative (a combination of low concentrations of both formaldehyde and glutaraldehyde in 0.05 M sodium cacodylate buffer,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1488 nt positions used for analysis) showing the relationships between strains SP1PR4^T, SP1PR5, *Terriglobus roseus* and related taxa. Bootstrap values >50% (based on 1000 replicates) are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

pH 7.3) (Hayt, 1981). Post-fixation was carried out in 1% osmium tetroxide in the same buffer for 1.5 h followed by dehydration in a graded ethanol series and embedding in Spurr's low-viscosity embedding media. Thin sections were prepared using a diamond knife and an Ultracut E ultramicrotome (Reichert Optische Werke). Thin sections were stained with 5% (w/v) uranyl acetate saturated solution in 50% ethanol for 15 min and then with 0.5% lead citrate solution in CO₂-free double-distilled water (Reynold's lead citrate stain) for 2 min. Observations and micrographs were made with a JEM-100CXII electron microscope (JEOL) (Bozzola & Russell, 1991) at the Electron Microscopy Facility, Nelson Biology Laboratory, Rutgers University, New Brunswick, USA. On R2A agar, strains SP1PR4^T and SP1PR5 formed small, circular, convex colonies with a diameter of approximately 1 mm. The pigment varied from light beige to light pink depending on the age of the culture. Cells of strains SP1PR4^T and SP1PR5 were Gram-negative, non-spore-forming, non-motile rods with a length of 1.5–3.0 µm and a diameter of 0.5–0.7 µm (Fig. 2). The cell-wall structure was typical of Gram-negative bacteria as seen in electron micrographs of ultrathin sections (Fig. 2a). Numerous outer-membrane vesicles with a size of approximately 0.05 µm were also regularly observed (Fig. 2b).

Growth of strains SP1PR4^T and SP1PR5 at 4–35 °C and pH 3–9 was tested on R2A agar or broth. SP1PR4^T grew at pH 4.5–7.5 and at 4–30 °C, with optimum growth at pH 6 and 25 °C. Strains SP1PR4^T and SP1PR5 were negative for oxidase and positive for catalase activities, as determined using Bactident oxidase strips and Bactident 3% hydrogen

peroxide solution (Merck), respectively. Carbon source utilization and enzymic activities of strains SP1PR4^T and SP1PR5 were compared to those of *T. roseus* DSM 18391^T by analysing all three strains in parallel. Assimilation of various carbon sources was tested after up to 7 days incubation at 20 °C using API 50 CH and API 20 NE (bioMérieux) kits, Biolog GN Microplates and in 96-well plates with VL55 mineral medium (pH 5.5; Sait *et al.*, 2002) supplemented with yeast extract (100 mg l⁻¹) and 10 mM carbon source. Yeast extract was required for good growth on single carbon sources. Growth of strains SP1PR4^T and SP1PR5 in liquid medium was flocculant and was scored positive if a visible increase in turbidity and/or flocs was detected when compared to a control well, lacking the carbon source. Enzymic activities of the strains were assayed after 48 h incubation using API ZYM kits (bioMérieux). Hydrolysis of different polysaccharides (starch, carboxymethyl cellulose, cellulose, xylan, lichenan, pectin, alginate, laminarin, pullulan, chitosan and chitin) was determined from CO₂ production after up to 20 days incubation at room temperature. CO₂ was measured from 0.1 ml gas samples using an Agilent HP 6890 series II GC (Hewlett Packard) equipped with a thermal conductivity detector and a Hayesep Q (80/100 mesh, 10 ft; Agilent Technologies) packed column. Carbon source utilization data are given in Table 1 and the species description.

The DNA base composition of SP1PR4^T was determined following the method of Mesbah *et al.* (1989). Genomic DNA was extracted and purified by phenol/chloroform extraction using a method modified from Kerkhof & Ward (1993). RNase A (50 µg ml⁻¹) was added and the mixture was incubated at 37 °C for 30 min followed by the addition of 50 µl 10% SDS and 50–100 µl proteinase K (100 µg ml⁻¹) and incubation at 37 °C for 1–12 h until loss of turbidity and viscosity was observed. The DNA G+C content (mol%) was determined by HPLC with the modifications described by Männistö *et al.* (2010). Salmon sperm DNA was used for calibration and *Escherichia coli* DNA was used as a control. The DNA G+C content of strain SP1PR4^T was 57.3 mol% (Table 1).

Cellular fatty acids of strains SP1PR4^T, SP1PR5 and *T. roseus* DSM 18391^T were analysed from cells grown on R2A agar (pH 6) at 20 °C for 5–7 days. Whole-cell fatty acids were methylated and analysed as described previously (Männistö & Häggblom, 2006) using an Agilent HP 6890 series II GC (Hewlett Packard) equipped with a flame-ionization detector. Identification of fatty acid methyl esters was further confirmed using an Agilent HP 6890 GC-MS equipped with an HP-5MS column (30 m × 0.25 mm ID, nominal film thickness 0.25 µm). Summed feature 3 was shown to contain only C_{16:1}ω7c, with no iso-C_{15:0} 2-OH detected. The major cellular fatty acids (>4%) of strain SP1PR4^T were iso-C_{15:0}, C_{16:1}ω7c, iso-C_{13:0} and C_{16:0} (Table 2).

The results of phylogenetic analysis and the cellular fatty acid compositions and DNA G+C contents placed strains

Fig. 2. Electron micrographs of ultrathin sections of vegetative cells of a 2-week-old culture of strain SP1PR4^T showing cell morphology (a) and outer-membrane vesicles indicated by arrows (b). Bars, 1 µm (a), 0.2 µm (b).

Table 1. Physiological characteristics of strains SP1PR4^T, SP1PR5 and *T. roseus* DSM 18391^T

Strains: 1, SP1PR4^T; 2, SP1PR5; 3, *T. roseus* DSM 18391^T. All data are from this study unless otherwise stated. All strains were positive for the hydrolysis of aesculin, laminarin, pectin and starch and for the utilization of *N*-acetylglucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, lactose, maltose, D-mannose, melibiose, L-rhamnose, raffinose, sucrose, turanose and D-xylose. All strains were negative for hydrolysis of alginate, cellulose, gelatin, chitin, chitosan, lichenan and pullulan and for the utilization of acetate, adipate, adonitol, amygdalin, D- and L-arabitol, arbutin, D- and L-alanine, caprate, citrate, dulcitol, erythritol, formate, D- and L-fucose, gluconate, 2-ketogluconate, 5-ketogluconate, sodium glucuronate, sodium glutamate, glycerol, glycogen, D-glycine, inulin, L-leucine, D-lyxose, malate, mannitol, methyl D-mannoside, methyl D-glucoside, methyl xyloside, L-ornithine, L-sorbose, D-sorbitol, D-tagatose, trehalose and xylitol. All strains were positive for the following enzymic reactions (API ZYM): acid and alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α - and β -galactosidase, α - and β -glucosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. All strains were negative for esterase (C4, C8), lipase (C14), valine arylamidase, arginine dihydrolase and urease activities, indole production, nitrate reduction and glucose fermentation. All strains were catalase-positive, oxidase-negative and non-motile. ND, Not determined.

Characteristic	1	2	3
Origin	Tundra soil	Tundra soil	Agricultural soil
Pigment	Light pink	Light pink	Pink
Growth temperature range (°C)	4–30	4–30	12–23*
pH range (optimum)	4.5–7.5 (6)	4.5–7.5	5–7 (6)*
Growth at 4 °C	+	+	–
DNA G + C content (mol%)	57.3	ND	59.8*
Utilization of:			
Inositol, L-arabinose, D-lyxose	–	–	+
Melezitose, D-ribose	+	+	–
Methyl D-mannoside	–	–	+
Enzymic activities (API ZYM):			
α -Chymotrypsin	+	+	–
Trypsin	–	–	+

*Data from Eichorst *et al.* (2007).

SP1PR4^T and SP1PR5 within the genus *Terriglobus* (Eichorst *et al.*, 2007). The cellular fatty acid compositions of strains SP1PR4^T and SP1PR5 were relatively similar to that of *T. roseus* DSM 18391^T (Table 2), the main difference being a higher relative abundance of iso-C_{13:0} and a corresponding lower abundance of iso-C_{15:0} in strains SP1PR4^T and SP1PR5 compared to *T. roseus* DSM 18391^T. However, fatty acid profiles could not readily differentiate these species. Strains SP1PR4^T and SP1PR5 could be distinguished from *T. roseus* DSM 18391^T by having a much lighter reddish pigment, the ability to utilize melezitose and ribose and the inability to utilize inositol, L-arabinose, D-lyxose and methyl D-mannoside (Table 1). Genotypic analyses, including 16S rRNA phylogeny, *rpoB* gene sequence similarities, different ERIC- and BOX-PCR patterns and a low DNA–DNA hybridization value (<20%), clearly distinguished strains SP1PR4^T and SP1PR5 from their closest relative *T. roseus* DSM 18391^T. Based on the phylogenetic, phenotypic and chemotaxonomic data, it was concluded that strains SP1PR4^T and SP1PR5 represent a novel species of the genus *Terriglobus*, for which the name *Terriglobus saanensis* sp. nov. is proposed.

Description of *Terriglobus saanensis* sp. nov.

Terriglobus saanensis (sa.a.nen'sis. N.L. masc. adj. *saanensis* pertaining to Mount Saana, Finland).

Cells are Gram-negative, non-motile, aerobic rods, ~0.5–0.7 × 1.5–3.0 µm. Colonies on R2A agar are light beige to light pink, circular, convex and smooth. Growth occurs at 4–30 °C and pH 4.5–7.5, with optimum growth at 25 °C and pH 6. Catalase-positive and oxidase-negative. Hydrolyses starch, pectin, laminarin and aesculin but not gelatin, cellulose, xylan, lichenan, sodium alginate, pullulan, chitosan or chitin. Utilizes the following substrates when tested in API 50 CH and API 20 NE systems and/or as 10 mM suspensions in VL55 buffer supplemented with yeast extract (100 mg l⁻¹): *N*-acetylglucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, lactose, D-mannose, melezitose, melibiose, L-rhamnose, raffinose, D-ribose, sucrose, turanose and D-xylose. Does not utilize acetate, adipate, adonitol, amygdalin, arbutin, D- or L-arabinose, D- or L-alanine, caprate, citrate, dulcitol, erythritol, formate, D-fucose, gluconate, 2-ketogluconate, 5-ketogluconate, sodium glucuronate, sodium glutamate, glycerol, glycogen, D-glycine, inositol, inulin, L-leucine, D-lyxose, malate, mannitol, methyl D-mannoside, methyl D-glucoside, methyl xyloside, L-ornithine, L-sorbose, D-sorbitol, D-tagatose, trehalose or xylitol. Produces acid and alkaline phosphatase, leucine arylamidase, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α - and β -galactosidase, α - and β -glucosidase, *N*-acetyl- β -glucosaminidase, β -glucuronidase, α -fucosidase and α -mannosidase but not esterase,

Table 2. Cellular fatty acid compositions of strains SP1PR4^T, SP1PR5 and *T. roseus* DSM 18391^T

Strains: 1, SP1PR4^T; 2, SP1PR5; 3, *T. roseus* DSM 18391^T. All strains were grown on R2A agar (pH 6) at 20 °C for 5–7 days. Values are percentages of the total fatty acid content expressed as means ($n=4$) with standard deviation in parentheses. tr, Trace ($\leq 0.3\%$); –, not detected.

Fatty acid	1	2	3
C _{14:0}	2.1 (0.1)	2.3 (0.3)	2.2 (0.3)
C _{15:0}	0.4 (0.2)	0.5 (0.0)	tr
C _{16:0}	8.4 (2.2)	5.4 (0.6)	4.0 (0.7)
C _{17:0}	0.5 (0.1)	0.5 (0.0)	tr
C _{18:0}	0.4 (0.2)	–	–
C _{14:1ω5c}	1.7 (0.6)	2.4 (0.2)	1.9 (0.1)
C _{15:1ω6c}	2.0 (0.6)	3.1 (0.1)	0.8 (0.7)
C _{16:1ω5c}	tr	–	tr
C _{16:1ω7c}	28.4 (1.2)	29.5 (2.1)	33.2 (1.7)
C _{17:1ω6c}	–	–	tr
C _{18:1ω5c}	–	–	0.6 (0.4)
C _{18:1ω7c}	0.4 (0.4)	–	tr
iso-C _{13:0}	9.8 (2.2)	12.4 (0.4)	4.9 (0.4)
iso-C _{15:0}	39.9 (2.8)	36.2 (1.9)	46.5 (3.1)
iso-C _{17:0}	1.7 (0.5)	1.1 (0.2)	1.9 (1.0)
iso-C _{17:1}	0.5 (0.1)	0.5 (0.1)	tr
anteiso-C _{15:0}	0.8 (0.1)	1.1 (0.1)	tr
anteiso-C _{17:0}	tr	0.5 (0.1)	tr
Summed feature 1*	–	–	2.3 (1.0)

* Summed feature 1 comprises iso-C_{15:1} H and/or C_{13:0} 3-OH.

lipase, trypsin, valine or cystine arylamidase. The major cellular fatty acids are iso-C_{15:0}, C_{16:1 ω 7c}, iso-C_{13:0} and C_{16:0}.

The type strain, SP1PR4^T (=DSM 23119^T =ATCC BAA-1853^T), was isolated from soil samples collected from a wind-exposed ridge of Mt. Saana, Finland. The DNA G + C content of the type strain is 57.3 mol%.

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