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**TAXONOMIC DESCRIPTION**

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# *Granulicella sibirica* sp. nov., a psychrotolerant acidobacterium isolated from an organic soil layer in forested tundra, West Siberia

Igor Y. Oshkin,[1] Irina S. Kulichevskaya,[1] W. Irene C. Rijpstra,[2] Jaap S. Sinninghe Damsté,[2,3] Andrey L. Rakitin,[4] Nikolai V. Ravin[4] and Svetlana N. Dedysh[1,*]

**Abstract**

An isolate of strictly aerobic, pale-pink pigmented bacteria, strain AF10$^T$, was obtained from an organic soil layer in forested tundra, Nadym region, West Siberia. Cells of strain AF10$^T$ were Gram-negative, non-motile rods that produced an amorphous extracellular polysaccharide-like substance and formed large cell aggregates in old cultures. These bacteria were chemoorganotrophic, mildly acidophilic and psychrotolerant, and grew between pH 3.5 and 7.0 (optimum, pH 4.5–5.0) and at temperatures between 2 and 30 °C. The preferred growth substrates were sugars and some polysaccharides. The major fatty acids were iso-$C_{15:0}$, $C_{16:0}$, $C_{16:1}\Delta 9c$ and 13,16-dimethyl octacosanedioic acid. The genome of strain AF10$^T$ was 6.14 Mbp in size and encoded a wide repertoire of carbohydrate active enzymes. The genomic DNA G+C content was 59.8 mol%. Phylogenetic analysis indicated that strain AF10$^T$ is a member of the genus *Granulicella*, family *Acidobacteriaceae*, but displays 94.4–98.0 % 16S rRNA gene sequence similarity to currently described members of this genus. On the basis of phenotypic, chemotaxonomic, phylogenetic and genomic analyses, we propose to classify this bacterium as representing a novel species of the genus *Granulicella*, *Granulicella sibirica* sp. nov. Strain AF10$^T$ (=DSM 104461$^T$=VKM B-3276$^T$) is the type strain.

The genus *Granulicella* belongs to the family *Acidobacteriaceae* of the phylum *Acidobacteria* and accommodates mildly acidophilic, strictly aerobic chemo-organotrophs, which possess hydrolytic capabilities and produce amorphous extracellular polysaccharide-like substances [1, 2]. At present, this genus includes 10 species with validly published names. *Granulicella paludicola*, *Granulicella aggregans*, *Granulicella pectinivorans* and *Granulicella rosea* originated from *Sphagnum*-dominated wetlands in northern Russia [1]. Strains representing *Granulicella arctica*, *Granulicella sapmiensis*, *Granulicella mallensis* and *Granulicella tundricola* were obtained from tundra soils of north-western Finland [3]. The type strains of *Granulicella cerasi* and *Granulicella acidiphila* were isolated from cherry (*Prunus yedoensis*) bark in Tsukuba, Japan [4] and the Guadiana pit lake at the Herrerías mine, Spain [5], respectively. Members of the genus *Granulicella* are common inhabitants of various low-temperature terrestrial environments, such as *Sphagnum*-dominated wetlands [6, 7] and acidic tundra soils [8–10]. In the course of our study on microbial diversity in soils of forested tundra, a bacterium with *Granulicella*-like cell morphology was isolated and designated strain AF10$^T$. A partial 16S rRNA gene sequence obtained from strain AF10$^T$ displayed high similarity (97–99 %) to a number of sequences retrieved in several cultivation-independent studies from various cold environments, i.e. Arctic glacier ice, highland grasslands and boreal forest soils (GenBank accession nos HQ622731, FJ624921, JN023629). At the same time, this sequence was divergent (94.4–98.0 % similarity) from 16S rRNA gene sequences of described members of the genus *Granulicella*. This study, therefore, was undertaken in order to determine the taxonomic position of strain AF10$^T$ and to verify its capability of growth at low temperatures.

Author affiliations: [1]Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow 119071, Russia; [2]NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, Utrecht University, 1790 AB Den Burg, The Netherlands; [3]Faculty of Geosciences, Department of Earth Sciences, Geochemistry, Utrecht University, Utrecht, The Netherlands; [4]Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow 119071, Russia.

*Correspondence: Svetlana N. Dedysh, dedysh@mail.ru

Keywords: Acidobacteria; Acidobacteriaceae; *Granuicella sibirica* sp. nov.; psychrotolerant bacterium; tundra soil.

Abbreviations: ANI, average nucleotide identity; EPS, extracellular polysaccharide-like substance; GH, glycoside hydrolase; GT, glycosiltransferase.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Granulicella sibirica* AF10$^T$ is MK053657. The annotated genome sequence of strain AF10$^T$ has been deposited in NCBI GenBank under the accession number RDSM00000000.

One supplementary table is available with the online version of this article.

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Strain AF10$^T$ was isolated from an acidic (pH 4.1–4.5) organic soil layer in forested tundra, Nadym region, West Siberia (65° 36′ 51.6″ N, 72° 43′ 35.5″ E). The isolation was performed using modified DSM medium No. 1284 containing (per litre distilled water): 0.6 g glucose, 0.1 g yeast extract, 0.1 g casamino acids, 0.1 g $NH_4NO_3$, 0.04 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$. The medium was solidified with 10 g $l^{-1}$ phytagel (Sigma–Aldrich). The medium pH was adjusted to 4.0–5.0 with 20–50 mg alginic acid $l^{-1}$. The inoculated plates were incubated at 20 °C for 4 weeks. One particular type of colony that developed on this medium was represented by small (1–2 mm in diameter after 4 weeks of incubation), irregularly circular, pale-pink, opaque colonies of gummy consistency that could be peeled from the medium surface. Cell material from these colonies was further re-streaked on the above described medium and the plates were incubated under the same conditions until the target bacterium, designated strain AF10$^T$, was obtained as a pure culture.

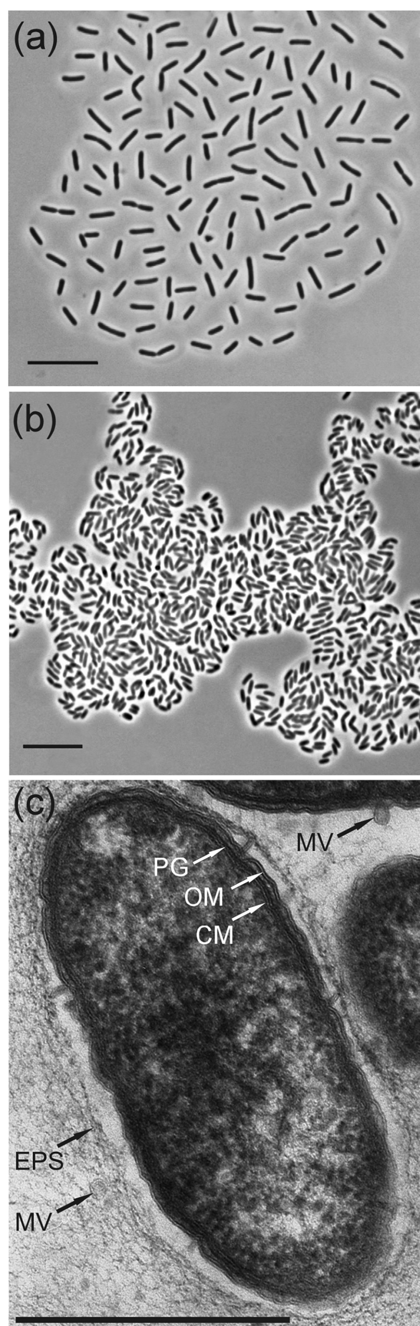


**Fig. 1.** Phase-contrast micrographs of cells of strain AF10$^T$ grown in liquid medium for 7 days (a) and 20 days (b); bar, 10 μm. (c) Electron micrograph of ultrathin section of a cell of strain AF10$^T$; bar, 1 μm. CM, cytoplasmic membrane; OM, outer membrane; PG, peptidoglycan layer; MV, membrane vesicles; EPS, extracellular polysaccharide-like substance.

Morphological observations and cell-size measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). Cell morphology was examined by using batch cultures grown to the exponential and stationary growth phases. Cells of strain AF10$^T$ were Gram-negative, non-spore-forming, non-motile rods that divided by binary fission, were 0.3–0.5 μm wide and 1.5–5 μm long (Fig. 1a). Young (up to 7–10 days old) cultures contained cells that occurred singly, in pairs or in short chains, and produced an amorphous extracellular polysaccharide-like substance (EPS), so that each cell was separated from other cells due to the extensive EPS production (Fig. 1a). The cells showed a tendency of forming aggregates upon ageing, so that old cultures contained large conglomerates of cells (Fig. 1b). Liquid cultures displayed white turbidity.

For preparation of ultrathin sections, cells of the exponentially growing culture of strain AF10$^T$ were collected by centrifugation and 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 with 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 [11]. After dehydration in an ethanol series, the samples were embedded into Epon 812 (Sigma-Adrich) epoxy resin. Thin sections were cut on an LKB-4800 microtome, stained with 3 % (w/v) uranyl acetate in 70 % (v/v) ethanol, and then stained with lead citrate [12] at 20 °C for 4–5 min. The specimen samples were examined with a JEM-1011 (JEOL) transmission electron microscope. Examination of thin-sectioned cells of strain AF10$^T$ revealed a typical Gram-negative structure of the cell wall. The cytoplasmic membrane, peptidoglycan layer and outer membrane were evident in ultrathin sections (Fig. 1c). The presence of outer-membrane vesicles that are commonly produced by members of the genus *Granulicella* was also detected. Staining of the EPS produced by cells of strain AF10$^T$ with ruthenium red [11] indicated that it was a polysaccharide-like substance.

Physiological tests were performed in tightly closed 120 ml serum bottles containing 10 ml liquid modified DSM medium No. 1284 with 0.5 g $l^{-1}$ glucose and 0.1 g $l^{-1}$ yeast

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extract. Growth of strain AF10$^T$ was monitored by measuring $OD_{600}$ for 2 weeks under a variety of conditions, including temperatures of 2–37 °C, pH 3.0–9.5 and NaCl concentrations of 0–5.0 % (w/v). Variations in the pH were achieved by mixing 0.1 M solutions of $H_2SO_4$ and KOH. Strain AF10$^T$ grew in the pH range of pH 3.5–7.0, with the optimum at pH 4.5–5.0. The temperature range for growth was 2–30 °C. The optimal growth with the specific growth rate $\mu$=0.130 $h^{-1}$ was observed at 20–25 °C. However, strain AF10$^T$ demonstrated good tolerance of low temperatures and grew relatively well down to 4 °C. The specific growth rates measured at 4 and 10 °C were 0.035 and 0.041 $h^{-1}$, respectively. NaCl inhibited growth at concentrations above 1.5 % (w/v).

Carbon source utilization and the ability of strain AF10$^T$ to degrade different biopolymers were determined using liquid mineral medium VL55 [13] with 50 mg $l^{-1}$ yeast extract in which glucose was replaced with one of the respective carbon sources or polymer substrates in a concentration of 0.05 % (w/v). The medium VL55 was chosen for these experiments because it was used to characterize several psychrotolerant members of the genus *Granulicella* isolated from tundra soils [3]. Growth intensity was the same on the modified DSM medium No. 1284 and the medium VL55. Oxidative and fermentative utilization of carbohydrates was determined by using the API 20 NE kit (bioMérieux). Catalase was tested using method 1 described by Gerhardt [14]. Oxidase was tested using a commercial kit (bioMérieux). Enzyme activities were examined by using the API ZYM kit (bioMérieux). The culture was also tested for growth under anoxic conditions in anaerobic jar by using AnaeroGen anaerobic system envelopes (Oxoid). Strain AF10$^T$ was a strictly aerobic chemoorganotroph that utilized a wide range of sugars and some polysaccharides, such as laminarin, lichenan, starch, pullulan and xylan (see Table 1 and the species description). It was oxidase- and urease-negative, but catalase-positive. No growth was observed under anoxic conditions. The tests for nitrate respiration and glucose fermentation were negative. The following enzyme activities were present: acid and alkaline phosphatases, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, $\alpha$- and $\beta$-galactosidases, $\alpha$- and $\beta$-glucosidases, *N*-acetyl-$\beta$-glucosaminidase, $\beta$-glucuronidase, $\alpha$-mannosidase and $\alpha$-fucosidase. The following enzymatic activities were negative: esterase (C4 and C8), lipase (C14), cystine arylamidase, trypsin and $\alpha$-chymotrypsin.

Susceptibility to antibiotics was determined on VL55 agar plates with 0.05 % (w/v) glucose using discs containing the following antibiotics (Oxoid): ampicillin (10 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (10 µg), novobiocin (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), lincomycin (10 µg) and rifampicin (10 µg). Strain AF10$^T$ was resistant to ampicillin, chloramphenicol, gentamicin, streptomycin and neomycin, but susceptible to lincomycin, novobiocin and kanamycin.

For lipid analyses, strain AF10$^T$ was grown on modified DSM medium No. 1284 and harvested in the late exponential growth phase. Lipids were analysed as described by Sinninghe Damsté *et al.* [15]. The PLFA (polar lipid derived fatty acids) profile of strain AF10$^T$ and those of other *Granulicella* species were highly similar and were defined by the predominance of 13,16-dimethyl octacosanedioic acid, iso-$C_{15:0}$, $C_{16:1}\Delta9c$ and $C_{16:0}$ fatty acids (Table S1, available in the online version of this article). This fatty acid pattern is characteristic for all currently described members of the genus *Granulicella* [2]. The only unique feature of the PLFA profile in strain AF10$^T$ was the minor presence of 6-methyl iso-diabolic acid, which, in addition to strain AF10$^T$, has so far only been found in *Paludibaculum fermentans* P105$^T$ [15].

Isoprenoid quinones were extracted according to Collins [16] and analyzed using tandem-type mass spectrometer LCQ Advantage Max and ionization mass spectrometer Finnigan Mat 8430. Similar to other taxonomically described members of the family *Acidobacteriaceae*, strain AF10$^T$ contained menaquinone-8 (MK-8) as the predominant isoprenoid quinone.

The genome of strain AF10$^T$ was sequenced using the Illumina HiSeq2500 platform (Illumina Inc., USA). The sequencing of a single-end (250 bp reads) TruSeq DNA library using HiSeq Rapid run version 2 sequencing reagents generated 1 774 910 reads. Upon adapter removal and quality trimming the reads were assembled into 16 contigs (N50 contig size of 1 421 513 bp; 54× average coverage) using SPAdes Genome Assembler version 3.11.1 [17]. The estimated genome size is 6 139 630 bp with an average G+C content of 59.8 mol%. Gene search and annotation were performed using the RAST server 2.0 [18], followed by manual correction. The annotated genome sequence of strain AF10$^T$ has been deposited in NCBI GenBank under the accession number RDSM00000000.

A total of 5289 protein-coding genes, two identical 16S–23S–5S rRNA operons and 48 tRNA genes were identified. The two 16S rRNA gene copies found in the genome were identical to 16S rRNA gene sequence determined by the Sanger sequencing of PCR-amplified gene fragment (GenBank accession number MK053657). The comparative analysis, which was carried out using the ARB program package [19], placed 16S rRNA gene sequence from strain AF10 $^T$ in the clade defined by several *Granulicella* species, i.e. *G. aggregans* TPB6028$^T$, *G. arcitica* MP5ACTX2$^T$, *G. sapmiensis* S6CTX5A$^T$, *G. pectinivorans* TPB6011$^T$, *G. tundricola* MP5ACTX9$^T$ and *G. rosea* TPO1014$^T$ (Fig. 2). The phylogenetically closest type strains were *G. pectinivorans* TPB6011$^T$ (97.96 % 16S rRNA gene sequence similarity), *G. sapmiensis* S6CTX5A$^T$ (97.68 %) and *G. aggregans* TPB6028$^T$ (97.61 %). We did not find loci for clustered, regularly interspaced, short palindromic repeats (CRISPRs).

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**Table 1.** Characteristics that differentiate strain AF10$^T$ from phylogenetically related species of the genus *Granulicella*

Strain: 1, AF10$^T$; 2, *G. aggregans*; 3, *G. rosea*; 4, *G. pectinivorans* (data from [1]); 5, *G. tundricola*; 6, *G. sapmiensis*; 7, *G. arctica* (data from [3]); 8, *G. paludicola* (type species of this genus; data from [1]). +, Positive; − negative; W, weakly positive; V, variable; ND, not determined or not reported.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| **Morphology:** | | | | | | | | |
| Cell length (μm) | 1.5–5.0 | 1.5–10 | 1.5–9.0 | 1.5–15 | 0.5–1.8 | 0.7–3.5 | 0.8–1.4 | 1.5–3.5 |
| Cell width (μm) | 0.3–0.5 | 0.8–1.5 | 0.5–1.0 | 0.8–1.0 | 0.5 | 0.5–0.7 | 0.5 | 0.4–0.6 |
| Colony colour | Pale pink | Pink | Pink | Red | Red | White | Red | Red |
| **Growth range:** | | | | | | | | |
| Temperature (°C) | 2–30 | 2–33 | 2–33 | 2–33 | 4–28 | 4–26 | 4–28 | 2–33 |
| pH | 3.5–7 | 3–7.5 | 3–7.5 | 3–7.5 | 3.5–6.5 | 3.5–7 | 3.5–6.5 | 3–7.5 |
| **Utilization of:** | | | | | | | | |
| **Sugars** | | | | | | | | |
| Galactose | + | + | − | + | + | + | + | + |
| Lactose | + | − | − | − | + | + | + | + |
| Lactulose | + | + | − | − | + | + | + | + |
| Maltose | + | + | + | − | + | + | + | + |
| Mannose | + | + | − | + | + | + | + | + |
| Melezitose | + | − | − | − | + | + | + | + |
| Raffinose | − | − | − | − | + | + | + | + |
| Rhamnose | + | + | − | − | ND | ND | ND | + |
| Ribose | − | + | − | − | − | + | − | − |
| Salicin | + | + | + | − | − | + | + | + |
| Sucrose | + | + | − | + | + | + | + | + |
| Trehalose | − | − | − | + | + | W | + | − |
| Cellobiose | + | − | − | + | + | + | + | + |
| Xylose | + | + | + | + | + | + | + | V |
| N-acetyl-D-glucosamine | + | + | − | + | + | − | + | + |
| **Acids** | | | | | | | | |
| Acetate | − | − | − | + | − | − | − | − |
| Gluconate | + | + | − | + | + | − | − | + |
| Glucuronate | − | + | + | − | − | W | − | V |
| Lactate | − | − | − | + | − | − | − | − |
| Malate | − | + | + | − | − | − | − | − |
| Pyruvate | − | + | + | − | − | − | − | − |
| Succinate | − | − | − | + | − | − | − | − |
| **Polyalcohols** | | | | | | | | |
| Mannitol | − | + | − | + | − | − | − | − |
| *myo*-Inositol | − | + | − | + | + | W | − | V |
| Sorbitol | − | + | − | − | − | − | − | − |
| Dulcitol | − | + | − | − | − | − | − | − |
| Oxidase | − | − | − | + | − | − | − | + |
| **Enzyme activities:** | | | | | | | | |
| Esterase (C4) | − | + | − | W | + | W | W | V |
| Esterase (C8) | − | + | + | + | + | W | W | − |
| Valine-arylamidase | + | + | − | + | + | − | − | + |
| $\alpha$-Chymotrypsin | − | + | − | + | + | + | W | V |
| $\alpha$-Galactosidase | + | − | − | + | + | + | + | + |
| $\alpha$-Glucosidase | + | + | − | + | + | + | + | + |
| *N*-Acetyl-$\beta$-glucosaminidase | + | + | − | + | + | + | + | + |
| Trypsin | − | − | − | + | + | − | − | − |
| **DNA G+C content (mol%)** | 59.8 | 59.3 | 58.3 | 59.1 | 59.9 | 56.0 | 57.5 | 57.3–57.4 |

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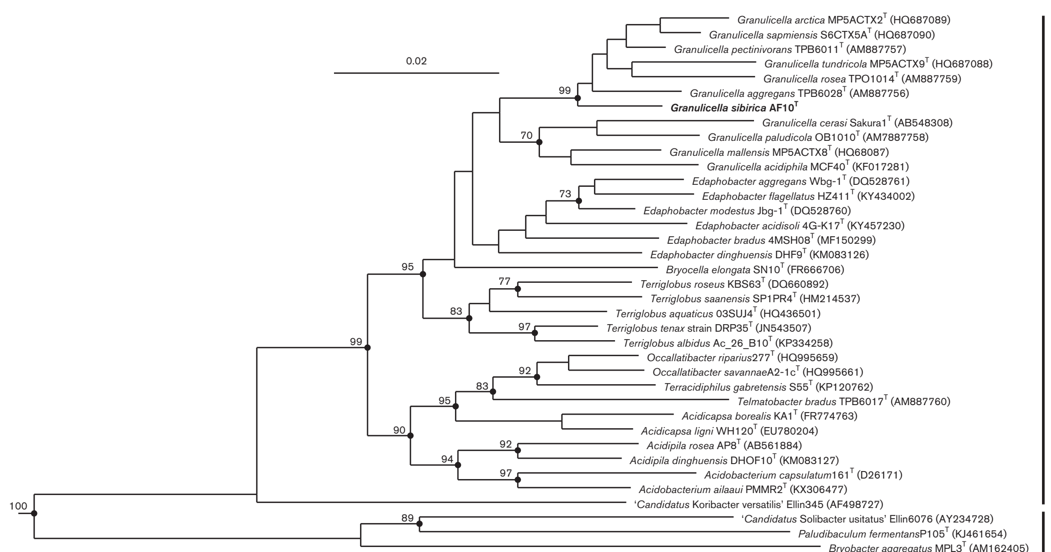


**Fig. 2.** 16S rRNA gene-based neighbour-joining tree showing the phylogenetic position of strain AF10$^T$ in relation to other representatives of the families *Acidobacteriaceae* (1) and *Bryobacteraceae* (2). The significance levels of interior branch points obtained in neighbour-joining analysis were determined by bootstrap analysis (1000 data resamplings). Bootstrap values of >70 % are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The root (not shown) was composed of three 16S rRNA gene sequences from members of the *Holophagae*, that is *Geothrix fermentans* H-5$^T$ (U41563), *Holophaga foetida* TMBS4$^T$ (X77215) and *Acanthopleuribacter pedis* FYK2218$^T$ (AB303221). Bar, 0.02 substitutions per nucleotide position.

Following the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes [20], we determined the overall genome similarities and the average nucleotide identity (ANI) of strain AF10$^T$ and the closely related organisms. Strain AF10$^T$ had the following overall genome similarities to the four publicly available genomes of *Granulicella* species: 20.8±2.3 % to *G. pectinivorans* DSM 21001$^T$, 20.8±2.3 % to *G. tundricola* MP5ACTX9$^T$, 20.4 ±2.3 % to *G. mallensis* MP5ACTX8$^T$ and 21.4±2.3 to *G. rosea* DSM 18704$^T$. These DNA–DNA hybridization values were estimated using formula 2 of the Genome-to-Genome-Distance-Calculator [21, 22] under parameter settings proposed elsewhere and are below 70 % cut-off value for species delineation with this method [23]. Accordingly, the ANI values (determined using ANI calculator: http://enve-omics.ce.gatech.edu/ani/) shared between the genome of strain AF10$^T$ and the genomes of other four representatives of the genus *Granulicella* are also below the threshold accepted for species discrimination: 78 % (*G. tundricola* MP5ACTX9$^T$), 78 % (*G. mallensis* MP5ACTX8$^T$), 79 % (*G. rosea* DSM 18704$^T$) and 79 % (*G. pectinivorans* DSM 21001$^T$) [20, 24].

KEGG-based annotation of the strain AF10$^T$ genome sequence classified 1881 proteins into 11 major functional categories. Most abundant categories were represented by carbohydrates metabolism and genetic information processing. The genes encoding metabolic pathways common for chemoorganotrophic bacteria, such as glycolysis, the citrate cycle, the pentose–phosphate pathway and oxidative phosphorylation, were identified in the genome of strain AF10$^T$.

The genome of strain AF10$^T$ encoded a wide repertoire of carbohydrate-active enzymes (CAZymes) including 131 glycoside hydrolases (GH) and 63 glycosyltransferases (GT) affiliated with 58 and 17 CAZy families, respectively. The most-represented families were GH2, GH105, GH29, GT2, GT4 and GT83. Utilization of aesculin, cellobiose, lactose, maltose, trehalose, and xylan can be explained by the occurrence of genes encoding GH2, GH5, GH13, GH15, GH43 and GH51-domain containing proteins. The cellulose synthase *bcsQABZC* operon found in the genome of strain AF10$^T$ could enable formation of observed amorphous extracellular polysaccharide structures. The presence of trehalose synthase and a pair of maltooligosyl trehalose synthase and maltooligosyl trehalose trehalohydrolase indicated that trehalose can be used as a storage polysaccharide and also contribute to cold tolerance of strain AF10$^T$. Protection against cold stress could also be enabled by cold-shock proteins of the CspA family, for which seven genes were identified in the genome. Similar mechanisms of cold protection were found in several other acidobacteria isolated from Arctic tundra soils [25].

In summary, strain AF10$^T$ displayed all features that are characteristic for members of the genus *Granulicella*, including moderate acidophily, good tolerance of low temperatures, aerobic lifestyle, preference for sugars as growth substrates, production of amorphous extracellular polysaccharide-like substances, and hydrolytic capabilities. However, according



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to comparative 16S rRNA gene- and genome-based analyses, our novel isolate was clearly distinct from earlier described *Granulicella* species. Characteristics that differentiate strain AF10$^T$ from the phylogenetically related species of the genus *Granulicella* are listed in Table 1. Based on these differences, we propose a novel species, *Granulicella sibirica* sp. nov., for strain AF10$^T$.

## DESCRIPTION OF *GRANULICELLA SIBIRICA*SP. NOV.

*Granulicella sibirica* (si.bi'ri.ca. N.L. fem. adj. *sibirica* originating from Siberia, referring to the site of isolation).

Cells are Gram-negative, non-motile, strictly aerobic rods, 0.3–0.5 µm wide and 1.5–5 µm long. Produces copious amounts of EPS. Tends to form large cell conglomerates in old cultures. Colonies are circular, pale-pink, opaque and of gummy consistency. Able to utilize the following carbon sources (0.05 %, w/v): D-glucose, D-fructose, D-galactose, D-mannose, melibiose, D-xylose, cellobiose, sucrose, *N*-acetyl-D-glucosamine, lactose, lactulose, leucrose, maltose, melezitose, D-rhamnose, salicin and gluconate. Unable to utilize D-arabinose, D-ribose, trehalose, raffinose, D-sorbose, D-fucose, arbutin, adonitol, sorbitol, dulcitol, mannitol, inulin, myo-inositol, acetate, lactate, sodium D-galacturonate, succinate, glucuronate, malate, pyruvate, butyrate, oxalate, propionate, formiate, fumarate, capronate, citrate, valerate, ethanol or methanol. Hydrolyses aesculin, laminarin, lichenan, starch, pullulan and xylan, but not pectin, sodium alginate, carboxymethylcellulose, cellulose, chitin, chitosan or fucoidan. Positive for acid and alkaline phosphatases, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, $\alpha$- and $\beta$-galactosidases, $\alpha$- and $\beta$-glucosidases, *N*-acetyl-$\beta$-glucosaminidase, $\beta$-glucuronidase, $\alpha$-mannosidase, and $\alpha$-fucosidase, but negative for esterase (C4 and C8), lipase (C14), cystine arylamidase, trypsin and $\alpha$-chymotrypsin. Oxidase- and urease-negative, but catalase-positive. Capable of growth at pH 3.0–7.0 (optimum, pH 4.5–5.0) and at 2–30 °C (20–25 °C). NaCl inhibits growth at concentrations above 1.5 % (w/v). Resistant to ampicillin, chloramphenicol, gentamicin, streptomycin and neomycin, but susceptible to lincomycin, novobiocin and kanamycin. Major fatty acids are 13,16-dimethyl octacosanedioic acid, $C_{16:1}$ Δ9 c, iso-$C_{15}$ and $C_{16:0}$.

The type strain, AF10$^T$ (=DSM 104461$^T$=VKM B-3276$^T$), was isolated from an organic soil layer in forested tundra, Nadym region, West Siberia (65° 36′ 51.6″ N, 72° 43′ 35.5″ E). The G+C content of the genomic DNA of the type strain is 59.8 mol% (genome sequence).

EMBL/GenBank accession (16S rRNA gene): MK053657.

EMBL/GenBank accession (genome): RDSM00000000.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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