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DIVERSITY OF CULTURED PROKARYOTES IN PERMAFROST SEDIMENT SAMPLES FROM WEST SPITSBERGEN ISLAND

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The diversity of anaerobic and aerobic microorganisms (prokaryotes) was studied in permafrost soil sampled at a depth of 0.5 to 3.7 m during the 2016 expedition of the State Scientific Centre of the Russian Federation the Arctic and Antarctic Research Institute (AARI). The estimated number of organotrophic microorganisms varies from 3.29·10 to $7.0 \cdot 10^4$ CFU·g^{-1} for aerobic, and from 3.0·10 to $2.3 \cdot 10^4$ cell·g^{-1} for anaerobic organisms. In separate anaerobically cultivated samples, methane and acetate were observed, while sulfate and iron reducing prokaryotes were detected in none of the samples. In the course of research, 60 strains of aerobic psychrophilic and psychotolerant bacteria were isolated. The taxonomic position of the isolated microorganisms was established by sequencing the 16S rRNA genes and using MALDI mass spectrometry. The created collection of strains consisted of representatives of the *Actinobacteria*, *Firmicutes*, *Betaproteobacteria* and *Gammaproteobacteria* phyla.

Permafrost sediments, West Spitsbergen, microbial communities, psychrophilic microorganisms, anaerobic prokaryotes

INTRODUCTION

The low-temperature high-latitude ecosystems and microbial communities inhabiting them are highly sensitive to global climate changes. A lack of balance between trophic interactions within the community can provoke a rise in greenhouse gas emissions, thereby potentially accelerating the rate of global warming. Recent research results have convincingly demonstrated that microbial communities within perennially frozen sediment (permafrost) are characterized by species richness. The novel bacteria and archaea taxa isolated from low temperature habitats include: *Arthrobacter psychrochitiniphilus* [*Wang et al., 2009*], *Carnobacterium pleistocenium* [*Pikuta et al., 2005*], *Clostridium algoriphilum* [*Shcherbakova et al., 2005*], *Exiguobacterium sibiricum* [*Rodrigues et al., 2006*], *Psychrobacter arcticus* [*Bakermans et al., 2006*], *P. glaciei* [*Zeng et al., 2016*], *Desulfovibrio arcticus* [*Pecheritsyna et al., 2012*], *Methanobacterium veterum* [*Krivushin et al., 2010*], *M. arcticum* [*Shcherbakova et al., 2011*], etc. Previously, it was shown that psychrophiles, i.e. cold-adapted microorganisms being, the sources of psychroactive enzymes are of great interest in terms of their biotechnological potential [*Marx et al., 2007; Margesin and Feller, 2010; Petrovskaya et al., 2010, 2012*], and that of held by identified genes of enzymes associated with the synthesis of antibiotics and antioxidants [*Hansen et al., 2007; Yuan et al., 2018*].

Earlier studies were focused primarily on the glacier microbiota [*Grzesiak et al., 2015*], and permafrost microbial communities [*Hansen et al., 2007; Singh et al., 2017*] of Spitsbergen Island. The dominant bacterial phyla group present in the West Spitsbergen permafrost-affected soil samples are: *Actinobacteria* (allocated to the genera *Arthrobacter*, *Cryobacterium*, etc.) and *Proteobacteria* (allocated to the genera *Pseudomonas*, *Psychrobacter*, etc.). A minor component of the microbiota was composed of *Firmicutes* (the genera *Bacillus*, *Paenibacillus*, *Virgibacillus*) and *Bacteroidetes* (the genera *Pedobacter*, *Spirosoma*, *Sphingobacterium*). A total of 38 representatives of these genera recovered from permafrost samples, have been identified by the researchers, along with sulfate reduction detected as the only anaerobic process [*Hansen et al., 2007*].

This paper sets out to expand the existing knowledge of the diversity of culturable prokaryotes on the example of permafrost samples from West Spitsbergen Island collected near the settlement of Barentsburg.

RESEARCH MATERIALS AND METHODS

Area and objects of study. The object of study is permafrost samples from core samples from seven wells drilled in the vicinity of Barentsburg (from 78°01′48″ to 78°06′11″ N and from 14°12′33″ to 14°19′25″ E) (Fig. 1).

Sample collection and storage. Both drilling and sampling operations were performed by the staff of the Soil Cryology Laboratory, Institute of Physicochemical and Biological Problems in Soil Science,

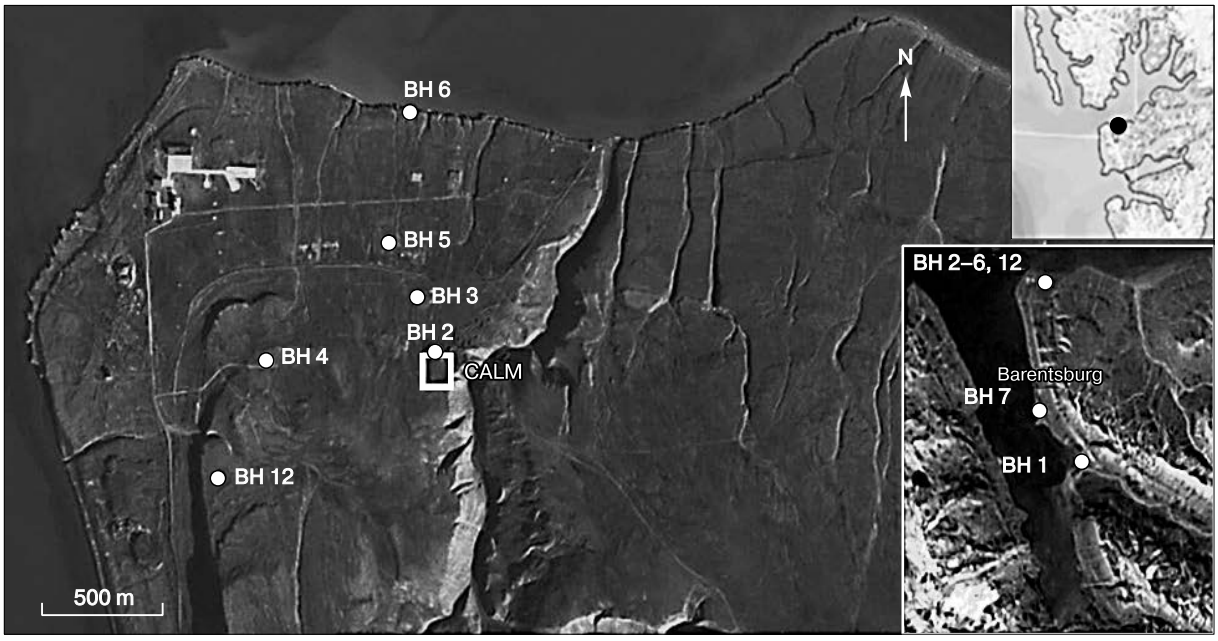

Fig. 1. Location of sampling points on the map of West Spitsbergen Island (modified after: [*Demidov et al., 2016*]).

Russian Academy of Sciences, during the 2016 AARI (Arctic and Antarctic Research Institute) expedition. Eight samples of sediments from seven boreholes were chosen for the microbiological study (Table 1). The thickness of the seasonally thawed layer (active layer, AL) in the vicinity of the boreholes varied from 1.15 to 2.6 m [*Demidov et al., 2016*]. Samples were collected from the active layer (samples 1 and 2), and from permafrost horizons (samples 3, 4, 5, 6, 7, 8).

The method of sterile sampling of permafrost using UKB-12/25 drilling equipment was previously described in detail in [*Gilichinsky et al., 1989; Shi et al., 1997*]. Before beginning the microbiological studies the samples had been stored at –20 °C in the laboratory conditions.

Microorganisms census. During the counting experiment to determine the maximum number of aerobic microorganisms (i.e. count of colony-forming units per gram of soil (CFU/g)), the standard nutrient (culture) media used for microorganisms recovered from permafrost [*Vishnivetskaya et al., 2000*] were: preliminary diluted 1:2 Tryptic Soy Broth (TSB) (supplied by Difco) with the addition of 15 g/L agar (Difco) – for heterotrophs; and R2A (Difco) – for aquatic oligotrophs from organics-depleted natural ecosystems. The cultivation was carried out at 7–8 and 20 °C, with the bacterial counts initiated on the 11th and 5th day, respectively.

We used the Hungate technique for cultivation of anaerobic bacteria [*Hungate, 1969*] at 7–8 and 20 °C. The count of anaerobic prokaryotes from various physiological groups was based on the liquid serial dilution method [*Gerhardt et al., 1994*], in Hangate test tubes with 10 mL total volume of medium. The number of anaerobic organotrophic bacteria was determined on the culture medium containing (g/L):

Table 1. **Characteristics of examined permafrost samples**

Sample No.	Depth, m	BH No.	Description	TDS, % of dry mass	pH	Concentrations of major ions in the water extract, mg/L				
						$F^- + Cl^-$	SO_4^{2-}	$Na^+ + K^+$	$Ca^{2+} + Mg^{2+}$	HCO^{3-}
1	0.5	6	Sand, gravel	0.024	6.4	17.0	1.0	21.3	0.7	2.1
2	1.25	4	Gravelly sandy loam	0.025	6.8	12.3	21.2	23.1	5.0	6.8
3	1.5	1	Sandy loam with gravel	0.040	7.2	16.7	24.4	31.4	3.5	8.7
4	1.75	2	Sand, clay-loam	0.007	6.7	14.7	3.2	20.0	1.1	4.1
5	2.2	3	Sand with pebble inclusions	0.014	6.7	14.6	8.2	22.3	1.6	4.9
6	2.9	5	Clay-loam	0.013	7.1	8.6	6.4	12.4	2.1	8.7
7	3.5	7	Sand, pebble and gravel	0.037	6.9	20.1	29.8	33.8	9.0	7.6
8	3.7	2	Clay	0.287	8.2	145.9	156.6	251.6	5.0	52.7

K_2HPO_4 (0.7), KH_2PO_4 (0.7), $MgSO_4 \cdot 7H_2O$ (0.1), $MnSO_4 \cdot 5H_2O$ (0.1), NH_4Cl (0.5), Na_2SO_4 (0.1), NaCl (0.9), ascorbic acid (1.0), peptone (2.0), glucose (2.5), trace element solution DSMZ 320 (1.0 mL), pH 7.0. The bacteria growth was inferred from variations in the broth medium turbidity, whose increase indicates an increase of the microbial cell mass.

A DSMZ 141 medium was used for both counts and isolation of methanogenic archaea (methanogens), while the presence of methanogens in the inoculum was determined from the presence of methane in the gaseous phase. The culture media for counts of acetogenic bacteria contained (g/L): NH_4Cl (0.1), $MgCl_2 \cdot 6H_2O$ (0.01), $CaCl_2$ (0.01), K_2HPO_4 (0.04), yeast extract (0.01), Tris-HCl (0.3), 2-bromoethanesulfonic acid BES (0.04), trace element solution DSMZ 318 (1.0 mL), resazurin (0.2), vitamin solution DSMZ 141 (10 mL), pH 7.0. A mixture of H_2/CO_2 (4:1) was used as a substrate for cultivation of acetogens. Bacteria growth was evaluated by acetate accumulation in the culture medium.

The culture medium (g/L) used for counts of sulfate-reducing bacteria (SRB) consisted of Na_2SO_4 (3.0), KH_2PO_4 (1.0), NH_4Cl (1.0), $CaCl_2$ (0.1), $MgSO_4 \cdot 7H_2O$ (0.25), KCl (0.5), yeast extract (0.5), trace element solution DSMZ 141 (2.0 mL), sodium lactate (1.0), pH 7.0. The growth of SRB that convert sulfate to sulfide was estimated by sulfide accumulation in culture medium.

Pfennig's medium modified for detection of iron-reducing bacteria contained (g/L): KH_2PO_4 (0.33), NH_4Cl (0.33), $MgCl_2 \cdot 6H_2O$ (0.33), $CaCl_2 \cdot 2H_2O$ (0.33), trace element solution (1.0 mL) [*Slobodkin and Wiegel, 1997*], vitamin solution (10.0 mL) [*Wolin et al., 1963*]. Sodium acetate (0.2) was used as electron donor and the only carbon source. To introduce electron acceptors, the media was supplemented with (mmol): Fe(III) citrate (20.0) and amorphous iron oxide hydroxide (AIOH) (10.0), which was prepared by titration of ferric chloride ($FeCl_3 \cdot 6H_2O$) solution with standard 10 % NaOH solution (wt/vol). The presence of iron reducers in the inoculum was determined by measuring the bivalent iron concentration in the culture medium.

All anaerobic inoculations were repeated three times, with the results recording after 60 days.

Obtaining pure cultures. Pure cultures of aerobic organotrophic bacteria were isolated by inoculation on solid culture media: 1/2 TSA (Difco) and R2A (Difco).

Microscopy techniques. Cell morphology was examined with phase contrast microscopy (PCM) at 1000 magnification using a transmitted light microscope Axiostar PLUS (Carl Zeiss, Germany).

Analytical methods. The optical density (OD) of culture medium was measured on a Spekol 221 spectrophotometer (Germany) using a wavelength of 600 nm. The *acetate* concentration was measured by high-performance liquid chromatography (HPLC) on a Knauer chromatograph (Germany) with an Inertsil ODS-3 column (5 μm, 250 × 4.6 mm; Dr. Maisch GmbH, Germany) at 210 nm and 35 °C with a flow rate 1 mL/min. The mobile phase composition was 20 mmol of orthophosphoric acid solution. The identification was performed using standard acid solutions at a concentration of 1 g/L (Sigma-Aldrich, USA) from retention times.

Methane concentration in the gas phase was determined by gas chromatography (Pye Unicam 304 GC (UK)) with flame ionization detection and using a glass column (length: 1 m, inner dia: 2 mm) with Porapak Q packing, mesh size 80/100 (Fluka, Germany). Sulfide detection was enabled by the standard Fischer method in the Cline modification [*Cline, 1969*]. The colorimetric method was applied to take into account *reduction* of Fe(III) by forming a stable magenta-colored divalent iron-ferrozine complex [*Lovley and Philips, 1986*].

Mass spectra of cells structures were obtained using a MALDI mass spectrometer (MS) Autoflex/26e3B (Bruker Daltonics, Germany) and a data processing system with the Compass™ software. Cells were isolated from the medium by centrifugation, washed with three volumes of 70 % HP ethanol, and resuspended. After ethanol removal, 50 μL of 70 % formate was added, with careful stirring, which was followed by adding 50 μL of acetonitrile. The mixture was stirred and centrifuged for 2 min at 13,000 rpm. Supernatant was spotted on the MALDI MS target, dried at room temperature, and coated with 1 μL of pre-prepared matrix solution (α-cyano-4-hydroxycoric acid).

DNA extraction, amplification and 16S rRNA gene sequencing. The chromosomal (genomic) DNA extraction and purification was based on the Marmur method [*Marmur, 1961*]. Amplification of nucleotide fragments of the 16S rRNA gene was carried out in a Tertsik amplifier (DNA-Technology, Russia). The total volume of the reaction mixture was 25 μL and included the following components: 1x Taq polymerase buffer (Silex, Russia); 10 ng of DNA matrix; 50 μmol of each dNTP (Silex, Russia); 0.25 μmol of primers 27f (3′-AGAGTTTGATC(A/C)TGGCTCAG-5′) and 1492r (3′-CGGTTTACCTTGTTACGACTT-5′) (Evrogen, Russia) [*Lane, 1991*], 1 unit of Taq polymerase (Eurogene, Russia). The amplification cycle consisted of initial denaturation for 3 min at 95 °C, subsequent 29 cycles-denaturation (95 °C, 30 s), primers annealing for 12 s (55 °C), DNA extension for 1 min 30 s (72 °C) and final extension for 3 min (72 °C). Results of the polymerase chain reaction (PCR) were evaluated by horizontal agarose gel (1 %) electrophoresis.

The complete nucleotide sequences of the 16S rRNA gene was determined at the Interinstitutional Center of collective usage "GENOM", Insti-

tute for Molecular Biology (Russian Academy of Sciences) using a set of the ABI PRISM BigDye™ Terminator v. 3.1. reagents. The reaction products were analyzed using the ABI PRISM 3730 automatic DNA sequencer (Applied Biosystems).

Phylogenetic analysis. The MEGA6 software was used for construction of phylogenetic trees [*Tamura et al., 2013*] on the basis of neighbor-joining (NJ), maximum likelihood (ML) and minimum evolution (ME) methods, which yielded similar dendrogram topologies, in this case. The resulting sequences are deposited in GenBank and labeled with numbers MK135907–MK135923.

RESULTS AND DISCUSSION

The scope of this study encompassed a wide range of permafrost occurrence depths and all the boreholes drilled into different geological structures. Of all the collected samples, those containing methane detected during the preliminary analysis were preferred as potentially indicative of the presence of spatially structured microbial communities with well-developed trophic relationships within permafrost affected soils.

Count of microorganisms. Quantitative characteristics of distributions of the aerobic and anaerobic representatives of permafrost microbial communities thriving on West Spitsbergen Island were studied on samples listed in Table 1.

The incubation temperature-dependent count of aerobic microorganisms varied between $1.61 \cdot 10^2$–$7.0 \cdot 10^4$ CFU/g at 7–8 °C and $3.29 \cdot 10$–$4.62 \cdot 10^4$ at 20 °C. The highest count of CFU/g was revealed in the active layer (sample 1) and at a depth of 2.9 m (sample 6) which amounted to $2.89 \cdot 10^4$ and $7.00 \cdot 10^4$ CFU/g, respectively (Table 2). For samples 4, 5 and 8, no growth of microorganisms was observed on any of the media under low-temperature conditions (7–8 °C), which was probably due to the lack of accessible organic carbon. The number of anaerobic organotrophic microorganisms determined by the serial dilution culture-most probable number (SDC-MPN) method were $3.0 \cdot 10$–$2.3 \cdot 10^4$ cells/g (at 7–8 °C) and $3.0 \cdot 10$–$2.5 \cdot 10^3$ cells/g (at 20 °C). Their maximum was $2.3 \cdot 10^4$ cells/g (sample 2) and minimum was $3.0 \cdot 10$ cells/g (sample 7). In samples 3, 4, 5, 7 and 8, the number of organotrophs did not depend on the incubation temperature, however in samples from the active layer (1, 2) and permafrost (6), the psychrophilic microorganisms count was an order of magnitude higher (Table 2).

There were no samples on the media used that contain sulfate- and iron-reducing bacteria. The counting experiment for methane-forming archaea and acetogens showed that methanogenesis was recorded in the dilution of ×10 when inoculating sample 3. In addition, acetate formed in the same dilution when inoculating samples 1, 7 and 8, despite the revealed significant differences in characteristics of these samples (Table 1). Thus, the number of acetogens and methanogens did not exceed tens of cells per gram of sediment (Table 2).

Previously, analysis of representative sampling of arctic soil bacterial communities showed that in buried soil and permafrost horizons, the number of bacteria can range from 10^2 to 10^8 cells per 1 g soil, locally reaching 10^8 cells/g [*Gilichinsky et al., 2008*]. The data obtained in this study correlate with the previous research results obtained for the West Spitsbergen bacterial permafrost community from the active layer [*Singh et al., 2017*] and the uppermost permafrost horizon [*Hansen et al., 2007*]. Thus, in the research conducted by Singh and colleagues [*Singh et al., 2017*] the count of culturable microorganisms did not exceed $7.6 \cdot 10^4$ CFU/g. In [*Hansen et al., 2007*], while the number of anaerobic bacteria was determined on a liquid medium R2A agar, which yielded bacterial count an order of magnitude higher, than the values obtained in our study ($7.3 \cdot 10^5$ cells/g) on samples from comparable soil horizons.

Table 2. **Number of cultivated prokaryotes in the studied permafrost samples**

Sample No.	Culturable microorganisms									
	Aerobic organotrophic prokaryotes, CFU/g				Anaerobic prokaryotes*, cell/g					
	R2A		1/2 TSA		Organotrophs		Acetogens		Methanogens	
	7–8 °C	20 °C	7–8 °C	20 °C	7–8 °C	20 °C	7–8 °C	20 °C	7–8 °C	20 °C
1	$1.2 \cdot 10^4$	$1.3 \cdot 10^4$	$1.2 \cdot 10^4$	$2.9 \cdot 10^4$	$5.6 \cdot 10^2$	5.6·10	5.6·10	0	0	0
2	$8.4 \cdot 10^2$	$1.0 \cdot 10^3$	$1.5 \cdot 10^3$	$9.7 \cdot 10^2$	$2.3 \cdot 10^4$	$2.5 \cdot 10^3$	0	0	0	0
3	$1.7 \cdot 10^2$	$3.9 \cdot 10^2$	$8.4 \cdot 10^2$	$8.4 \cdot 10^2$	4.9·10	4.9·10	0	4.9·10	4.9·10	4.9·10
4	0	7.4·10	0	$1.5 \cdot 10^2$	4.5·10	4.5·10	0	0	0	0
5	0	3.3·10	0	6.6·10	5.0·10	5.0·10	0	0	0	0
6	$7.0 \cdot 10^4$	$2.5 \cdot 10^4$	$7.0 \cdot 10^4$	$4.6 \cdot 10^4$	$4.9 \cdot 10^3$	$4.9 \cdot 10^2$	0	0	0	0
7	$4.8 \cdot 10^2$	$1.2 \cdot 10^2$	$1.6 \cdot 10^2$	$3.6 \cdot 10^2$	3.0·10	3.0·10	3.0·10	0	0	0
8	0	0	0	$5.4 \cdot 10^2$	$5.3 \cdot 10^2$	$5.3 \cdot 10^2$	$5.3 \cdot 10^2$	0	0	0

*Sulfate- and Fe(III)-reducing bacteria have not been detected in the samples.

Table 2 demonstrates the largest count of aerobic microorganisms (samples 1 and 6) and anaerobes (samples 2 and 6). In sample 1 collected from the active layer, the number of agar plate-grown colonies was the largest at 20 °C, while microorganisms growth was more active at 7–8 °C in sample 6 (permafrost). A relatively high count of microorganisms in the active layer, as compared to the underlying sediments, was noted previously in [*Spirina and Fyodorov-Davydov, 1998*], which is accounted for accessibility of the organic matter and enhanced biological activity during the summer months.

Obtaining pure cultures of aerobic bacteria. A total of 60 strains of aerobic psychrophilic and psychrotolerant bacteria were isolated during reinoculation onto solid medium. The first stage of research involved the rapid analysis of MALDI mass spectrometry (MS) method for identification of the obtained cultures, which allowed grouping identical strains and recognizing bacteria that represent taxa (Table 3). Based on the data obtained, strains EMg2, EMg3, YMg6, BMg2, BPb1 were assigned to the genus *Arthrobacter*, and strains EMg4, BPb2, Ami3 – to the genera *Massilia*, *Agromyces* and *Corynebacterium*, respectively. Given that the MALDI MS database contained mostly medical strains, this precluded identification of most of the environmental isolates.

16S rRNA gene sequence with 561 to 1403 b.p. (base pairs) were determined for 17 strains (Table 4). The bacteria isolates assigned to the genera *Arthrobacter*, *Cryobacterium*, *Frondihabitans*, *Micrococcus* (phylum *Actinobacteria*), *Carnobacterium*, *Bacillus*, *Sporosarcina* (phylum *Firmicutes*), *Massilia*, *Psychrobacter*, *Pseudomonas* (*Beta-* and *Gammaproteobacteria*) had similarity ranging from 98.5 to 100 % with other representatives known within these genera.

Table 3. **Taxonomic position of isolated strains from MALDI MS data**

Sample No.	Temperature, °C	Strain	Closest species (similarity coefficient*)
1	7–8	BPb1	*Arthrobacter pascens* (1.52)
1	7–8	BPb2	*Agromyces salentinus* (1.654)
3	20	PMd1	*Micrococcus luteus* (1.628)
6	20	BMg2	*Arthrobacter oxydans* (1.678)
6	20	EMg2	*Arthrobacter gangotriensis* (1.642)
6	20	EMg3	*Arthrobacter polychromogenes* (1.677)
6	20	EMg4	*Massilia timonae* (1.626)
6	20	YMg6	*Arthrobacter sulfonivorans* (1.716)
8	7–8	DPi1	*A. sulfonivorans* (1.646)
8	20	AMi3	*Corynebacterium falsenii* (1.616)

* The similarity coefficient (SC) value >1.5 indicates that the strain is within the specified genus, the SC value >2.0 indicates that the strain is within the species.

Dendrograms based on comparison of 16S rRNA gene sequences (Fig. 2) show that among actinomycetes of *Arthrobacter* spp. strains are representatives of already known species of this genus, while PMd1, CMb6 and LPf1 strains can be described as novel species of the genera *Micrococcus*, *Cryobacterium* and *Frondihabitans*, respectively (Fig. 2, *A*). Among the strains belonging to the *Firmicutes* phylum, the YPi4 and OMh2 strains can be ranked as novel species of the genera *Carnobacterium* and *Sporosarcina* (Fig. 2, *B*). Proteobacteria ZMg10, HPg4, GPg3 and BMg1 probably represent two novel species of the genus *Psychrobacter*, whereas strain CPi6 is expected to be described as a novel species of the genus *Massilia*.

Thus, as many as 27 strains of aerobic bacteria were found to be representative of the genus using

Table 4. **Taxonomic position of isolated aerobic microorganisms according to the 16S rRNA gene sequencing results**

Sample No.	Strain	Fragment length, b.p.	Closest culturable species (GenBank number)	Similarity, %
1	FPh4	689	*Arthrobacter oryzae* KV-651^T(AB279889)	99.3
1	OMh2	1140	*Sporosarcina globispora* 785^T (X68415)	98.5
2	APf1	561	*Arthrobacter psychrochitiniphilus* GP3^T (AB588633)	99.3
2	LPf1	1317	*Frondihabitans australicus* E1HC-02^T (FM998017)	99.1
3	CMb6	1403	*Cryobacterium arcticum* SK1^T (GQ406814)	98.7
3	CMb7	848	*Cryobacterium psychrotolerans* 0549^T (DQ515963)	99.8
3	PMd1	660	*Micrococcus endophyticus* YIM 56238^T (EU005372)	98.5
3	UMb2	1359	*Arthrobacter oryzae* KV-651^T (AB279889)	98.7
6	BMg1	1397	*Psychrobacter glacincola* ACAM 483^T (AJ312213)	99.3
6	GPg2	845	*Bacillus albus* N35-10-2^T (KJ812440)	99.6
6	GPg3	934	*Psychrobacter glaciei* BIc20019^T (FJ748508)	99.7
6	GPg4	797	*Pseudomonas migulae* CCUG 43165^T(AF074383)	100
6	HPg4	811	*Psychrobacter glaciei* BIc20019^T (FJ748508)	99.8
6	ZMg10	1373	*Psychrobacter glaciei* BIc20019^T (FJ748508)	99.5
7	CPi6	1024	*Massilia aurea* AP13^T (AM231588)	98.0
7	DPi1	681	*Arthrobacter pascens* VKM Ac-1116^T (X80740)	99.9
7	YPi4	1143	*Carnobacterium inhibens* K1^T (Z73313)	98.5

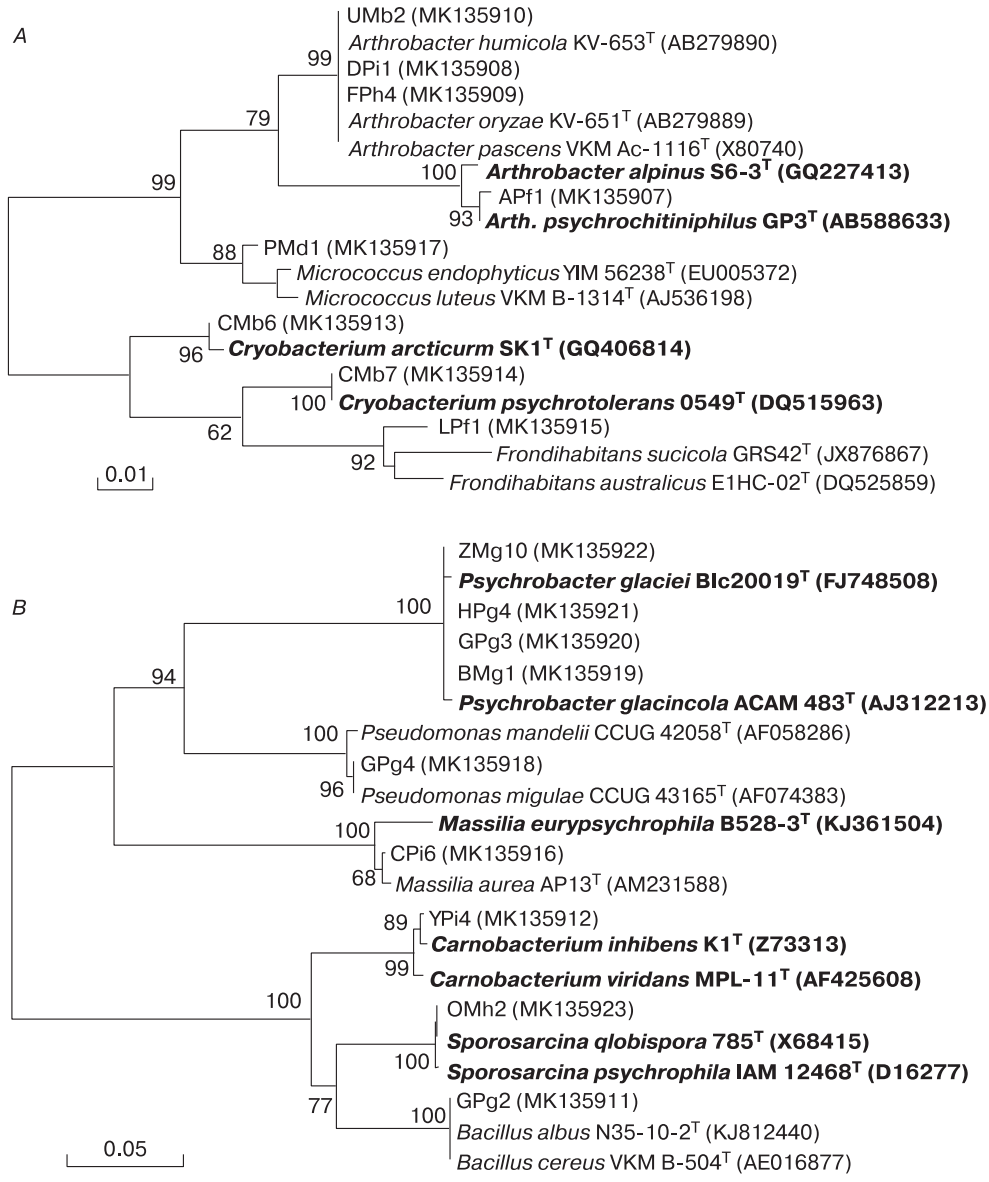

Fig. 2. Position of isolates among representatives of the phyla *Actinobacteria* (*A*), *Proteobacteria* and *Firmicutes* (*B*) on phylogenetic dendrograms based on the analysis of nucleotide sequences of the 16S rRNA gene using the "nearest neighbor" method (bootstrap = 1000).

Sequence numbers in NCBI are given in parentheses; closely related species from permanently low-temperature ecosystems are highlighted in bold type.

16S rRNA and MALDI MS gene sequencing techniques. Among the obtained isolates, some of the recognized representatives of *Actinobacteria* (58 %), *Proteobacteria* (23 %) and *Firmicutes* (19 %) showed a close phylogenetic affinity with those previously isolated from glaciers and sediments of West Spitsbergen Island.

16S rRNA gene sequences of the *Cryobacterium* sp. CMb6 (MK135913) and *Psychrobacter* sp. BMg1 (MK135919) strains were found to be close to the *Cryobacterium* sp. Asd. M3-6 (FM955863) and *Psychrobacter glacincola* Kongs-17 (HE800823) sequences which resulted from the studies of glaciers and fjords on Spitsbergen, with 98.8 and 99.1 % similarity, respectively. Figure 2 shows that many of the bacteria studied had been arranged in clusters with novel species isolated from cold-temperature habitats.

The most interesting bacteria from the perspective of further research are: ZMg10 strain assigned to the genus *Psychrobacter*, which is widespread in per-

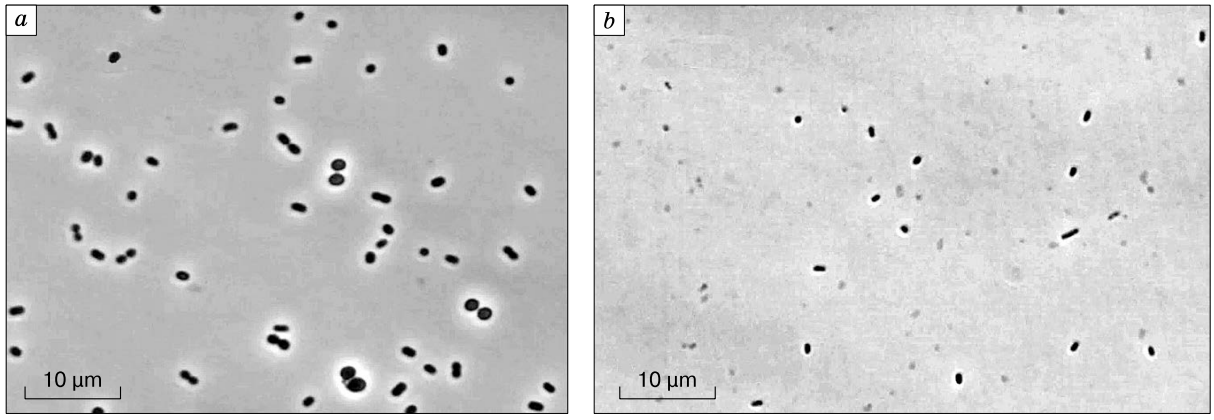

Fig. 3. Micrographs of the ZMg10 (*a*) and LPf1 (*b*) strain cells, phase contrast.

mafrost, and LPf1 strain assigned to the genus *Frondihabitans*, whose representatives have not been hitherto isolated from perennially frozen sediment.

The strain ZMg10 cells are immovable gram-negative cocci 1.5–2.0 μm in diameter (Fig. 3, *a*). The strain was isolated on 1/2 TSA medium at 20 °C, and later demonstrated good culturability on meat-peptone agar. On the plate, it formed convex gray-colored colonies with a smooth edge. When cultured on liquid media, this strain produced a precipitate which is hard to be dissolve. Analysis of the 16S rRNA gene sequence of strain ZMg10 (MK135922) showed that its closest relative (99.9 % identical) is the *Psychrobacter glaciei* BIc20019^T isolated from Lovenbrin glacier, West Spitsbergen Island [*Zeng et al., 2016*]. Despite a high similarity of the 16S rRNA genes, ZMg10 strain probably denotes a novel species of the genus, inasmuch as all species of the genus *Psychrobacter* are characterized by almost identical sequences of these genes.

Strain LPf1 belongs to the genus *Frondihabitans* and is the pioneering member of this genus identified in permafrost. The strain cells appear as small cocci (1.5–2.0 μm diameter) and then as short rods (1.0 by 2.0–3.0 μm), showing coccus-to-rod morphogenesis from the exponential growth phase to the stationary growth phase (Fig. 3, *b*). While growing on the plates, the strain produced smooth convex, cream-colored colonies. Initially, the strain was isolated on 1/2 TSA medium at 7–8 °C.

The 16S rRNA gene of LPf1 strain proved 99.1 % identical to its closest relative *Frondihabitans australicus* E1HC-02^T (DQ525859) suggests that this isolate probably belongs to a novel species. The previously discovered carotenoid-producing actinobacteria of the genus *Frondihabitans* were often associated with lichens which can harbor diverse bacterial families, and showed high protease and lipase activity in psychrophilic conditions, which makes them promising objects with biotechnological potential [*Lee et al., 2014*].

CONCLUSION

The number of cultured aerobic and anaerobic microorganisms was determined in the process of microbiological study of permafrost samples from West Spitsbergen Island collected near the settlement of Barentsburg. Novel methanogenic archaea and bacteria forming acetate from H_2 and CO_2 have been detected in the permafrost samples. A total of 60 strains isolated from of aerobic psychrophilic and psychrotolerant bacteria were identified, 27 of which were found to be representatives on the genus level.

The data obtained fundamentally contribute to our understanding of the structure of microbial communities in arctic permafrost environments. Further research will allow to study the isolated microorganisms in greater detail and evaluate their perspectives for biotechnological applications.

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