

Culturable Bacterial Communities Isolated from Cryo-Arid Soils: Phylogenetic and Physiological Characteristics

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Abstract—The results of the phylogenetic analysis and analysis of the physiological characteristics of metabolic activity of strains isolated from frozen sedimentary rocks collected in the Antarctic and the Severnaya Zemlya archipelago are reported in this article. A comparable abundance of cultured cells and close morphological diversity of colony morphotypes were revealed in all samples. Representatives of the *Actinobacteria* and *Firmicutes* phyla were predominant in the cultured bacterial communities. These communities were characterized by mesophilic and neutrophilic optima, as well as by the wide ranges of temperatures and pH suitable for metabolic activity. Moderate halotolerance in the presence of sodium or potassium chloride, as well as the high inhibitory effect of sodium hydrogencarbonate and low inhibitory effect of magnesium sulfate, were revealed. The communities were highly resistant to the presence of 5% magnesium perchlorate in the culture medium. Strains resistant to antibiotics in the composition of the medium were revealed: The isolated strains were the most resistant to ampicillin, chloramphenicol, and cephalixin.

Keywords: culturable bacterial communities, Antarctica, Dry Valleys, Arctic, antibiotic resistance, temperature, pH, halotolerance

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INTRODUCTION

Large territories of our planet are characterized by low temperatures. The World Ocean, most of which (90%) has the temperature below +5°C, occupies approximately 71% of the Earth's surface (Hartmann, 2015). The polar regions, including the Antarctic continent and the cold zones of the Arctic, occupy about 20% of the Earth's surface (Steven et al., 2006; Jansson and Taş, 2014). Ambient temperature is one of the most important parameters that control microbial activity in the natural environment. The temperature range for growth varies in different microorganisms: bacteria capable of growth and metabolic activity at around 0°C and lower temperatures were isolated from diverse natural and anthropogenic cold habitats (Rothschild and Mancinelli, 2001). The lower temperature limit for the metabolic activity of prokaryotes, known to date, is –18°C (Rivkina et al., 2000).

At temperatures below –20°C, the growth of prokaryotic cells is probably impossible due to its' vitrification (Clarke, 2014). In this state, microorganisms can persist for a long time. Under conditions favorable for metabolism, they are able to become in metabolic active state. At the same time, thermophilic and hyperthermophilic prokaryotic species that can grow

at +80°C or higher temperatures are known (Stetter et al., 1990).

An important biospheric function of permafrost is the cryopreservation of cells of various microorganisms (Bacteria, Archaea, and Fungi) in the state of metabolic dormancy (Rivkina et al., 1998). This is facilitated by the structural and mineralogical heterogeneity of heterophase media, such as soil and sedimentary rocks, in the horizontal and vertical directions, which results in the extremely high diversity of microlocal habitats in limited areas (Jansson and Taş, 2014).

Nucleotide sequences of the 16S rRNA gene, obtained from the Arctic and Antarctic soil samples, belong to bacteria of various phyla and functional groups (mainly anaerobic), including various methanogenic, sulfur-oxidizing, iron-reducing, and denitrifying bacteria. The bacterial diversity in permafrost rocks is significantly higher than that of archaea and fungi. *Proteobacteria*, *Firmicutes*, *Chloroflexi*, and *Acidobacteria* are the most typical phyla. Moreover, the metagenomic data obtained for permafrost soils and rocks often contain nucleotide sequences of the previously unknown phyla (Jansson and Taş, 2014). Analysis of total DNA revealed that the genes associated with various processes of carbon and nitrogen cycles,

including those encoding chitinases, cellobiases, β glycosidases, and β -galactosidases, were widespread in the microbial communities of permafrost soils. Genes associated with the processes of emission, transformation, and microbial degradation of greenhouse gases, such as CO₂, CH₄, and N₂O, were also identified (Yergeau et al., 2010; Taş et al., 2014).

Phylogenetic analysis of the surface and underlying permafrost layers of the West Antarctic revealed two predominant groups belonging to the *Proteobacteria* and *Actinobacteria* phyla in all studied samples. According to the analysis of clonal libraries of total DNA from the ice sheet of Central Antarctica, the following genera predominated in all samples: *Arthrobacter*, *Nocardioides*, *Bacillus*, *Caulobacter*, *Comamonas*, *Flavobacterium*, *Pseudomonas*, and *Sphingobacterium*. Anaerobic denitrifying (2–18 cells/g), methanogenic (2–22 cells/g), and sulfate-reducing (10²–10³ cells/g) bacteria were also identified (Gili-chinsky et al., 2007).

Analysis of the surface soil of the Antarctic Dry Valleys by molecular genetic methods revealed the presence of *Gammaproteobacteria* (25%), *Betaproteobacteria* (9%), *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* in the samples. Representatives of the genera *Rhodococcus*, *Methylobacterium*, *Sphingomonas*, and *Bacillus* (Goordial et al., 2016) were isolated from the same samples in pure cultures. The molecular genetic study of the taxonomic diversity of bacteria from the Antarctic soils (Yergeau et al., 2007; Zeng et al., 2013) indicated the presence of the genera *Arthrobacter*, *Corynebacterium*, *Micrococcus*, *Brevibacterium*, *Bacillus*, *Pseudomonas*, *Achromobacter*, *Nocardia*, *Flavobacterium*, *Streptomyces*, *Alcaligenes*, *Chromobacterium*, *Aeromonas*, and *Planococcus* (de los Ríos et al., 2004; Bulat, 2016).

In samples of snow collected near Druzhnaya and Leningradskaya Antarctic stations, the presence of bacteria (10³ cells/mL) was shown by epifluorescence microscopy; 45.8% of them were metabolically active. *Acinetobacter* sp., *Bacillus* sp., *Ochrobactrum* sp., *Pseudomonas* sp., and *Rhodococcus* sp. were able to form macrocolonies on nutrient media at +4°C. Moreover, all isolates were capable of rapid growth at +37°C; therefore, a wide temperature range was suitable for their growth (Lopatina et al., 2013).

Among heterotrophic aerobic bacteria isolated from the soils of polar latitudes at low temperatures, representatives of the following genera were dominant: *Arthrobacter*, *Corynebacterium*, *Cellulomonas*, *Microbacterium*, and *Brevibacterium*. Their abundance sometimes reached 10⁷ colony forming units (CFU) per gram of soil (Chattopadhyay, 2006).

Representatives of the *Acetobacterium*, *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Cellulomonas*, *Flavobacterium*, *Methanosarcina*, *Methylobacter*, *Micrococcus*, *Nitrobacter*, *Nitrosomonas*, *Pseudomonas*, *Rhodococcus*, and *Streptomyces* prevail in the composition of the

aerobic heterotrophic bacterial communities of the High Arctic soils. The total abundance of the cultured bacteria was approximately 4 × 10⁷ cells/g of soil, and a direct relationship was found between microbial diversity and the amount of organic matter in the samples (Wagner et al., 2005).

In the soil samples collected in the Canadian High Arctic, bacteria of the phylum *Firmicutes*, known for their ability to form highly resistant spores, as well as bacteria of the *Actinobacteria* and *Proteobacteria* phyla, characterized by high resistance to desiccation (including cryogenic drying) and oxidative stress (Steven et al., 2008), were identified in the dominant positions.

The snow and ice surfaces, as well as the soil or mineral surface layers of glaciers outside the polar latitudes, are most affected by frequent fluctuations in temperature and water availability. Different authors (Foght et al., 2004; Hallbeck, 2009) reported bacterial abundance in the ice and snow surface layers of various glaciers in the range from 0.01 to 2.7 × 10⁶ CFU/g (mL). The numbers of the reproductive cells in the modern and ancient surface ices aged up to 750000 years were found to vary from <10² to 10⁶–10⁷ CFU/g (Christner et al., 2003).

However, to date, the physiological characteristics of bacteria from cryo-arid ecosystems and their metabolic potential remains insufficiently studied. We studied cultured communities of aerobic heterotrophic bacteria isolated from the Antarctic surface and ancient frozen sedimentary rocks, as well as from the surface permafrost of the Severnaya Zemlya archipelago and their resistance to various physicochemical and biotic stress factors.

MATERIALS AND METHODS

The objects of this research were culturable bacteria isolated from the ancient permafrost sedimentary rocks of Antarctica and the surface permafrost of the Severnaya Zemlya archipelago.

The Permafrost sedimentary rock of the Antarctic desert (A-6/99 sample) was collected from borehole 6/99 drilled in the Beacon Valley lowlands (77°50' S, 160°36' E; at an altitude of 1270 m above sea level) from a depth of 1.3–1.5 m. The age of the rocks was not more than 70000 years (unpublished data from the Geological Survey of Canada). The permafrost sedimentary rock of the Antarctic Dry Valleys (Ho-3 sample) was collected from the borehole located at the mouth of the Taylor Valley (77°35' S, 163°24' E; at an altitude of 50 m above sea level) from a depth of 1.2–1.5 m. The sample was described in detail in the study carried out by D.A. Gilichinsky et al. (2007). The surface permafrost of Komsomolets Island of the Severnaya Zemlya archipelago (Sz sample) was collected at the western tip of the island near the Yuny Strait (80°19' N, 92°5' E) from a depth of 0–2 cm.

Prior to analysis, the samples were stored frozen at -20°C . The isolation of aerobic heterotrophic bacteria was performed using the glucose-peptone-yeast (GPY) medium containing various carbon sources and growth factors (Belov et al., 2018). The series of tenfold diluted soil suspension was directly plated on the solid nutrient media (Wood and Krieg, 1989). The inoculated dishes were incubated for 14 days at $+10$ and $+25^{\circ}\text{C}$.

For the amplification of the 16S RNA gene, DNA preparations of the isolated cultures were obtained by boiling the two- or three-day-old biomass in a TAE buffer with subsequent homogenization using sterile glass beads (see complete protocol in Belov et al. (2019)). The target gene was amplified using the 27f + 1492r, 537r + 805r or 27f + 537r primer pairs, which are listed in the order of their use, if the PCR product could not be obtained with the preceding primer pair (see complete protocol in Belov et al. (2019)).

Resistance to stress factors was determined using liquid culture technique. Stress agents were added to the medium composition. To assess the resistance to temperature, the inoculated plates were incubated in thermostats at $+2$ and $+4^{\circ}\text{C}$ for 60 and 30 days, respectively, as well as at $+10$, $+25$, $+37$, $+45$, and $+50^{\circ}\text{C}$ for 10 days. Phosphate (pH 2–7) and Tris-alkaline (pH 8–12) buffer systems were used to estimate the tolerance to pH. Resistance to the presence of salts was determined at concentrations of 2, 5, 10, 15, and 20% (wt/vol) of sodium or potassium chlorides, sodium bicarbonate, and magnesium sulfate; magnesium perchlorate concentrations studied were 0.5, 1, 2, 5, 10, and 15% (wt/vol). Resistance to ampicillin, kanamycin, tetracycline, doxycycline, cephalexin, chloramphenicol, and rifampicin was studied on the media containing $100\ \mu\text{g}/\text{mL}$ of one of these antibiotics. All tests were performed in triplicate with sterility controls. For all analyses, excluding low temperature cultivation, the incubation period was 10 days (Belov et al., 2018).

RESULTS

The samples were characterized by a high abundance of cultured cells. In the sample from the Antarctic desert (A6/99), close numbers of cultured bacteria were revealed under mesophilic and psychrophilic conditions: $(2.4 \pm 1.33) \times 10^5$ and $(2.29 \pm 1.25) \times 10^5$ CFU/g, respectively. In the sample of permafrost sedimentary rock from the Dry Valleys of Antarctica (Ho-3), the abundance of cells cultured at $+25^{\circ}\text{C}$ at the level of $(5.27 \pm 4.02) \times 10^5$ CFU/g and its decrease to $(8.67 \pm 1.24) \times 10^2$ CFU/g at $+10^{\circ}\text{C}$ were revealed. The surface permafrost collected on Komsomolets Island of the Severnaya Zemlya archipelago (Sz) was characterized by the following numbers of cultured bacteria: $(1.93 \pm 3.95) \times 10^5$ CFU/g at $+25^{\circ}\text{C}$ and $(4.00 \pm 3.53) \times 10^5$ CFU/g at $+10^{\circ}\text{C}$ (Fig. 1).

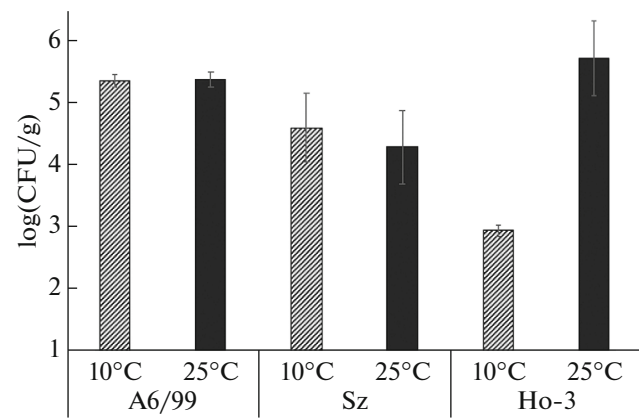


Fig. 1. Abundance of cultured bacterial cells in samples of permafrost sedimentary rocks from the Antarctic desert (A6/99), Antarctic Dry Valleys (Ho-3), and surface permafrost of the Severnaya Zemlya archipelago (Sz). Error bars show confidence interval with 95% significance level.

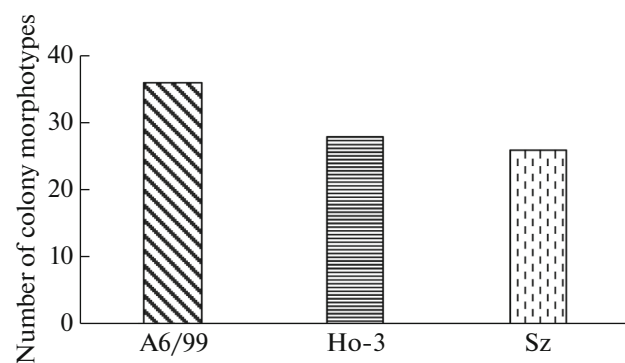


Fig. 2. Indicators of diversity of morphological types of bacterial colonies isolated from studied samples.

The cultured bacterial communities obtained in this study were characterized by moderate indices of bacterial colonies diversity. The most morphologically diverse culturable bacterial community was isolated from the Antarctic desert sample (36 morphological types of colonies). Twenty-eight morphotypes of bacterial colonies were isolated from the sample collected in the Dry Valleys, and twenty-six morphotypes of bacterial colonies were observed in the culturable bacterial community, which was isolated from the surface permafrost of Komsomolets Island (Fig. 2).

Gram-positive bacteria (representatives of the *Actinobacteria* and *Firmicutes* phyla) prevailed in all culturable communities: approximately 80% of colony forming units (Fig. 3). No connection between the community composition and the temperature of cultivation, or between the structure of the communities and the geographical location of the sampling point, was found.

Representatives of the *Bacillus* genus predominated in the culturable bacterial community isolated from

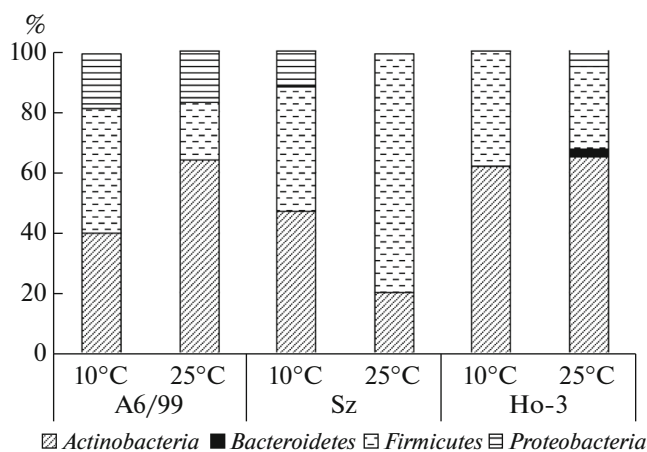


Fig. 3. Structure of cultured bacterial communities of studied samples.

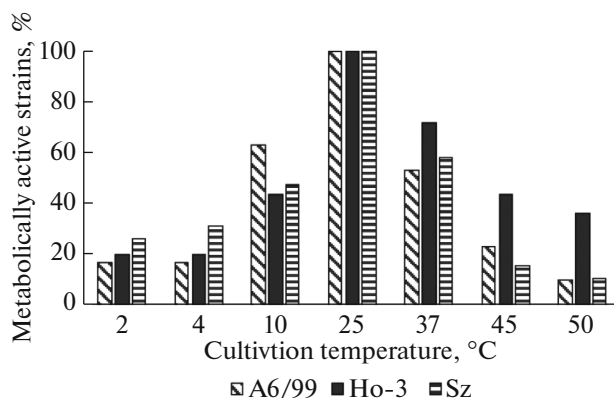


Fig. 4. Resistance to temperature of cultivation of strains isolated from studied samples.

the Antarctic desert sample at low temperatures (+10°C). Representatives of the genera *Arthrobacter*, *Brevibacterium*, *Geodermatophilus*, *Leucobacter*, and *Pantoea*, as well as of the species *Dietzia cinnamea* and *Shinella kummerowiae*, were also identified in this community. Bacteria of the *Rhodococcus* and *Bacillus* genera predominated in the communities isolated from this sample under mesophilic conditions. Representatives of the genera *Brevibacterium*, *Leucobacter*, *Microbacterium*, *Paracoccus*, *Pseudoarthrobacter*, and *Streptomyces*, as well as *Gordonia terrae*, *Kocuria rosea*, *Leucobacter aridicollis*, and *Stenotrophomonas maltophilia*, were identified.

Bacillus also predominated in the culturable bacterial community isolated from the ancient permafrost sample of the Dry Valleys of Antarctica at low temperatures (+10°C). Representatives of the genera *Arthrobacter*, *Rhodococcus*, and *Streptomyces*, as well as the species *Arthrobacter oxydans*, *Bacillus simplex*, *Microbacterium barkeri*, *Planomicrobium glaciei*, and *Stenotrophomonas maltophilia*, were also identified.

Bacteria of the genus *Bacillus* (*Bacillus infans* and *Bacillus* sp.) were identified as dominants in the community growing at +25°C; *Bacillus simplex*, *Bacillus subtilis*, *Lysinibacillus sphaericus*, *Paracoccus* sp., *Rhodococcus* sp., and *Streptomyces* sp. were identified also in this community.

The cultured bacterial community isolated from the surface soil of the Komsomolets Island of the Severnaya Zemlya archipelago at low temperatures was characterized by the predominance of bacteria of the species *Bacillus* sp. and *Microbacterium kitamiense* (in equal proportions); *Leucobacter aridicollis* and *Bacillus simplex* were subdominant. *Arthrobacter ginisoli* and *Arthrobacter* sp. were also detected as the minor components. The community isolated from the same sample at +25°C was characterized by the predominance of bacteria of the species *Micrococcus* sp., *Brevibacterium sediminis*, *Arthrobacter gisengisoli*, and *Leucobacter aridicollis*. *Arthrobacter* sp., *Bacillus pumilus*, *Bacillus simplex*, *Bacillus* sp., *Microbacterium paraoxydans*, *Microbacterium* sp., *Pedobacter* sp., *Rhizobium* sp., *Shingobacterium* sp., and *Stenotrophomonas maltophilia* were isolated as minor components.

The high proportion of representatives of the genus *Bacillus* in all studied communities is noteworthy, as well as the isolation of representatives of the genera *Arthrobacter*, *Dietzia*, *Geodermatophilus*, *Microbacterium*, *Pantoea*, *Planomicrobium*, *Shinella*, and *Stenotrophomonas* only during low-temperature cultivation. Table 1 shows detailed data on the composition of cultured communities.

The studied bacterial communities were characterized by mesophilic values of the temperature optimum (+25°C), at which all isolated strains were able to reproduce, and by broad temperature ranges for their metabolic activity: from +2 to +50°C. Approximately 20% of the strains isolated from the Antarctic samples could reproduce at +2 and +4°C. At these temperatures, 26 and 32% of the cultures isolated from the permafrost of Severnaya Zemlya archipelago were able to proliferate in these conditions, respectively. The most thermotolerant properties were revealed for the cultures isolated from the frozen soils of the Dry Valleys of Antarctica: 36% of isolates reproduced at +50°C. The cultures isolated from the Antarctic desert and the permafrost of Severnaya Zemlya were less resistant to high temperatures: 10 and 11% of the studied cultures, respectively, reproduced at +50°C (Fig. 4).

The cultures studied had a neutrophilic optimum for growth and pH-tolerant features: the growth was observed in the pH range from 3 to 12. It is noteworthy that a high proportion (37% of isolates) of acidotolerant strains was detected in the cultured bacterial community, which was isolated from the soil of the Antarctic desert. In all studied communities, 20% of isolates retained their metabolic activity at pH 12 (Fig. 5).

On media supplemented with sodium or potassium chlorides, moderately halotolerant properties of bac-

Table 1. Structure of cultured bacterial communities in studied samples

Taxonomic affiliation	A6/99		Ho-3		Sz	
	10°C	25°C	10°C	25°C	10°C	25°C
<i>Arthrobacter ginsengisoli</i>	0%	0%	0%	0%	10%	13%
<i>Arthrobacter oxydans</i>	0%	0%	1%	0%	0%	0%
<i>Arthrobacter</i> sp.	9%	0%	15%	0%	12%	5%
<i>Bacillus infantis</i>	0%	0%	0%	26%	0%	0%
<i>Bacillus pumilus</i>	0%	0%	0%	0%	0%	5%
<i>Bacillus simplex</i>	0%	0%	3%	13%	17%	3%
<i>Bacillus</i> sp.	41%	19%	38%	23%	21%	3%
<i>Bacillus subtilis</i>	0%	0%	0%	13%	0%	0%
<i>Brevibacterium sediminis</i>	0%	0%	0%	0%	0%	14%
<i>Brevibacterium</i> sp.	6%	4%	0%	0%	0%	0%
<i>Dietzia cinnamnea</i>	7%	0%	0%	0%	0%	0%
<i>Geodermatophilus</i> sp.	8%	0%	0%	0%	0%	0%
<i>Gordonia terrae</i>	0%	2%	0%	0%	0%	0%
<i>Kocuria rosea</i>	0%	2%	0%	0%	0%	0%
<i>Leucobacter aridicollis</i>	0%	6%	0%	0%	19%	10%
<i>Leucobacter</i> sp.	6%	3%	0%	0%	0%	0%
<i>Lysinibacillus sphaericus</i>	0%	0%	0%	5%	0%	0%
<i>Microbacterium barkeri</i>	0%	0%	4%	0%	0%	0%
<i>Microbacterium kitamiense</i>	0%	0%	0%	0%	21%	0%
<i>Microbacterium paraoxydans</i>	0%	0%	0%	0%	0%	5%
<i>Microbacterium</i> sp.	0%	14%	0%	0%	0%	5%
<i>Micrococcus</i> sp.	0%	0%	0%	0%	0%	17%
<i>Pantoea</i> sp.	9%	0%	0%	0%	0%	0%
<i>Paracoccus</i> sp.	0%	2%	0%	5%	0%	0%
<i>Pedobacter</i> sp.	0%	0%	0%	0%	0%	5%
<i>Planomicrobium glaciei</i>	0%	0%	11%	0%	0%	0%
<i>Pseudarthrobacter</i> sp.	5%	1%	0%	0%	0%	0%
<i>Rhizobium</i> sp.	0%	0%	0%	0%	0%	5%
<i>Rhodococcus</i> sp.	0%	25%	2%	5%	0%	0%
<i>Shinella kummerowiae</i>	9%	0%	0%	0%	0%	0%
<i>Sphingobacterium</i> sp.	0%	0%	0%	0%	0%	5%
<i>Stenotrophomonas maltophilia</i>	0%	17%	12%	0%	0%	5%
<i>Streptomyces</i> sp.	0%	5%	14%	10%	0%	0%

teria isolated from the studied samples were found. The strains isolated from the soil of the Antarctic Dry Valleys were the least resistant to the presence of sodium or potassium chlorides: 56% of the strains were able to reproduce in the medium containing 2% NaCl. In the presence of 5% NaCl, 60% of the strains in the communities isolated from samples A6/99 and Sz were retaining their metabolic activity (Fig. 6). Potassium chloride had a less pronounced inhibitory effect on the studied strains (the average resistance to the presence of this salt was two times

higher). In all the bacterial communities, strains that could reproduce in a saturated solution of this salt were identified (Fig. 7).

The presence of magnesium sulfate in the medium had a weak inhibitory effect on the studied communities: 60% of the isolates from the samples of the Antarctic desert and surface permafrost of Severnaya Zemlya, as well as 45% of the isolates from the frozen sedimentary rock of the Dry Valleys, retained their metabolic activity on the medium containing 20% magnesium sulfate (Fig. 8). The presence of low con-

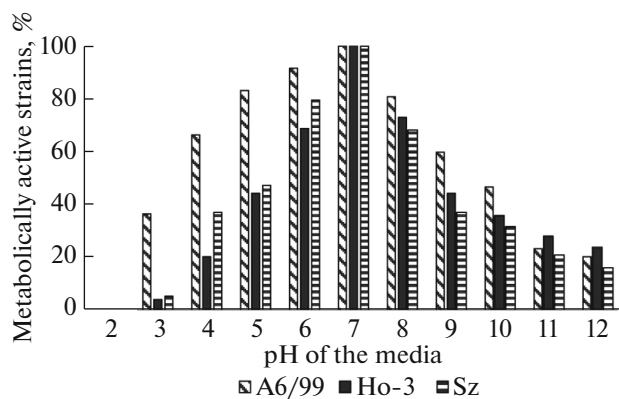


Fig. 5. Resistance to pH of the strains isolated from studied samples.

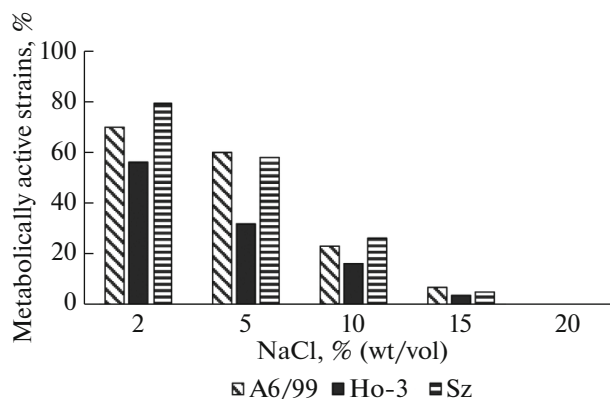


Fig. 6. Resistance to sodium chloride of strains isolated from studied samples.

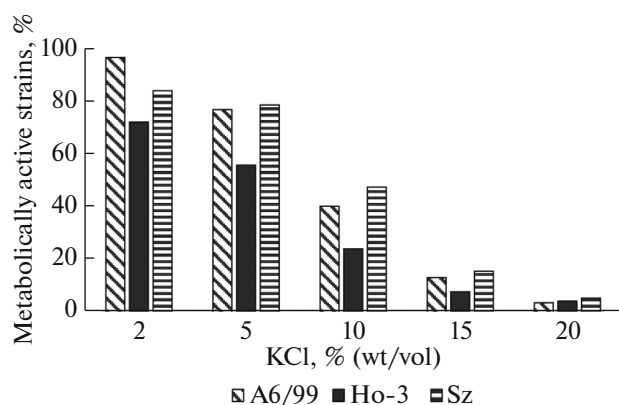


Fig. 7. Resistance to potassium chloride of strains isolated from studied samples.

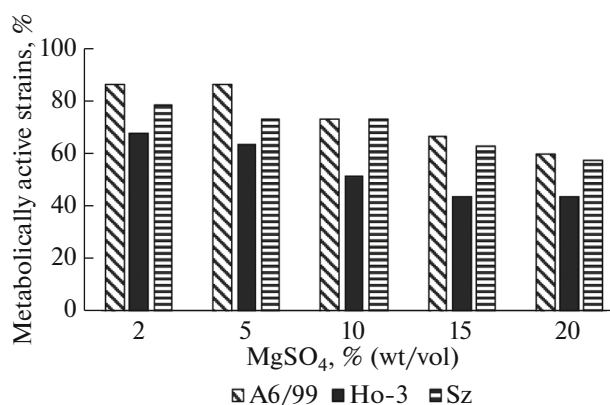


Fig. 8. Resistance to magnesium sulfate of strains isolated from studied samples.

concentrations (2%, wt/vol) of sodium hydrocarbonate significantly inhibited all studied cultures. At the same time, less than 20% of the strains from all communities were able to reproduce on medium containing 5% sodium hydrocarbonate. Single strains, which were stable in the presence of 10% sodium hydrocarbonate, were identified in the culturable communities that were isolated from the permafrost of the Antarctic Desert and the Severnaya Zemlya permafrost (Fig. 9). The lowest resistance to the presence of magnesium perchlorate in the medium was shown by bacteria isolated from the permafrost of the Antarctic Dry Valleys, while the maximum resistance to this salt was found for isolates from the soil of the Antarctic desert. The metabolic activity of pure cultures isolated from all the studied samples was observed at a magnesium perchlorate concentration of up to 5% in the medium (Fig. 10).

Bacteria capable of reproduction on media containing antibiotics were identified in all cultured bacterial communities. High proportions of bacteria resistant to ampicillin, chloramphenicol or cephalixin in the studied communities were revealed. In addition to

the resistance to the antibiotics listed above, strains that were resistant to tetracycline and kanamycin were found in all communities. In the communities isolated from the Antarctic frozen rocks, 10% of bacteria were resistant to doxycycline. Rifampicin-resistant bacterial strains were identified only in the cultured community isolated from the permafrost of the Antarctic Dry Valleys (Fig. 11).

DISCUSSION

The revealed numbers of the culturable bacteria indicate the enrichment of these samples with viable culturable bacterial cells both in the deep and in the surface layers. The observed numbers of culturable cells are in agreement with previous studies data (Christner et al., 2003; Foght et al., 2004; Hallbeck, 2009).

Close values for abundance of cells cultured at +10 and +25°C in the samples from the Antarctic desert and the surface permafrost of Komsomolets Island indicate widespread physiological adaptations in these

bacterial communities to existence at low temperatures. At the same time, the discovery of a predominantly mesophilic development strategy of the community from the permafrost of the Dry Valleys of Antarctica can be interpreted as an adaptive mechanism to the seasonal cycles of this ecotope. The availability of water and, therefore, the possibility of metabolic processes in this region of Antarctica increase with increased temperatures due to the extremely low humidity of this region. The segment of the complex capable of *in vitro* reproduction at +10°C is likely to be more xerotolerant, and therefore able to reproduce at low moisture availability, while the metabolic activity of the main segment of the cultured community requires higher values of moisture availability, which can be reached *in situ* at higher ambient temperatures.

The diversity values obtained indicate the effects of environmental (rather than geographical) factors, which have determined the composition of the community. Close environmental conditions and close diversity of bacterial communities characterize the studied samples. The geographical location of the sampling site is therefore not the factor that determines the diversity of bacterial communities, although they were collected in the polar regions of different hemispheres.

The predominance of the *Actinobacteria* and *Firmicutes* phyla representatives in all studied communities can be explained by the ability of representatives of these phyla to switch to a state of metabolic dormancy, and their long-term preservation in a metabolically inactive state, as well as by the wide distribution of the psychrotolerant species and species resistant to oxidative stress among them (Steven et al., 2008). Moreover, similar results were previously obtained using molecular genetic methods (Steven et al., 2008, Jansson and Taş, 2014). The absence of obvious correlations between the composition of the communities and the temperature of cultivation, as well as between the structure of communities and the geographical location of the sample collection, additionally confirms the hypothesis of the predominant role of the ecological factor in the development of bacterial communities.

The prevalence of bacteria of the *Bacillus* genus in all studied communities may be explained by their ability to form spores that are highly resistant to various environmental stressful effects (Nicholson et al., 2000).

Bacteria of the *Bacillus* genus predominated in the communities isolated from the sample of the Antarctic desert (A6/99) under psychrophilic conditions (+10°C); representatives of the genera *Arthrobacter*, *Brevibacterium*, *Geodermatophilus*, *Leucobacter*, *Pantoea*, and *Pseudoarthrobacter*, as well as the species *Dietzia cinnamea* and *Shinella kummerowiae* were identified in the culture. Under mesophilic conditions (+25°C), representatives of the genera *Rhodococcus*

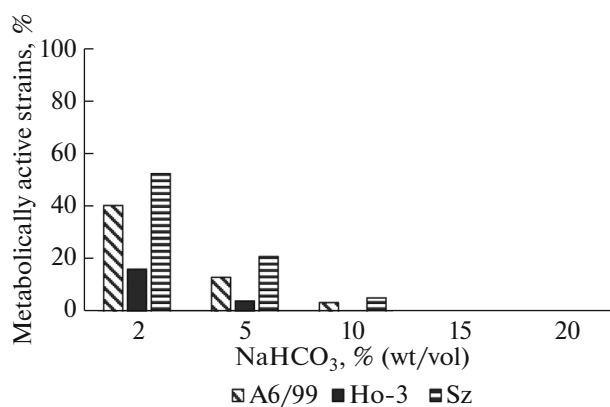


Fig. 9. Resistance to potassium bicarbonate strains isolated from studied samples.

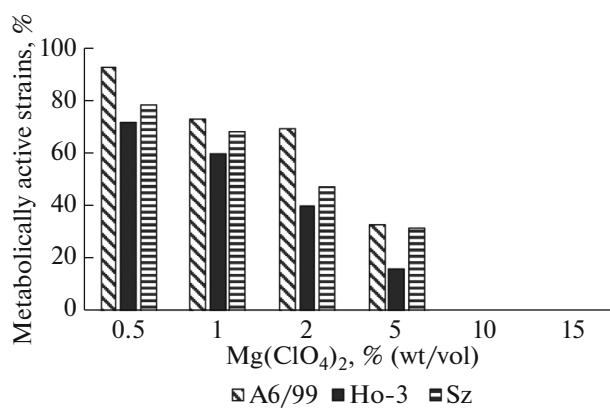


Fig. 10. Resistance to magnesium perchlorate of strains isolated from studied samples.

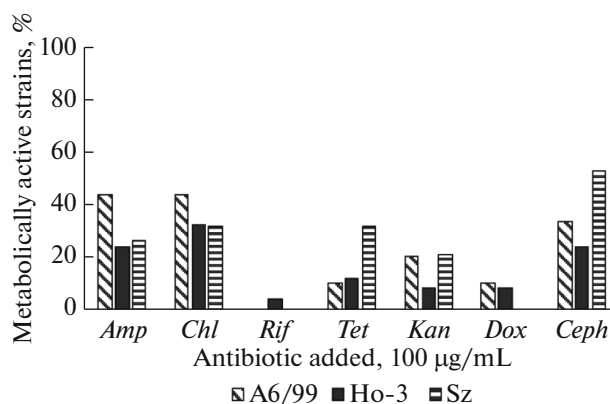


Fig. 11. Antibiotic resistance of strains isolated from studied samples.

and *Bacillus* prevailed in the cultured communities isolated from the same sample; bacteria of the *Brevibacterium*, *Leucobacter*, *Microbacterium*, *Paracoccus*, *Pseudoarthrobacter*, and *Streptomyces* genera, as well as the species *Gordonia terrae*, *Kocuria rosea*, *Leucobacter aridicollis*, and *Stenotrophomonas maltophilia* were determined in the culture.

Bacteria of the genus *Bacillus* also predominated in the cultured bacterial community isolated from the ancient permafrost of the Dry Valleys of Antarctica at low temperatures of cultivation (+10°C); representatives of the genera *Arthrobacter*, *Rhodococcus*, and *Streptomyces*, as well the species *Arthrobacter oxydans*, *Bacillus simplex*, *Microbacterium barkeri*, *Planomicrobium glaciei*, and *Stenotrophomonas maltophilia*, were identified. Bacteria of the genus *Bacillus* (*Bacillus infans* and *Bacillus* sp.) predominated in the community which was cultured at +25°C; *Bacillus simplex*, *Bacillus subtilis*, *Lysinibacillus sphaericus*, *Paracoccus* sp., *Rhodococcus* sp., and *Streptomyces* sp. were identified.

The cultured bacterial community isolated from the surface soil of Komsomolets Island of the Severnaya Zemlya archipelago at low temperatures was characterized by the predominance of bacteria of *Bacillus* sp. and *Microbacterium kitamiense* (in equal proportions); *Leucobacter aridicollis* and *Bacillus simplex* were identified as subdominant species. *Arthrobacter ginissoli* and *Arthrobacter* sp. were also determined in the culture. A community isolated from the same sample at a mesophilic temperature optimum (+25°C) was characterized by the predominance of bacteria of the species *Micrococcus* sp., *Brevibacterium sediminis*, *Arthrobacter gisengisoli*, and *Leucobacter aridicollis*; *Arthrobacter* sp., *Bacillus pumilus*, *Bacillus simplex*, *Bacillus* sp., *Microbacterium paraoxydans*, *Microbacterium* sp., *Pedobacter* sp., *Rhizobium* sp., *Shingobacterium* sp., and *Stenotrophomonas maltophilia* were isolated as minor components.

It is noteworthy that representatives of the genera *Arthrobacter*, *Dietzia*, *Geodermatophilus*, *Microbacterium*, *Pantoea*, *Planomicrobium*, *Shinella*, and *Stenotrophomonas* were identified only during low-temperature culturing, while they are also capable of growing at higher temperatures in axenic cultures.

Representatives of the genera from the studied samples were previously isolated from cryogenic and permafrost soils and rocks (Wagner et al., 2005; Bai et al., 2006; Bulat, 2006; Chattopadhyay, 2006; Zhang et al., 2007; Gilichinsky et al., 2008; Bull, 2011; Vishnivetskaya et al., 2011; Petrovskaya et al., 2012; Zhu et al., 2013; Suzina et al., 2015; Goordial et al., 2016; Singh et al., 2017; Efimenko et al., 2018; Sepehr et al., 2018; Humphrey et al., 2019). Moreover, the species *Arthrobacter oxydans*, *Bacillus pumilus*, *Dietzia cinnamea*, *Leucobacter aridicollis*, *Microbacterium barkeri*, *Planomicrobium glaciei*, and *Stenotrophomonas maltophilia*, identified in the microbial communities from the studied frozen soils, were previously isolated from the soils of the hot arid Sahara, Gibson, and Mojave deserts (Belov et al., 2018; Belov et al., 2019).

Mesophilic and neutrophilic physiological profiles of the studied communities with wide range of metabolic activity were previously determined in the bacterial communities isolated from the soils of the arid Sahara (Egypt) and Gibson (Australia) deserts (Belov

et al., 2018). We can therefore conclude that the tolerance of the metabolic state of bacterial cells (rather than the formation of specialized psychrophilic and thermophilic or acidophilic and alkalophilic subpopulations within the bacterial complex) is an adaptive strategy under the conditions of high stress load of various origins. The detection of a high proportion of thermotolerant strains in the sample collected in the Dry Valleys of Antarctica can be regarded as evidence of the preserving role of permafrost (Kryazhevskikh et al., 2012), and its capacity to preserve bacterial cells from past geological epochs.

The retention of metabolic activity of more than a quarter of the strains from all studied communities in the presence of 5% $Mg(ClO_4)_2$ is the most interesting result from the study of the resistance to various water-soluble salts. Antarctica and the microorganisms inhabiting various ecosystems of this continent are considered as the model objects in astrobiology studies (Gilichinsky et al., 2007), and the presence of oxidizing compounds in the regolith of various planets (in particular, the presence of perchlorates in the regolith of Mars), has been previously shown (Clark and Van Hart, 1981). The high resistance to in vitro presence of perchlorate can be interpreted as the possibility of reproduction of Earth-type bacteria in the regolith of Mars. However, due to the complex granulometric and mineralogical composition of the regolith, additional model experiments are required to clarify the effect of the presence of perchlorates in soils on bacterial communities.

On the one hand, the detection of antibiotic-resistant bacterial forms in the permafrost of polar ecosystems is additional evidence of the ecological role of antibiotics as signal metabolites of prokaryotes (Romero et al., 2011) and, on the other hand, it suggests that the permafrost bacterial communities are a reservoir of the determinants of antibiotic resistance. Therefore, these communities require further research: the study of the mechanisms of antibiotic resistance and the search for the producers of new antagonists (Andersson, 2003).

To summarize this research, the in vitro study of the bacterial communities from the cryo-arid soils of the Antarctic and Arctic revealed the polyextremotolerant features of the studied communities, indicating their potential ability to maintain metabolic activity in situ under aggressive environmental conditions. All studied communities were taxonomically diverse and dominated by representatives of *Actinobacteria* and *Firmicutes* phyla (representatives of the genera *Bacillus*, *Rhodococcus*, and *Arthrobacter*). The adaptive potential revealed in this study is quite interesting for the study of physiological mechanisms of anabiosis and involvement of these ecosystems in the global cycles of chemical elements in the biosphere, as well as for the development of new biotechnological processes.

LEGEND

A6/99 is a sample of permafrost sedimentary rock of the Antarctic desert.

Ho-3 is a sample of permafrost sediment rock, collected in the Antarctic Dry Valleys.

Sz is a sample of surface permafrost collected in the Komsomolets Island, Severnaya Zemlya archipelago.

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