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EXPERIMENTAL ARTICLES

The Prokaryotic Community of Subglacial Bottom Sediments of Antarctic Lake Untersee: Detection by Cultural and Direct Microscopic Techniques

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Abstract—The heterotrophic mesophilic microbial component was studied in microbial communities of the samples of frozen regolith collected from the glacier near Lake Untersee collected in 2011 during the joint Russian–American expedition to central Dronning Maud Land (Eastern Antarctica). Cultural techniques revealed high bacterial numbers in the samples. For enumeration of viable cells, the most probable numbers (MPN) method proved more efficient than plating on agar media. Fluorescent in situ hybridization with the relevant oligonucleotide probes revealed members of the groups *Eubacteria* (*Actinobacteria, Firmicutes*) and *Archaea*. The application of the methods of cell resuscitation, such as the use of diluted media and prevention of oxidative stress, did not result in a significant increase in the numbers of viable cells retrieved from subglacial sediment samples. Our previous investigations demonstrated the necessity for special procedures for efficient reactivation of the cells from microbial communities of replace with buried soil and permafrost samples collected in the Arctic zone. The differing responses to the special resuscitation procedures may reflect the differences in the physiological and morphological state of bacterial cells in microbial communities subject to continuous or periodic low temperatures and dehydration.

Keywords: subglacial sediments, Antarctics, prokaryotes, cell numbers, cell resuscitation, FISH
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Due to the potential threat of global warming, investigation of the microbial component of the perennially frozen ecosystems of the Arctic and Antarctic regions, as well as of the biogeochemical potential of these ecosystems, becomes a high priority [1–4]. Elucidation of the phylogenetic (taxonomic) structure of microbial communities is also required for determination of the temporal boundaries of microbial survival. Of particular interest are the ecosystems of Antarctica characterized by multiple temporary or permanent factors unfavorable for cell metabolism and reproduction [5]. After the existence of viable microorganisms in an Antarctic glacier was confirmed [6, 7], investigations with the use of cultural, biogeochemical, and molecular biological techniques were aimed at the study of microbial diversity in various Antarctic ecosystems. These included perennially and temporarily ice-covered lakes of the Antarctic oases Dry Valleys and Bunger Hills [8–10], as well as eutrophic and oligotrophic maritime lakes on the Byers Peninsula [11], the water column of Lake Vida [12], soils and rocks of the Dry Valleys [13–15], and sediments under the Kamb ice sheet [16].

However, the microbiological and biogeochemical processes occurring in some unique Antarctic ecosystems (e.g., the low-mineral oligotrophic Lake Untersee and associated continental glacier and subglacial sediments) remain poorly studied. During the course of the Joint US–Russian expedition in 2011 to Central Dronning Maud Land (Eastern Antarctica), water samples and bottom and subglacial sediments were collected from the lake. We have started a series of studies of the microbial component of these ecosystems using direct microscopic, cultural and molecular biological techniques.

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Table 1. Description of the samples collected from under the Lake Untersee glacier

No.	Sample description	Sampling site		
		coordinate position	altitude above sea level, m	depth, cm
1	Dry frozen ground from under the snow bed	71°19.382′ S 13°27.200′ E	643	0 (surface)
2	Moist semi-frozen ground, sand			30
3*	Frozen ground under the ice flow	71°19.368′ S 13°27.231′ E	613	0
4*	Frozen ground under the ice flow			0

* The sampling sites are located at a distance of 5 m from each other.

Our interest in the samples of subglacial ground was mainly due to the lack of information about the efficiency of survival of microorganisms in these environments. The surface systems, left behind by a retreating glacier, are characterized by short-term activity/rest cycles of microbial cells in the course of the freezing–thawing cycles. Survival of microorganisms (or at least of the vast majority of them) after long-term cryoconservation in perennially frozen ground is due to their anabiotic state. Although detection of psychrophilic and psychrotoperant bacteria is a logical step in microbiological studies of Antarctic ecosystems, the mesophilic component of microbial communities, which could form before freezing (or before the glacier was formed) and survive cryoconservation as dormant cells, was of particular interest. The numbers of viable cells is used as a criterion of survival efficiency; however, standard media and cultivation conditions are often insufficient for complete enumeration of viable cells. The authors have successfully reactivated the resting forms obtained from laboratory cultures of heterotrophic bacteria and permafrost samples with the use of different media and enumeration techniques. Hence, this group of microorganisms was of considerable interest due to their survival strategy implemented in Antarctic subglacial ground and was the most convenient model for testing the methods for complete enumeration of viable cells.

The goal of this work was to evaluate the numbers of viable heterotrophic mesophilic bacteria inhabiting the perennially or temporarily frozen ground under the tongue of the glacier flowing to Lake Untersee using various methods of cultivation and direct microscopy, as well as by means of FISH with fluorescently labeled oligonucleotide probes targeting various groups of prokaryotes.

MATERIALS AND METHODS

The subjects of study were 4 ground samples collected in the course of the 2011 expedition to the area under the right flank of the glacier flowing into Lake Untersee (see description in Table 1) The samples were collected into screw-capped sterile plastic 50-mL tubes, hermetically sealed, and stored at 0°C in the dark until the end of the expedition; after being delivered to the laboratory, the samples were stored at 4°C in the dark.

Preparation of the samples. Weighed portions of ground were resuspended in sterile saline (1 : 10, wt/vol). Large mineral particles were then precipitated from the supernatant, and serial dilutions were plated onto agarized or into liquid media.

To obtain enrichment cultures, samples (1 g) were placed in 250-mL flasks with 50 mL of liquid medium and incubated with agitation (180 rpm) at 28°C (optimal for mesophilic microorganisms, the main object of our search) for 5 days.

Obtaining of microbial fractions from the samples. To determine the total numbers of potentially viable cells ("live", according to the Live/Dead test), the microbial fractions obtained from the samples (1 g) resuspended in 10 mL of saline were used. Large particles were precipitated by centrifugation (225 *g*, 2 min); the supernatant was recentrifuged (4000 *g*, 2 min); and the sediment was resuspended in the physiological saline (1 mL).

For enumeration of metabolically active cells by FISH, the microbial fractions obtained from the samples (5 g) resuspended in 25 mL of distilled water were used. The suspensions were sonicated (22 kHz, 0.44 A, 2 min); large particles were precipitated by centrifugation (330 *g,* 10 min); the supernatant was recentrifuged (7400 *g*, 5 min); and the sediment was resuspended in 0.5 mL of PBS buffer (pH 7.0).

Enumeration of the cultivable cells of aerobic heterotrophic mesophilic bacteria. In our study, we used the following media: (1) standard liquid Luria–Bertani (LB) medium; (2) tenfold diluted liquid LB medium; (3) solid LB medium (1.5% agar); (4) semisolid LB medium (0.2% agar); (5) soil extract (SE); (6) Lochhead soil agar and soil extract. Soil extract was obtained by resuspending air-dry alluvial soil (100 g) from the Oka River (Pushchino, Russia) in 150 mL of water with subsequent agitation of the obtained suspension at 180 rpm for 60 min. Then, soil particles were removed by centrifugation (7400 *g*, 5 min). Lochhead soil agar contained the following: SE, 1 L; K_2HPO_4, 0.2 g; and agar, 1.5% (wt/vol).

Table 2. Cell numbers of heterotrophic mesophilic bacteria (CFU/g, LB agar) and the total number of potentially viable cells (Live/Dead test) in the samples of subglacial ground

Sample	Number of cultivable cells, CFU/g	Total number of "live" cells in the microbial fractions, cells/g
1	$(6.5 \pm 0.7) \times 10^5$	$(1.3 \pm 0.4) \times 10^8$
2*	$(1.0 \pm 0.2) \times 10^4$–$(1.7 \pm 0.2) \times 10^5$	$(4.6 \pm 0.7) \times 10^8$
3	$(1.1 \pm 0.2) \times 10^5$	$(2.1 \pm 0.5) \times 10^7$
4	$(1.2 \pm 0.3) \times 10^5$	$(3.3 \pm 0.7) \times 10^7$

* For this sample, the numbers of CFU from different experiments varied more extensively than for other samples, which is probably due to its heterogeneity.

The colony-forming capacity of the cells (CFU/g) was determined by plating the 10^n-fold diluted material from enrichment cultures or suspensions (100 μL) on Petri dishes with agarized media, with subsequent incubation at 28°C for 2–4 weeks.

The number of viable cells capable of growth in liquid media was estimated by MPN test using sterile 48-well Corning plates (100 μL of suspension + 900 μL of liquid LB medium or semisolid LB agar). The plates were incubated under static conditions at 28°C for 4–30 days. The titer of viable cells (MPN/g) was calculated using the standard tables. Tenfold dilutions of the suspensions were used as inocula for both solid and liquid media.

The total counts of cells in microbial fractions were determined by direct count under an Axioplan fluorescence microscope (Carl Zeiss) in at least 15 microscope fields. The suspensions were stained (at 37°C for 10 min in the dark) with the Live/Dead Baclight kit® L-13152 (Molecular Probes) for the differentiation of "live" and "dead" cells.

FISH enumeration of metabolically active prokaryotic cells. Microbial fractions obtained from the ground samples and suspensions of enrichment cultures (after 5-day growth in the liquid LB medium) were concentrated 100-fold, fixed with formaldehyde, and treated by the method described by Amann et al. [17, 18] with modifications [19]. For hybridization, a set of Cy3-labeled rRNA-targeted oligonucleotide probes (Syntol, Russia) were used for detection of the representatives of the phylogenetic groups of prokaryotes, including *Bacteria* (EUB338 and EUB338-I), *Firmicutes* (LGC354A), *Actinobacteria* (HGC69a), and *Archaea* (ARCH915) [17]. After hybridization, the preparations were additionally stained with aqueous solutions of acridine orange and examined under an Axioskop 2 PLUS fluorescence microscope (Carl Zeiss, Germany) using Filterset 15 (λ = 546 nm) to reveal Cy3-labeled oligonucleotide probes and Filterset 09 (λ = 450–490 nm) to reveal cells stained with acridine orange. The numbers of the targeted groups of microorganisms in the samples were determined by counting the number of probe-hybridized cells from one well in 50 microscope fields with subsequent recalculation for 1 g of the sample.

Microscopic examinations of enrichment cultures or colonies grown on solid media were carried out under a phase-constrast Zetapan microscope (Reichert, Austria).

The data presented are the mean values with calculated standard deviations. Cells were enumerated in triplicate in the course of two independent series of experiments. The cells that responded positively to the probes were enumerated in four replicates for each sample of subglacial ground or for the enrichment culture obtained from it.

RESULTS AND DISCUSSION

In our study, samples of subglacial ground, perennially or temporarily frozen, and collected near the Antarctic Lake Untersee (Table 1) were the objects of search for viable microbial cells subjected to cryoconservation or freezing–thawing cycles. These components of the cryosphere have not been previously studied in detail. On the one hand, it was considered essential to study the population density of prokaryotes (in this work), heterotrophic aerobic mesophilic microorganisms; on the other hand, the cell numbers in the samples of subglacial ground subjected to different factors of natural cryoconservation were also of particular interest.

Using the standard methods of sample preparation and plating on LB agar, comparatively high numbers of colony-forming cells of aerobic heterotrophic mesophilic bacteria (10^4 to 6.5×10^5 CFU/g) were detected in all the four ground samples (Table 2). The bacterial population density in the samples of subglacial ground was comparable to those reported for some Antarctic ecosystems; namely, soils of the Schirmacher Oasis (10^3 to 10^6 CFU/g) [20]; algal mats of the Dry Valleys (10^4 to 10^7 CFU/g) [21], sediments and soils near the Maitri station (number of anaerobic microorganisms 10^2 to 3×10^5 CFU/g) [22]. Some perennially frozen ecosystems of the Antarctic cryosphere are characterized by low numbers of cultivable prokaryotic cells: 10–10^4 CFU/g [1] in the deep horizons of Antarctic permafrost and several CFU per 1 L

of melted glacier waters [6, 7] or water from the accretion ice of Lake Vostok [23].

In the microbial fractions obtained from the studied samples of subglacial ground, the total numbers of "live" cells determined by the Live/Dead test varied within the range of 10^7–5×10^8 cells/g (Table 2). These fractions consisted of nondividing cocci and straight or curved rods, single or arranged in clusters. Some cells in the microbial fractions were associated with small mineral particles and could be detected only by fluorescence microscopy.

Importantly, the total numbers of "live" cells was several orders of magnitude higher than the number of cells producing colonies on LB agar. The greatest discrepancy (3–4 orders of magnitude) between these values was demonstrated for sample 2, whereas, in samples 1, 3, and 4, it varied within a range of two orders of magnitude (Table 2). The absolute values of the total number of cells in sample 2 were higher that those in the samples of frozen ground (Table 2), which was probably due to the natural reactivation of the cells and their subsequent growth, as the sample was collected during ground thawing.

The low ratio between CFU titers and the total number of cells determined by direct microscopic count is typical not only of the subglacial ground samples (Table 2), but also of other components of the Antarctic cryosphere [1, 6, 7, 16]. The discrepancy between the numbers of colony-forming cells and the total number of microorganisms may be the result of several factors. First, the majority of cells might be dormant or nonculturable, as was previously revealed by direct microscopy of frozen sediments [24, 25]. Hence, special activation procedures are required for the germination of dormant cells [26]. Second, the standard media and cultivation conditions may be insufficient for development of the major part of the microbial community. Third, some visually intact cells might be incapable of growth. To gain more detailed insight into the numbers of viable bacteria cells in the samples of subglacial ground, other media and counting techniques were tested [26, 27].

The combined method for retrieval of bacterial cells from the samples involved the use of (1) tenfold diluted LB broth in order to prevent "substrate-accelerated death"; (2) semisolid agarized LB medium in order to limit oxygen access and to prevent oxidative stress; (3) soil extract and agar with organic compounds of soils. The number of cultivable cells was determined repeatedly at different periods of incubation for the number of colony-forming units (CFU/g) on solid nutrient media and by the method of limiting dilutions in liquid media (MPN titer calculation, MPN/g).

In the studied samples of subglacial ground, the number of colony-forming units on different solid media varied from 3×10^3 to 6.5×10^5 CFU/g (Table 3). About 13–30% of all colonies emerged after 3-day cultivation; other colonies were detected after prolonged incubation of inoculated samples for 14 days (prolongation of incubation up to 1 month did not result in an increase in the number of CFU). As noted above, sample 2 showed variations in the CFU number when inoculated on different media. However, no significant differences were detected in the numbers of CFU depending on the compositions of solid media.

The number of viable cells determined by the MPN method as cell growth in liquid or semisolid media after 1-month incubation at 28°C varied within a range of 10^6–10^8 MPN/g (Table 2), which was 1–3 orders of magnitude higher than the number of CFU in the same samples. For some samples of subglacial ground (3 and 4), inoculation of tenfold diluted liquid LB media, soil extract, or semisolid agarized media was a more efficient way of assessment of the numbers of viable cells than plating (Fig. 1).

Our data correspond well with the results obtained by other authors. For instance, enumeration of anaerobic and spore-forming bacteria in the perennially frozen soils of Spitsbergen was more efficient with the use of MPN than by CFU enumeration [28]. Optimization of the medium composition and the incubation temperature made it possible to detect viable microorganisms in marine sediments of the Antarctic continental shelf (10^6–10^7 MPN/g) [29], as well as to detect 20 to 40 times more viable (colony-forming) cells in the accretion ice of Lake Vostok [21]. An increase in the numbers of detected viable cells (by several orders of magnitude) in the samples of perennially frozen subglacial sediments collected in the Kolyma Lowland was due to the use of diluted media in MPN tests, as well as to the application of special methods for sample preparation [25].

The efficiency of cell enumeration by cultural methods was determined as the ratio between the numbers of cultivable cells (the numbers of CFU or MPN per 1 g of sample) and the total number of "live" cells (Live/Dead test) in microbial fractions (Figs. 1a–1d).

When comparing the efficiency of enumeration of viable cells by cultural methods (plating or MPN test, with different media and incubation times), the following circumstances should be considered:

(1) We should a priori assume that we cannot detect all potentially "live" cells, including the dormant forms, by direct counting, since some cells are adhered to soil particles or arranged in biofilm structures which cannot be stained with the Live/Dead dye.

(2) Only microorganisms growing on a specific selective medium may be detected (in this study, aerobic heterotrophic mesophilic prokaryotes).

(3) For the activation of the dormant cells that are difficult to germinate, a combination of resuscitation methods should be used.

(4) The numbers of viable cells determined by MPN vary within a broad range ($0.1 \times N$ to $10 \times N$,

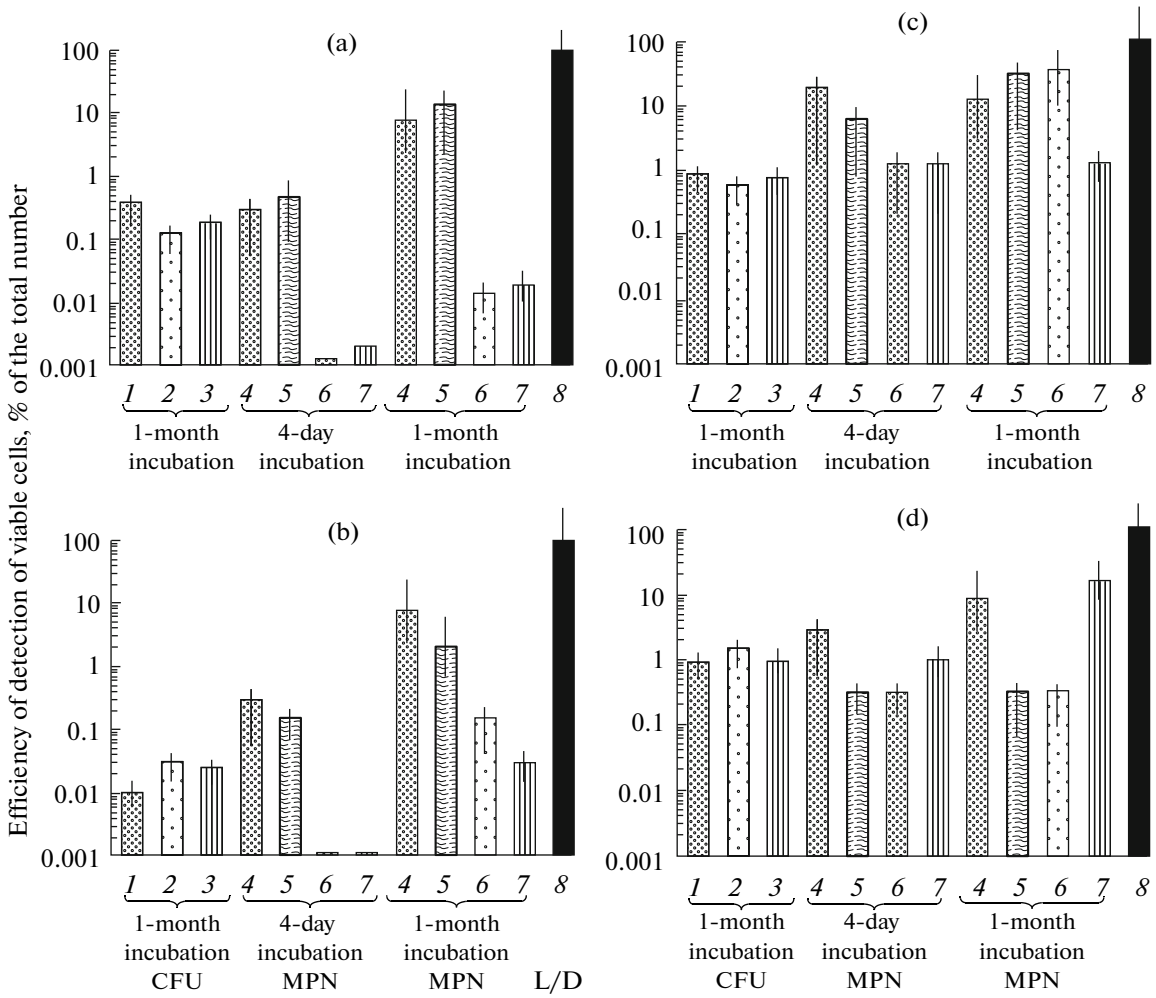

Fig. 1. Share of cultivable cells (CFU/g or MPN/g) in the total number of "live" cells (Live/Dead test), %; (a–d) are samples 1–4 of Antarctic subglacial ground, respectively. Growth media types: LB agar (*1*); tenfold diluted LB agar (*2*); soil agar (*3*); LB broth (*4*); semisolid agarized LB medium (*5*); tenfold diluted LB agar (*6*); and soil extract (*7*).

where N is the MPN titer per 1 g of sample). Therefore, on the plots (Fig. 1) depicting the efficiency of different methods for cell enumeration, we used the MPN values according to the bottom boundary line.

On nutrient-rich and diluted agarized LB media, the number of colony-forming cells in the samples of subglacial ground reached 0.01–0.1% of the total cell number (Figs. 1a–1d, columns 1–3). The effectiveness of MPN test with short-term incubation (4 days) varied from 0.1% for samples 1 and 2 (Figs. 1a, 1b columns 4–7), to several percents for samples 3 and 4 (Figs. 1c, 1d). The application of MPN test with prolonged incubation (1 month) of inoculated liquid media was found to be most efficient for enumeration of cultivable heterotrophic mesophilic bacteria (up to 10%) (Fig. 1).

It should be noted that enumeration of cultivable bacteria in sample 2 had relatively low efficiency, although the absolute values (MPN titers) were higher than in other samples (Fig. 1). It may be suggested that, in the course of freezing–thawing cycles, many cells had insufficient time to activate the mechanisms protecting them against stress factors (environmental changes) and become "uncultured". However, the use of diluted media and reduction of the damaging effect of oxygen did not, on the whole, result in a sharp increase in the efficiency of detection of viable cells in the samples of Antarctic subglacial ground. Pretreatment of suspensions with low-molecular-weight regulators functioning as messengers for intercellular communication may prove successful for the activation of microbial cells from perennially frozen ecosystems of the cryosphere. This is evidenced by the results of our earlier investigations into the conditions of efficient resuscitation of the bacterial communities of Arctic fossil soils and permafrost [27].

Hence, despite high numbers of cultivable cells permanently or temporarily exposed to low temperatures or dehydratation, various special procedures of sample preparation and cell activation are required for

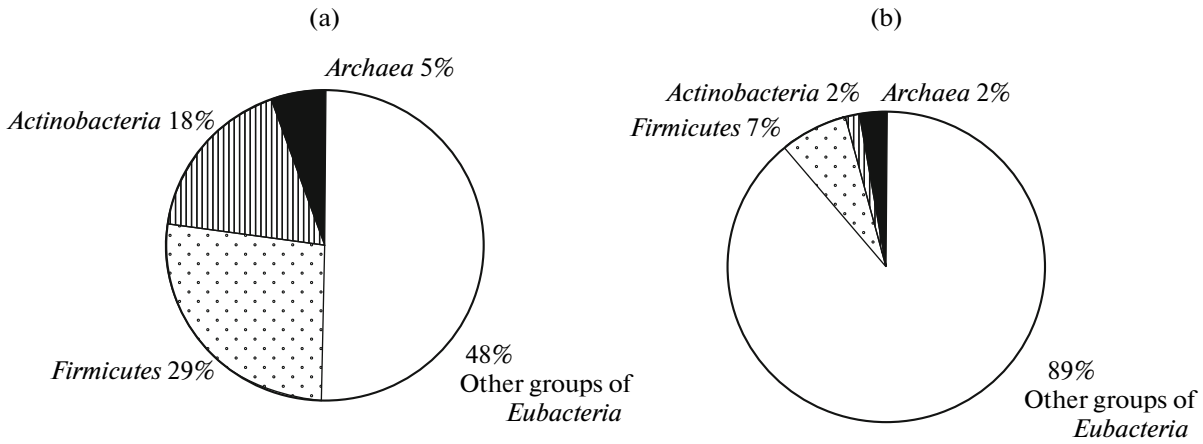

Fig. 2. The ratio between representatives of different groups of prokaryotes detected by FISH in the microbial fraction of sample 1 (a) and in the obtained enrichment culture (LB broth) (b).

more efficient monitoring of the microbial composition of these ecosystems. The practicality of these procedures is based on the physiological and morphotypic properties of the microbial communities inhabiting perennially frozen ecosystems. For perennially frozen subsoil deposits (and other such objects of the cryosphere), the predominance of cystlike dormant cells was demonstrated by direct microscopy (electron microscopy of ultrathin sections) [24, 25]. Moreover, in the microbial fractions of these objects, as well as of buried paleosols, isolated after thawing and humidification, the proportion of metabolically active cells detected by means of FISH was 5%; after resuscitation, it reached 75% [27].

The numbers of cells revealed in this study in the microbial fractions of sample 1 by means of FISH were 5.9×10^7 cells/g (*Eubacteria*), 10^7 cells/g (*Actinobacteria*), 1.7×10^7 cells/g (*Firmicutes*), and 3.2×10^6 cells/g (*Archaea*) (Table 4). The total number of metabolically active prokaryotic cells was 6.2×10^7 cells/g, which corresponded to 47% of the total number of "live" cells and which was 2 orders of magnitude higher than the number of CFU/g (Table 2). It seems likely that, in the course of thawing of subglacial ground, the majority of the cells regained their metabolic activity; however, special conditions were required to induce their growth. The evidence considered above demonstrates that prokaryotic cells from the microbial communities of subglacial ground and permafrost deposits responded differently to the onset of favorable conditions.

Despite the fact that application of different solid media did not improve the detection of colony-forming cells, it revealed higher diversity of the types of colony morphology in the samples of Antarctic subglacial ground. For instance, the microscopic examinations of specimens from colonies on soil agar inoculated with sample 1 revealed various morphological types of non-spore-forming bacteria: cocci of different sizes, curved rods, as well as club-shaped and branching cells. At the same time the diversity of morphotypes obtained on LB agar inoculated with the same sample was less pronounced: they were represented mainly by spore-forming cells and motile non-spore-forming rods. It is notable that some isolates obtained on diluted agarized LB media or soil agar (samples 3 and 4) did not survive transfer to nutrient-rich media: for their maintenance, diluted media were required.

In the enrichment cultures isolated from the samples incubated in LB broth (28°C, 4 days), the total number of cultivable cells varied from 1.3×10^7 to 5.2×10^8 CFU/mL (or from 7×10^8–3×10^{10} CFU/g, when calculated per 1 g of the sample). The enrichment cultures obtained in LB broth were characterized by the predominance of spore-forming rods and motile cells. The use of liquid LB medium was found to be nonoptimal for the development of actinobacteria, which was confirmed by the results of FISH (Table 4). For instance, in the enrichment cultures obtained from sample 1 incubated in LB broth, the percentage of *Actinobacteria* cells hybridized with the relevant probe was 2% of the total number of eubacterial cells (Fig. 2). This was an order of magnitude lower than the percentage of actinobacteria (18%) in the microbial fraction of sample 1 (Fig. 2), from which the enrichment culture was obtained. The occurrence frequency of the *Firmicutes* in this culture was also lower (7%) than the numbers of these bacteria in the microbial fraction of the sample (29%) (Table 4). Apparently, representatives of other phyla of the domain *Bacteria* had an advantage in growth and development in the liquid LB medium.

Despite the fact that investigation of the diversity of microorganisms in the samples of subglacial ground was beyond the scope of this research, the results obtained may be useful for a more detailed study of the diversity of *Actinobacteria*, which is of particular interest to the scientists studying Antarctic ecosystems.

Table 3. Numbers of cultivable (at 28°C) aerobic heterotrophic mesophilic bacteria in the samples of subglacial ground determined by plating and MPN tests after plating

No.	Brief description	Numbers of colony-forming cells (CFU/g) on different media (1-month incubation)			Numbers of viable cells* (MPN/g) estimated by growth in liquid media for 4 days (top) and 1 month (bottom)			
		LB agar	10-fold diluted LB agar	soil agar	LB broth	semisolid LB medium	10-fold diluted LB broth	soil extract
1	Frozen ground	$(6.5 \pm 0.7) \times 10^5$	$(2.1 \pm 0.8) \times 10^5$	$(2.9 \pm 0.7) \times 10^5$	7.5×10^5 1.2×10^7	2.1×10^4 2.3×10^7	2.3×10^3 2.3×10^4	4.6×10^3 2.9×10^5
2**	Semi-frozen ground	$(1.0 \pm 0.2) \times 10^4$– $(1.7 \pm 0.2) \times 10^5$	$(3.0 \pm 0.1) \times 10^3$– $(5.6 \pm 0.8) \times 10^5$	$(1.0 \pm 0.3) \times 10^4$– $(4.0 \pm 0.8) \times 10^5$	1.5×10^6 1.2×10^8	7.5×10^5 2.8×10^7	4.3×10^3 7.5×10^5	1.5×10^4 1.5×10^5
3	Frozen ground	$(1.1 \pm 0.2) \times 10^5$	$(7 \pm 1.0) \times 10^4$	$(8.0 \pm 1.5) \times 10^4$	2.3×10^6 2.3×10^6	7.3×10^5 3.6×10^6	1.5×10^5 2.1×10^7	1.5×10^5 1.5×10^5
4	Frozen ground	$(1.2 \pm 0.2) \times 10^5$	$(2.0 \pm 0.4) \times 10^5$	$(1.2 \pm 0.2) \times 10^5$	3.6×10^5 1.1×10^6	3.6×10^4 3.6×10^4	3.0×10^4 3.0×10^4	1.2×10^5 2.3×10^6

* The same tenfold dilutions of the suspensions were used to inoculate both solid and liquid media.

** For this sample, the numbers of CFU from different experiments varied more extensively than for other samples, which is probably due to its heterogeneity.

Table 4. Numbers of metabolically active cells (fluorescent in situ hybridization, FISH) in the microbial fractions of sample 1 of subglacial ground and in the obtained enrichment culture (LB broth, 5 days)

Target group	Cell numbers*	
	microbial fraction, cells/g	enrichment culture, cells/g of the sample
Eubacteria	$(5.9 \pm 1.23) \times 10^7$	$(1.9 \pm 0.2) \times 10^{10}$
Archaea	$(3.2 \pm 0.5) \times 10^6$	$(4.2 \pm 0.3) \times 10^8$
Firmicutes	$(1.73 \pm 0.34) \times 10^7$	$(1.3 \pm 0.4) \times 10^8$
Actinobacteria	$(1.05 \pm 0.21) \times 10^7$	$(3.0 \pm 0.6) \times 10^8$

* Cell numbers were determined in tenfold concentrated suspensions.

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REFERENCES

1. Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E., and Vishnivetskaya, T., The deep cold biosphere: facts and hypothesis, *FEMS Microbiol. Rev.*, 1997, vol. 20, pp. 277–290.
2. Cowan, D.A. and Tow, L.A., Endangered Antarctic environments, *Annu. Rev. Microbiol.*, 2004, vol. 58, pp. 649–690.
3. Yergeau, E., Bokhorst, S., Kang, S., Zhou, J., Greer, C.W., Aerts, R., and Kowalchuk, G.A., Shifts in soil microorganisms in response to warming are consistent across a range of Antarctic environments, *ISME J.*, 2012, vol. 6, no. 3, pp. 692–702.
4. Wadham, J.L., Arndt, S., Tulaczyk, S., Stibal, M., Tranter, M., Telling, J., Lis, G.P., Lawson, E., Ridgwell, A., Dubnick, A., Sharp, M.J., Anesio, A.M., and Butler, C.E., Potential methane reservoirs beneath Antarctica, *Nature*, 2012, vol. 488, no. 7413, pp. 633–637.

5. Tindall, B.J., Prokaryotic diversity in the Antarctic: the tip of the iceberg, *Microb. Ecol.*, 2004, vol. 47, no. 3, pp. 271–283.
6. Abyzov, S.S., Bobin, N.E., and Kudriashov, B.B., Microbiological analysis of glacial series of Central Antarctica, *Biol. Bull. Acad. Sci. USSR*, 1979, vol. 6, pp. 828–836.
7. Abyzov, S.S., Duxbury, N.S., Bobin, N.E., Fukuchi, M., Hoover, R.B., Kanda, H., Mitskevich, I.N., Mulyukin, A.L., Naganuma, T., Poglazova, M.N., and Ivanov, M.V., Super-long anabiosis of ancient microorganisms in ice and terrestrial models for development of methods to search for life on Mars, Europa and other planetary bodies, *Adv. Space Res.*, 2006, vol. 38, pp. 1191–1197.
8. Wharton, R., Mckay, C., Clow, G., and Andersen, D., Perennial ice covers and their influence on Antarctic lake ecosystems, *Antarctic Res. Ser. ANTSA*, 1993, vol. 459, pp. 53–70.
9. Galchenko, V.F., Sulphate reduction, methane generation and oxidation in different Bunger Hills reservoirs (Antarctica), *Microbiology* (Moscow), 1994, vol. 63, no. 4, pp. 388–396.
10. Belyaev, A.S., Chernykh, N.A., Gal'chenko, V.F., and Ivanov, M.V., Detection of methylotrophs in natural samples by amplification of a fragment of the *moxF* gene, *Microbiology* (Moscow), 1995, vol. 64, no. 6, pp. 667–669.
11. Villaescusa, J.A., Casamayor, E.O., Rochera, C., Velázquez, D., Chicote, A., Quesada, A., and Camacho, A., A close link between bacterial community composition and environmental heterogeneity in maritime Antarctic lakes, *Int. Microbiol.*, 2010, vol. 13, no. 2, pp. 67–77.
12. Mosier, A.C., Murray, A.E., and Fritsen, C.H., Microbiota within the perennial ice cover of Lake Vida, Antarctica, *FEMS Microbiol. Ecol.*, 2007, vol. 59, no. 2, pp. 274–288.
13. Pointing, S.B., Chan, Y., Lacap, D.C., Lau, M.C., Jurgens, J.A., and Farrell, R.L., Highly specialized microbial diversity in hyper-arid polar desert, *Proc. Natl. Acad. Sci. USA*, 2009, vol. 106, no. 47, pp. 19964–19969.
14. Babalola, O.O., Kirby, B.M., Le Roes-Hill, M., Cook, A.E., Cary, S.C., Burton, S.G., and Cowan, D.A., Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils, *Environ. Microbiol.*, 2009, vol. 11, no. 3, pp. 566–576.
15. Cary, S.C., McDonald, I.R., Barrett, J.E., and Cowan, D.A., On the rocks: the microbiology of Antarctic Dry Valley soils, *Nat. Rev. Microbiol.*, 2010, vol. 8, no. 2, pp. 129–138.
16. Lanoil, B., Skidmore, M., Priscu, J.C., Han, S., Foo, W., Vogel, S.W., Tulaczyk, S., and Engelhardt, H., Bacteria beneath the West Antarctic ice sheet, *Environ. Microbiol.*, 2009, vol. 11, no. 3, pp. 609–615.
17. Amann, R.I., Krumholz, L., and Stahl, D.A., Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology, *J. Bacteriol.*, 1990, vol. 172, pp. 762–770.
18. Amann, R.I., Ludwig, W., and Schleifer, K.-H., Phylogenetic identification and in situ detection of individual microbial cells without cultivation, *Microbiol. Rev.*, 1995, vol. 59, pp. 143–169.
19. Manucharova, N.A., *Identifikatsiya metabolicheski aktivnykh kletok prokariot v pochvakh s primeneniem molekulyarno-biologicheskogo flyuorestsentnomikroskopicheskogo metoda analiza (FISH)* (Identification of Metabolically Active Prokaryotic Cells in Soils by Molecular Biological Fluorescence Microscopic Technique Fluorescence in situ Hybridization (FISH)), Moscow: Universitet i shkola, 2008.
20. Shivaji, S., Chattopadhyay, M.K., and Ray, M.K., Bacteria and yeasts of Schirmacher Oasis, Antarctica: taxonomy, biochemistry and molecular biology, *Proc. NIPR Symp. Polar Biol.,* 1994, vol. 7, pp. 173–184.
21. Antibus, D.E., Leff, L.G., Hall, B.L., Baeseman, J.L., and Blackwood, C.B., Cultivable bacteria from ancient algal mats from the McMurdo Dry Valleys, Antarctica, *Extremophiles*, 2012, vol. 16, no. 1, pp. 105–114.
22. Ramteke, P.W., Diversity of anaerobes around Indian Antarctic station "Maitri", *Sci. Rep. 18th Indian Expedition to Antarctica. Dept. Ocean Dev. Techn. Publ.*, 2002, no. 16, pp. 293–298.
23. Christner, B.C., Mosley-Thompson, E., Thompson, L.G., and Reeve, J.N., Isolation of bacteria and 16S rDNAs from Lake Vostok accretion ice, *Environ. Microbiol.*, 2001, vol. 3, pp. 570–577.
24. Dmitriev, V.V., Suzina, N.E., Rusakova, T.G., Gilichinskii, D.A., and Duda, V.I., Ultrastructural characteristics of natural forms of microorganisms isolated from permafrost grounds of Eastern Siberia by the method of low-temperature fractionation, *Doklady Biol. Sci.,* 2001, vol. 378, no. 6, pp. 304–306.
25. Soina, V.S., Mulyukin, A.L., Demkina, E.V., Vorobyova, E.A., and El-Registan, G.I., The structure of resting microbial populations in soil and subsoil permafrost, *Astrobiology*, 2004, vol. 4, no. 3, pp. 348–358.
26. Mulyukin, A.L., Demkina, E.V., Kriazhevskikh, N.A., Suzina, N.E., Vorob'eva, L.I., Duda, V.I., Galchenko, V.F., and El-Registan, G.I., Dormant forms of *Micrococcus luteus* and *Arthrobacter globiformis*, non platable on standard media, *Microbiology* (Moscow), 2009, vol. 78, no. 4, pp. 407–418.
27. Kriazhevskikh, N.A., Demkina, E.V., Manucharova, N.A., Soina, V.A., Galchenko, V.F., and El-Registan, G.I., Reactivation of dormant and nonculturable bacterial forms from paleosoils and subsoil permafrost, *Microbiology* (Moscow), 2012, vol. 81, no. 4, pp. 435–445.
28. Hansen, A.A., Herbert, R.A., Mikkelsen, K., Jensen, L.L., Kristoffersen, T., Tiedje, J.M., Lomstein, B.A., and Finster, K.W., Viability, diversity and composition of the bacterial community in a high Arctic permafrost soil from Spitsbergen, Northern Norway, *Environ. Microbiol.*, 2007, vol. 9, no. 11, pp. 2870–2884.
29. Bowman, J.P., McCammon, S.A., Gibson, J.A.E., Robertson, L., and Nichols, P.D., Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments, *Appl. Environ. Microbiol.*, 2003, vol. 69, no. 5, pp. 2448–2462.

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