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EXPERIMENTAL ARTICLES

Survival of Micromycetes and Actinobacteria under Conditions of Long-Term Natural Cryopreservation

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Abstract—Almost all of the investigated samples of the Arctic and Antarctic permafrost sediments of different genesis with ages from 5–10 thousand to 2–3 million years were found to contain viable micromycete and bacterial cells. The maximum amounts of viable cells of fungi (up to 10^4 CFU/g air-dried sample) and bacteria (up to 10^7–10^9 CFU/g air-dried sample) were present in fine peaty sediment samples taken from different depths. The identified micromycetes belonged to more than 20 genera of the divisions *Basidiomycota*, *Ascomycota*, and *Zygomycota*, and some represented mitosporic fungi. Thawing the samples at 35 and 52°C allowed the number of detected fungal genera to be increased by more than 30%. Aerobic heterotrophic prokaryotes were dominated by coryneform, nocardioform, and spore-forming microorganisms of the order *Actinomycetales*. Analysis of the isolated fungi and actinomycetes showed that most of them originated from the microbial communities of ancient terrestrial biocenoses.

Key words: permafrost, mycelial fungi, actinobacteria, identification.

It is presently accepted that microorganisms can retain their viability under temperatures below zero for a long period of time [1–3]. The microbiological and molecular biological analyses of permafrost sediments showed that they contain viable cells of many phyletic lines, such as actinobacteria, proteobacteria, and monosporous bacteria [1, 2, 4]. The aerobic prokaryotes detected in permafrost sediments by culture methods are dominated by non-spore-forming bacteria of the order *Actinomycetales* [4]. Data on the abundance and taxonomic composition of viable eukaryotic microorganisms, including micromycetes, in permafrost are scarce [5–7]. It is believed that eukaryotes, particularly fungi, remain viable in permafrost sediments over a period of 10–40 thousand years, although prokaryotes can remain viable for longer periods of time [1]. However, some researchers have also revealed viable eukaryotes (algae and yeasts) in the early Pleistocene permafrost [5, 6].

The preservation of microorganisms in nature is limited by a number of detrimental factors, including the natural degradation of biomacromolecules, the absence of cell reproduction, the effect of radiation, unfavorable temperature conditions, etc. [3]. Lowered ambient temperatures slow down biological processes in cells (the effect is greater the lower the temperature); this substantially lengthens the time period during which cells remain viable. The lethal damage inflicted on cells by supercooling or freezing is associated with the degradation of cellular structures and biomolecules and is caused by the harmful effects of ice crystals formed outside and inside cells, cell dehydration, the shifts of pH and electrolyte concentrations from physiological values, and so on [8, 9]. In nature, these effects are mitigated, to a certain degree, by the presence of natural cryoprotectants (sugars, proteins, and others [8–10]), which are synthesized autogenously or by other members of the microconsortia. An unfrozen film of water on the surface of frozen organomineralic sediment particles with adsorbed microbial cells also contributes to their cryoprotection [11].

As laboratory practices show, a sufficient number of cells die during the thawing of cryopreserved samples because of the cell damage caused by the recrystalliza-

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Table 1. The number of viable bacteria and mycelial fungi in permafrost sediments (in CFU/g air-dried sample)

Sample	Hole/year	Region	Sediment	Depth, m	Age, 10^3 years	Bacteria resuscitated on CBA (1 : 10)	Fungi resuscitated			
							on Cz at 4°C	on Cz at 25°C	on MA at 4°C	on MA at 25°C
Kolyma Lowland										
26	6/91	Chukoch'ya river mouth	Sandy loam with sulfides, Marine sediments	4.80	5–10	$(1.3–2.6) \times 10^5$	$\mathbf{(0.1–1.6) \times 10^2}$	0.0	$(0–0.4) \times 10^2$	$\mathbf{(0.4–0.6) \times 10^2}$
27	6/91			12.30	5–10	$(0.8–1.2) \times 10^4$	0.0	$\mathbf{(0.4–2.8) \times 10^2}$	$\mathbf{(0.5–2.4) \times 10^2}$	$(0–6.7) \times 10^2$
9	4/91		Muddy loam	14.70	100	ND	$\mathbf{(0.9–1.6) \times 10^2}$	$(0–3.4) \times 10^2$	$\mathbf{(0.4–3.1) \times 10^2}$	$\mathbf{(0.6–2.8) \times 10^2}$
7	4/91			16.80	100	$(1.2–2.0) \times 10^4$	0.0	$\mathbf{(1.0–5.0) \times 10^2}$	$\mathbf{(1.7–8.6) \times 10^2}$	$\mathbf{(1.3–6.6) \times 10^2}$
11	5/94	Chukoch'ya river mid-stream	Light loam	12.60–12.80	200–600	ND	$(0–0.8) \times 10^2$	$(0–2.6) \times 10^2$	$\mathbf{(0.8–3.9) \times 10^2}$	$\mathbf{(1.7–1.9) \times 10^2}$
35	2/94		Muddy, sandy loam	11.0–11.5	1800–3000	$(0.2–3.5) \times 10^6$	$(0–1.0) \times 10^2$	$\mathbf{(0.2–1.1) \times 10^2}$	0.0	$(0–1.1) \times 10^2$
37	2/94		Peaty sand	27.50–27.60	1800–3000	ND	$\mathbf{(1.1–1.8) \times 10^2}$	$\mathbf{(0.7–2.2) \times 10^2}$	$\mathbf{(0.4–4.3) \times 10^2}$	$(0–1.4) \times 10^2$
38	2/94		Peaty, sandy loam	45.30–45.80	1800–3000	$(1.7–2.8) \times 10^9$	$\mathbf{(1.3–2.7) \times 10^4}$	$\mathbf{(1.2–2.9) \times 10^4}$	$\mathbf{(2.6–5.9) \times 10^4}$	$\mathbf{(1.2–3.1) \times 10^4}$
12	17/91	Khaller-chinskaya tundra	Medium sand	3.50	20–30	ND	0.0	$\mathbf{(0.3–1.4) \times 10^2}$	$(0–1.2) \times 10^2$	$\mathbf{(1.4–3.0) \times 10^2}$
15	17/91			10.20	20–30	ND	0.0	$(0–2.6) \times 10^2$	$\mathbf{(1.5–3.7) \times 10^2}$	0.0
13	17/91			17.50	20–30	ND	$(0–1.20) \times 10^2$	$\mathbf{(0.3–1.4) \times 10^2}$	$(0–0.8) \times 10^2$	$(0–1.6) \times 10^2$
21	1/93	Malaya Kon'kovaya river flood-plain	Muddy, sandy loam	4.00	15–30	$(2.3–4.0) \times 10^7$	$(0–8.0) \times 10^2$	$(0–3.9) \times 10^2$	$\mathbf{(1.3–6.5) \times 10^2}$	$(0–1.6) \times 10^2$

Table 1. (Contd.)

Sample	Hole/year	Region	Sediment		Depth, m	Age, 10^3 years	Bacteria resuscitated on CBA (1 : 10)	Fungi resuscitated		
								on Cz at 4°C	on Cz at 25°C	on MA at 4°C
30	1/97	Malaya Kon'kovaya river valley	Light loam	13.00	40	$(0.2–4.4) \times 10^7$	0.0	0.0	0.0	$(0–0.5) \times 10^2$
24	1/92		Fine sand	48.80	3000	ND	0.0	0.0	**$(0.3–2.2) \times 10^2$**	$(0–0.7) \times 10^2$
Canadian Arctic										
1	Taglu	McKenzie river delta	Muddy, sandy loam	17.90–18.05	10–2500	ND	ND	**$(0.1–0.7) \times 10^2$**	ND	$(0–0.7) \times 10^2$
2	Taglu		Gravelly sand	107.18–107.23	10–2500	ND	ND	**$(0.2–5.6) \times 10^2$**	ND	$(0–0.6) \times 10^2$
3	Taglu			206.86–206.93	10–2500	ND	**$(1.3–6.3) \times 10^2$**	$(0–3.1) \times 10^2$	**$(2.3–5.2) \times 10^2$**	$(0–1.6) \times 10^2$
4	Taglu			303.96–304.03	10–2500	ND	**$(1.5–2.1) \times 10^3$**	**$(1.5–7.5) \times 10^2$**	**$(1.1–5.7) \times 10^2$**	**$(1.9–10.7) \times 10^2$**
5	Unipkat		Muddy, sandy loam	20.50–20.55	170	ND	**$(0.8–1.1) \times 10^2$**	$(0.4–0.5) \times 10^2$	**$(0.7–1.5) \times 10^2$**	**$(0.9–2.3) \times 10^2$**
Antarctica										
16	4/95	Mayers dry valley	Gravelly coarse quartz–feldspar sand	1.40–1.44	30	ND	**$(0.3–1.4) \times 10^2$**	**$(0.7–3.3) \times 10^2$**	**$(0.3–1.5) \times 10^2$**	$(0–1.3) \times 10^2$
18	4/95			2.01–2.04	30	$(1.8–2.1) \times 10^5$	**$(0.6–9.3) \times 10^2$**	**$(0.8–5.5) \times 10^2$**	**$(2.2–9.6) \times 10^2$**	**$(2.8–3.0) \times 10^2$**
22	3/95	Tailor dry valley		3.02–3.06	170	ND	$(0–0.6) \times 10^2$	$(0–0.4) \times 10^2$	$(0–1.6) \times 10^2$	$(0–0.6) \times 10^2$
23	3/95			11.92–11.96	170	ND	$(0–0.6) \times 10^2$	**$(0.1–1.3) \times 10^2$**	$(0–1.2) \times 10^2$	$(0–1.9) \times 10^2$

Note: Confident numbers of fungi are given by boldface letters. ND stands for "not determined." CBA (1 : 10), tenfold diluted corynebacterial agar; Cz, Czapek agar; and MA, malt agar.

Table 2. Fungal genera revealed in the Arctic and Antarctic ancient sediments

Sample	Sediment age, thousand years	Fungal genera
26	5–10	*Alternaria, Botrytis, Chaetophoma, Cladosporium, Penicillium, Stachybotrys*
27	5–10	*Alternaria, Aureobasidium, Chaetomium, Penicillium*
21	15–30	*Botrytis, Geotrichum, Mycelia sterilia* (w, d), *Penicillium, Verticillium*
12	20–30	*Aspergillus, Cladosporium, Engyodontium, Geotrichum, Mycelia sterilia* (w), *Papulaspora, Penicillium*
13	20–30	*Mycelia sterilia* (w), *Penicillium*
15	20–30	*Aspergillus, Mycelia sterilia* (w), *Penicillium*
16	30	*Arthrinium, Genus* sp. (*Basidiomycetes*), *Chaetomium, Cladosporium, Mycelia sterilia* (w, d), *Penicillium*
18	30	*Cladosporium, Mycelia sterilia* (d, w), *Penicillium*
30	40	*Mycelia sterilia* (w)
9	100	*Aspergillus, Cladosporium, Geotrichum, Mucor, Mycelia sterilia* (w), *Penicillium*
7	100	*Aspergillus, Genus* sp. (*Basidiomycetes*), *Mycelia sterilia* (w), *Penicillium, Trichoderma*
5	10–30	*Aspergillus, Eurotium, Geotrichum, Mycelia sterilia* (w), *Penicillium*
22	170	*Dipodascus, Fusarium, Mycelia sterilia* (w)
23	170	*Aspergillus, Genus* sp. (*Basidiomycetes*), *Cladosporium, Mycelia sterilia* (d, w), *Oidiodendron*
11	200–600	*Aspergillus, Cladosporium, Geotrichum, Monodictys, Mycelia sterilia* (w), *Papulaspora, Penicillium*
1	10–30	*Cladosporium, Geotrichum, Mycelia sterilia* (w), *Penicillium, Sporotrichum*
2	2500	*Aspergillus, Mycelia sterilia* (w)
3	2500	*Alternaria, Cladosporium, Engyodontium, Mycelia sterilia* (d, w), *Penicillium, Trichoderma*
4	2500	*Alternaria, Aspergillus, Bispora, Chaetomium, Cladosporium, Mycelia sterilia* (d, w)
35	1800	*Mycelia sterilia* (w), *Penicillium*
37	2500	*Aspergillus, Cladosporium, Geotrichum, Mycelia sterilia* (d, w), *Paecilomyces, Penicillium*
38	3000	*Botrytis, Cladosporium, Geomyces, Penicillium, Ulocladium*
24	3000	*Cladosporium, Penicillium*

Note: "*Mycelia sterilia* (w)" stands for an unidentified to a generic level fungus of the family *Mucedinaceae* that produces white sterile mycelium but does not develop fruiting bodies necessary for proper identification. "*Sterile mycelium* (d)" stands for an unidentified to a generic level fungus of the family *Dematiaceae* that produces dark sterile mycelium.

tion of intracellular ice (at slow thawing rates), oxidative and osmotic stresses, and phase-transition phenomena (the latter are especially harmful to cellular membrane structures) [9, 12]. The mitigation of harmful effects and the maintenance of conditions favorable for the repair of damaged cells and their growth promote the recovery of a greater number of viable cells after long-term natural cryopreservation.

The aim of the present work was to study, by microbiological methods, the number and taxonomic diversity of viable microorganisms in the ancient Arctic and Antarctic microbiocenoses, with special emphasis on micromycetes, which are studied little in this respect, and actinobacteria, which are believed to be dominant among the recoverable prokaryotes of permafrost sediments.

MATERIALS AND METHODS

Permafrost samples were collected in the Arctic regions of Russia and Canada, where the average annual temperature of permafrost sandy loams are –7 to –12°C, and in Antarctica, where this temperature is below –20°C. The permafrost age was from 5000 to 3 million years (Table 1). Sampling methods and the control of sample sterility were described earlier [2]. Before inoculation to nutrient media, samples were stored at –20 or –70°C. Material for inoculation was taken with a scalpel from the axial part of frozen core samples. To recover fungi, 0.5-g portions of a core sample were placed in test tubes with 5 ml of water heated to room temperature (20°C), as well as to 35 and 52°C. Following one minute, the suspension was shaken at room temperature for 10 min. The tenfold dilutions of

Table 3. Effect of resuscitation conditions on the range of micromycete genera revealed in permafrost sediments

Genus	Number of samples in which the genus was revealed			
	Cz		MA	
	4°C	25°C	4°C	25°C
Alternaria	2	1		2
Arthrinium			**1**	
Aspergillus	1	5	4	4
Aureobasidium			**1**	
Genus sp. (*Basidiomycetes*)	1	1	1	1
Bispora			**1**	
Botrytis	2			1
Chaetomium	1		1	1
Chaetophoma			**1**	
Cladosporium	6	3	6	5
Dipodascus				**1**
Engyodontium			1	1
Eurotium		**1**		
Fusarium				**1**
Geomyces	1	1	1	1
Geotrichum	1		3	3
Monodictys			**1**	
Mucor				**1**
Mycelia sterilia (dark)	1	3	5	1
*Mycelia sterilia (*white)	9	11	8	11
Oidiodendron			**1**	
Papulaspora		1		1
Paecilomyces		**1**		
Penicillium	7	13	11	8
Sporotrichum		**1**		
Stachybotrys				**1**
Trichoderma		1		1
Ulocladium			**1**	
Verticillium		**1**		

Note: The genera whose representatives were revealed only in one sample under all resuscitation conditions are given by boldface letters. For other notes, see Tables 1 and 2.

this suspension were inoculated, in triplicate, on Czapek agar and malt agar, to which lactic acid was added at a concentration of 4 ml/l to suppress the unwanted growth of bacterial cells. The inoculated plates were incubated at 4 and 25°C. The grown colonies were examined and enumerated on the 21st and 30th days, respectively. The data obtained were statistically processed using the Excel 97 program [13]. The isolates obtained were reinoculated on malt agar and stored at 4°C. Micromycetes were identified on the basis of their cultural and morphological properties using the respective manuals [14, 15].

To recover bacteria, the tenfold dilutions of the above sediment suspension were inoculated, in triplicate, on the tenfold diluted corynebacterial agar [4]. The inoculated agar plates were incubated at 4 and 24°C. The grown colonies were enumerated after 3 weeks of incubation. Three to five representatives of each of the dominant bacterial morphotypes were identified using the respective methods and manuals [4, 16–18].

To control the degree of the sterility of the sterile tap water used for the preparation of sample suspensions, the water was inoculated on agar plates, which were then incubated at 4 and 25°C. To control the sterility of air in the microbiological box, open petri dishes with agar medium were exposed to the air for 10 min and then were incubated at 4 and 25°C.

RESULTS

Number of Micromycetes in the Ancient Arctic and Antarctic Sediments

Twenty-one of the twenty-three samples of sediments from 5–10 thousand to 1.8–3 million years old were found to contain (with a probability of 0.95) viable cells of mycelial fungi. Their number varied from 0.2×10^2 to 4.2×10^4 CFU/g air-dried sample (Table 1). As a rule, greater numbers of micromycetes were recovered from fine sediments, provided that the recovery was performed using malt agar incubated at 4°C. In some core samples, particularly those withdrawn from the Taglu hole (Canada) and hole 17/91 (the Kolyma Lowland), the number of recoverable micromycetes did not change with the depth of sampling. By contrast, the number of viable micromycetes increased with the depth of sampling in the cores withdrawn from holes 4/91, 6/91, 2/94 (the Kolyma Lowland), and hole 4/95 (Antarctica). The maximum number of micromycetes was recovered from the core sample withdrawn from hole 2/94 (the Chukoch'ya River region). This core represented muddy, peaty, and sandy loam 1.8 to 3 million years old.

Taxonomic Diversity of Micromycetes in the Ancient Arctic and Antarctic Sediments

The 213 identified micromycete isolates were found to belong to 25 genera of the divisions *Basidiomycota*, *Ascomycota*, and *Zygomycota*, and some represented mitosporic fungi (Table 2) [19]. The most frequently encountered micromycetes belonged to the genera *Penicillium* (detected in 18 sediment samples), *Cladosporium* (detected in 13 samples), and *Aspergillus* (detected in 10 samples). Nineteen samples gave rise to white sterile mycelium, identified only to the family level (*Mucedinaceae*), and 7 samples gave rise to dark

Table 4. Major groups of actinobacteria isolated from the Arctic sediments

Group	Morphological characteristics	Chemotaxonomic markers
Arthrobacter spp.	Rod–coccus cycle	Lysine; MK-9(H_2)
Micrococcaceae	Cocci (single, in pairs, and in clusters)	Lysine; short-chain menaquinones
Rhodococcus and related taxa	Rudimentary branched mycelium	*Meso*-DAP, mycolic acids, arabinose, and galactose
Streptomyces spp.	Aerial mycelium with long spore chains	LL-DAP
Microbacterium spp.	Irregular rods (2×0.5 μm)	Ornithine; MK-10 and MK-11
Cryobacterium spp.	Irregular rods (2×0.5 μm)	DAB; MK-11 and MK-12
Brevibacterium spp.	Rod–coccus cycle	Peptidoglycan Alγ; MK-8(H_2)
Nocardioides spp.	Substrate mycelium is fragmented	LL-DAP; MK-8 (H_4)

Note: MK, menaquinone; DAP, diaminopimelic acid; and DAB, diaminobutyric acid.

sterile mycelium, which was assigned to the family *Dematiaceae*. All these micromycetes were detected in 30% of the sediment samples, including the most ancient ones. Each of the representatives of the genera *Arthrinium, Aureobasidium, Bispora, Eurotium, Fusarium, Chaetophoma, Monodictys, Mucor, Oidiodendron, Paecilomyces, Stachybotrys, Sporotrichum*, and *Ulocladium* was found only in one of the samples investigated. The most ancient sediments (1.8 to 3 million years old) bore viable fungi of the genera *Penicillium, Cladosporium, Geomyces, Geotrichum, Aspergillus, Paecilomyces, Botrytis*, and *Ulocladium*, as well as fungi that produce dark and white sterile mycelia.

Effect of the Thawing Rate and Cultivation Conditions on the Range of Isolated Micromycetes

It is known that the thawing rate affects the ability of microorganisms to be recovered after deep cooling. For instance, the thawing of samples at a high temperature (45–52°C) greatly augmented the yield of viable cells of some eukaryotes as compared with their thawing at 20°C [20]. In our experiments, the thawing of samples at 35°C led to the recovery of the fungal genera (*Arthrinium, Chaetomium*, and *Ulocladium*) that could not be recovered at lower temperatures. The thawing of sediment samples at 52°C further enlarged the range of recovered genera: fungi of the genera *Aureobasidium, Bispora, Oidiodendron*, and *Paecilomyces* could be isolated only at this temperature.

Analysis showed that malt agar is more preferable than Czapek agar for the recovery of rare fungal genera: malt agar allowed 11 of the 15 rare fungal genera to be recovered (*Arthrinium, Aureobasidium, Bispora, Chaetophoma, Mucor, Oidiodendron, Stachybotrys, Ulocladium*, and others), whereas only four genera (*Eurotium, Sporotrichum, Verticillium*, and *Paecilomyces*) were recovered using Czapek agar. The total number of fungal genera recovered using malt agar (25 genera) was also higher than the number of the genera recovered on Czapek agar (17 genera).

The incubation temperature also influenced the range of recovered fungi: incubation at 4°C led to the recovery of the genera *Arthrinium, Aureobasidium, Bispora, Chaetophoma, Monodictys, Oidiodendron*, and *Ulocladium*. Incubation at 25°C led to the recovery of *Sporotrichum, Mucor, Paecilomyces, Verticillium, Stachybotrys*, and others. The fungi that produce dark mycelium and/or spores were recovered predominantly at 4°C (about 60% of the total number of fungi isolated at this temperature), whereas light-colored micromycetes were recovered primarily at 25°C.

The Abundance and Taxonomic Composition of Bacteria in the Arctic Sediments

The abundance and taxonomic composition of bacteria in the Arctic sediments were studied in samples differing in age, particle size, and the total carbon content. Viable bacteria were revealed in all of the sediment horizons studied in amounts ranging from 10^5 to 10^7 cells/g air-dried sample (Table 1). The maximum number of bacterial cells (up to 2.8×10^9 cells/g) and their widest taxonomic diversity were detected in the early Pleistocene muddy, peaty, and sandy loam 1.8–3.0 million years old from hole 2/94 (the Chukoch'ya river region) and in the late Pleistocene loam and sandy loam sediments 15–40 thousand years old (the Malaya Kon'kovaya river region) (Table 1). There was no essential difference in the range of bacteria isolated at incubation temperatures of 4 and 24°C.

The isolated bacteria belonged to diverse taxonomic groups: gram-negative and gram-positive bacteria, spore-forming and non-spore-forming (predominantly, coryneform) bacteria, cocci, and those forming developed mycelium (the order *Actinomycetales*) [21]. The latter comprised 50–90% of the total number of detected prokaryotes and were arbitrarily assigned to

8 taxonomic groups (Table 4). The most abundant bacterial group was that of the genus *Arthrobacter.* This group was heterogeneous with respect to the cultural and morphological characteristics of its members and the composition of their peptidoglycans and the cell wall sugars, indicating the presence of several species in this group. The group of coccoid bacteria of the family *Micrococcaceae* (the genera *Micrococcus, Kosuria*, and others) and the group of bacteria of the suborder *Corynebacterinea* (the genera *Rhodococcus, Gordona*, and *Mycobacterium*) with a thick lipopolysaccharide layer in their cell wall [16, 21] were also abundant. Representatives of the genera *Microbacterium, Brevibacterium*, and *Nocardioides* were encountered less frequently. It should be noted that microbacteria, which are fairly susceptible to unfavorable environmental conditions, as a rule, were isolated in associations with bacteria of the genus *Rhodococcus*. The sediment samples from holes 1/93, 1/97, 6/91, and 2/94 (the Kolyma Lowland) contained many mycelial sporoactinomycetes of the genus *Streptomyces* (up to 50% of all isolates from these samples). The psychrophilic bacteria of the genus *Cryobacterium*, which are characterized by a wide growth temperature range (from 3 to 25°C) with an optimum at 15–18°C, were detected in the most ancient sediments.

DISCUSSION

The data presented show that the permafrost sediments of different geneses and ages preserve the remnants of ancient microbial communities, including bacteria and microscopic fungi. The population densities of microorganisms in ancient and more recent sediments of one type varied within the same limits. However, it is evident that greater amounts of viable cells could be recovered from fine sediments, where the initial number of microbial cells was presumably higher and the physicochemical conditions of their freezing and subsequent long-term storage at low temperatures were most beneficial. The observed differences in the number of prokaryotic and eukaryotic microorganisms in various sediments were found to be independent on the depth or, which is the same, the age of sediments but reflected the specific conditions of their formation and transition to the permanently frozen state.

The analysis of the taxonomic composition of the recovered microorganisms allowed some other factors promoting their survival in permafrost to be revealed. The majority of the recovered fungi are those which asexually produce small single spores. Such fungi are obviously the most resistant to long-term cryopreservation. Some recovered genera (*Bispora, Alternaria*, and *Ulocladium*) are oligosporous and have melanin in their cell wall. About 70% of such isolates were recovered at an incubation temperature of 4°C. This is not surprising, since melanin is believed to make cells resistant to ultraviolet irradiation and extreme temperatures [22]. For instance, most fungi found in Antarctica and in the cold Pamirs deserts are melanin-pigmented [23].

The genera *Arthrinium, Aureobasidium, Botrytis, Dipodascus, Fusarium, Geotrichum, Oidiodendron*, and *Stachybotrys*; and the actinomycetes *Rhodococcus fascians, Brevibacterium* sp., and *Microbacterium* sp. are usually isolated from plant materials and are phytopathogens [14–16]. The tendency of these microorganisms to live in nutritionally rich terrestrial biocenoses explains their predominance in sediment samples rich in organic carbon and their preferential recovery from frozen sediments on malt agar. The presence of plant substrates in sediments and the high density of associated microcommunities presumably create more beneficial conditions for microbial survival when these sediments become frozen.

The great number of viable actinobacteria in permafrost sediments may be due to the unique structural, biochemical, and molecular biological organization of these microorganisms: the formation of mycelial thallome, the specific composition of their cellular lipids and cell wall, the stability of their double-strand DNA due to a high content of the G+C nucleotide pairs, the large genome and its high redundancy, and so on. All this provides for a high survival of actinomycetes under the conditions that are fairly lethal to other bacteria [24].

Some isolates are likely adapted to active life at low (but above zero) temperatures. Indeed, the abundance and diversity of fungi isolated at 4°C appear to be higher than those of the fungi isolated at 25°C. For instance, the psychrotolerant species of the genus *Cladosporium*, whose enzymes are active at relatively low temperatures [28], can easily be isolated at 4°C but not at 25°C. Furthermore, we succeeded in isolating the psychrophilic actinomycetes of the genus *Cryobacterium*, whose only known representative was isolated earlier from Antarctic permafrost [18]. The microorganisms isolated from permafrost are distinguished by having a great number of lipid granules in cells (basidiomycetes) [5], a high content of unsaturated fatty acids in lipids (*Cryobacterium*) [18, 25], a great amount of capsular material during cultivation at low temperatures [1, 12], and so on. The microorganisms adapted to low temperatures must be more resistant to seasonal variations in the environment, to related phase transitions, and to the action of harmful factors associated with the natural cryopreservation of microorganisms and their subsequent resuscitation under laboratory conditions.

Of interest is the fact that the thawing of sediment samples at 35 and 52°C allowed the number of detected fungal genera to be increased by more than 30%. This shows the expediency of the attempts to further optimize conditions for the resuscitation of frozen ancient microorganisms.

Thus, the presented data show that both prokaryotic and eukaryotic microorganisms may long be preserved under conditions of natural cryopreservation. The preservation of microorganisms in permafrost sediments depends on the original composition and density of ancient microbial consortia, the degree of microbial adaptation to low temperatures, and on the protective properties of the environment. All these factors are related to the evolution of biocenoses in the ancient times, the geological history of sediments, the conditions of their transition to the permanently frozen state, rather than to their age and, hence, the depth of accommodating sediments. In spite of the limited number of nutrient media used for the resuscitation of frozen microorganisms, we succeeded in the isolation of microorganisms, including eukaryotes, whose age is several million years. This does not seem to be a limit.

The resuscitated microorganisms can be not only of theoretical but also of practical interest, since their adaptation to low temperatures might have led to the evolution of enzymes with unique properties. Furthermore, the possibility cannot be excluded that these microorganisms retained the ability to produce some valuable metabolites that are not synthesized by modern microorganisms [3].

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