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Review

Biological investigations of the Antarctic ice sheet: review, problems and projects

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Abstract: The Antarctic Icecap has attracted considerable attention of scientists for many years. Many microbiological and general biological studies of the ground surface, snow and glacial cover have been performed. In recent decades, it has been discovered that the Antarctic ice sheet is a unique depository of paleoclimatic and paleomicrobiological information that reflects previous ecological events on our planet. Thus, dust particles, spores, plant pollen, and different microorganisms, which were brought onto the glacier surface by wind, appear to be gradually buried in the glacier layers and preserved in the frozen state for hundreds thousands years. These investigations have become especially important in the era of space research, many scientists consider the Antarctic as the most appropriate model to solve some methodical problems in astrobiology. Further developments and improvements of drilling methods have made it possible to penetrate into the deepest levels of the Antarctic Icecap, including the lower layers of so-called accretion ice above the sub-glacial Lake Vostok. The initial glaciological and microbiological investigations were mainly focused on the glacier ice and gave very useful information on the history of the Earth's climate and the distribution of dust particles and microorganisms in the ice sheet during different periods. However, studies of the deep accretion ice and the sub-glacial Lake Vostok are expected to provide more insight into relict microbial forms and give rise to new investigations of the Antarctic ice cover. These works should include searches for microbial life in the accretion ice and Lake Vostok, and requires new biological techniques, new multidisciplinary approaches and international cooperation.

Nowadays, biological investigations of the Earth's polar regions are of paramount importance in view of current ecological problems. Of no less significance is the search for possible extraterrestrial life, since permafrost and glaciers are widely spread in the Universe; this is why, researchers have paid great attention to the Antarctic as a model for astrobiology.

key words: Antarctic icecap, biology, microorganisms, Lake Vostok

Introduction

Glaciers of the Antarctic region are regarded as a unique depositary of paleoclimatical and paleomicrobiological information that reflects past ecological events on our planet (Abyzov *et al*., 1998b). After being deposited onto glacier surfaces as atmospheric fallout, dust particles, plant pollen, and various microorganisms became gradually submerged into deep levels of the glacier, along with air bubbles, and preserved there for hundreds of thousand's of years.

In the central part of the East Antarctic glacier from the meaning unclear Vostok station, where the thickness of the ice cap is about 3750 m (Lipenkov *et al*., 2000); deep drilling and sampling of ice cores have been conducted for about 30 years (Abyzov, 1993; Abyzov *et al*., 1998a). In 1998, the bore hole reached a depth of –3623 m. Drilling was stopped approaching the surface of a subglacial lake. Since 1974 scientists from the Institute of Microbiology (Russian Academy of Sciences), St. Petersburg Mining Institute, and Arctic and Antarctic Research Institute have carried out microbiological studies of Antarctic ice cores using the specially developed procedure of aseptic sampling. The most recent of these microbiological investigations was conducted joint by with the National Institute of Polar Research, Japan (NIPR) and Grenoble Glaciological Laboratory in France.

At the first stage of investigations by classical microbiological methods such as inoculating of the ice core samples in nutrient media, it was experimentally proved that ancient ice is well suited to the study of super-long anabiosis of microorganisms belonging to various taxonomic groups (Abyzov *et al*., 1979). Optical and electron microscopy made it possible to find a variety of well preserved and intact bacteria, actinomycetes, yeast and mycelial fungi cells in ice core samples taken from deep levels (Abyzov *et al*., 1998a, b; Karl *et al*., 1999; Priscu *et al*., 1999).

Microbiological investigations have demonstrated the presence of microbial cells in all layers of the Central Antarctica ice sheet (Abyzov *et al*., 1998a, b). In addition unicellular algae, represented mainly by the Diatomeae, were found at many depths upon examination under luminescence and scanning electron microscope (Abyzov *et al*., 1999). These algae are common inhabitants of Antarctic seas, superficial lakes, and ice and snow (Bardin *et al*., 1969).

This work focuses on specific features of near-bedrock levels and the influence of the large subglacial Lake Vostok on accretion ice formation in the bottom glacier layers.

Brief microbiological characterization of Icecap layers

Previous experiments have proved the presence of viable pro-kariotic and eukaryotic microorganisms ice layers with depths from 0 to 2405 m (Abyzov, 1993), and the number of viable microbial cells was shown to decline gradually with increasing depth. Results of direct counting point to other regularities in distribution of the total bacterial cell number, which correlate with periods of climate warming and cooling, but not with the age of ice layers. These regularities, observed for 1500–2750 m deep layers are considered in detail in Abyzov *et al*. (1998a).

The data on the number of microbial cells at depths from 1000 to 3611 m are

Table 1. The number of viable microbial cells in ice core from different ice sheet horizons from 1000 m to 3611 m.

Horizon, m	The number of studied samples	Percentage of samples with the content of microbial cells		
		$N \times 10^2$ per 1 m*l* of thawed water:	$N \times 10^3$ per 1 m*l* of thawed water:	
		N=1.0–9.0	N=1.0–2.0	N>2.0
1000–1249	5	100	0	0
1500–1975	23	26	70	4
2002–2474	27	41	18	41
2500–2974	20	50	30	20
3002–3344	15	93	7	0
3538–3611	10	100	0	0
	Total: 100			

summarized in Table 1. According to results of glaciological studies (Lipenkov and Barkov, 1998), ice in the basal glacier sheet that was formed due to consecutive burial and compaction of atmospheric precipitates is observed to a depth of 3538 m. The lower are composed of so-called accretion ice with meaning unclear changed under influence of the lake water.

All the studied samples contained bacterial cells whose number was in the range from 100 to 900 ml^{-1} of thawed water. As seen from Table 1, among almost all the samples representing the overall ice thickness, there were specimens in which the number of cells was close either to the lowest or the highest values of the above range. Over 50% of the samples taken from 1500–3000 m contained no less than 10^3 microbial cells per 1 m*l* of thawed water.

Upon seeding nutrient media with samples taken from 0 to 2500 m, more than 160 strains of viable microorganisms belonging to various taxonomic groups were isolated (Abyzov, 1993). Some selected strains from the collection of isolated microorganisms were phylogenetically analyzed in the Biological Oceanography Laboratory of Hiroshima University (Abyzov *et al*., 2003).

As revealed from the studies, the layer of accretion ice from 3538 to 3611 m, above Lake Vostok, had an insignificant number of microorganisms (Table 1). According to the data of glaciologists (Lipenkov *et al*., 2000), the age of these layers is hundreds of thousands or even millions of years. Regarding the viability of microorganisms that rarely occur at these depths, it is unfortunately impossible to draw a definite conclusion because attempts to isolate bacteria or other microorganisms from 300000-year-old ice deeper than 2400 m and to select the media or substrates optimal for the growth of these microbes have failed. Meanwhile, the observed uptake of C^{14}-labeled protein hydrolyzate added to the samples of ice cores taken from a depth of about 3000 m points to biological activity (Abyzov *et al*., 1998b), suggesting the presence of metabolically active and viable cells in the oldest ice layers (Table 2).

The uptake of the radiolabeled substrate was observed in almost all 17 samples of the core. Although the uptake rates were very low, slight heterotrophic activity was revealed. In this case, there was a clear tendency to decrease in the activity of C^{14}-labeled

Table 2. The uptake of organic substrate (^{14}C-labeled protein hydrolyzate) in samples of thawed water from ancient ice sheet horizons.

N	Depth, m	Incubation time, h	The rate of C^{14}-labeled protein hydrolyzate uptake, V_{max}, μg protein/*l* per 1 h
1	1585	24	0.0037
2	1685	24	0.0031
3	1864	20	0.0003
4	1885	17	0.0007
5	1914	20	0.0005
6	1938	17	0.0060
7	1964	3	0.0024
8	1984	23	0.0025
9	2015	18	0.0017
10	2135	6	0.0000
11	2216	18	0.0005
12	2235	24	0.0009
13	2283	24	0.0004
14	2376*	18	0.0003
15	2425*	24	0.0010
16	2500*	24	0.0006
17	2750*	20	0.0002

* These data from Abyzov *et al*., 1998b.

substrate consumption by microorganisms that existed in the anabiotic state for long periods. Nevertheless, direct proof of biological activity in cells that maintained viability in the oldest layers of ice is a prominent advantage of the radioisotope technique.

More thorough examinations of fluorescamine-stained specimens, fixed on membrane filters, make it possible to investigate a morphological diversity of prokaryotes (coccoid and rod-like bacteria, actinomycetes, cyanobacteria) and eukaryotes (yeast, mycelial fungi, unicellular algae) (Fig. 1).

By their morphological features, microbial cells that were revealed in ice layers resemble the known representatives of the contemporary microbial world. The majority of microflora are small cocci and short rods, whereas the other types of microorganisms occur in lesser quantities and their distribution in the ice sheet is irregular. However, some ice sheet layers, such as that at 3299 m depth, had a relatively large number of bacterial cells (10^2–10^3 cells), having the form of long rods and belonging to distinct morphological groups (Fig. 2).

Using epufluorescence and scanning electron microscopy made it possible to observe a great variety of non-bacterial objects such as small-sized unicellular algae and pieces of their shells, as well as spores and fungal cells at many depths. These objects are interesting due to their relatively large size of several or tens of microns so that it was possible to distinguish between the intracellular structural components and, in some cases, to determine the genus or even the species of some cells based on their visualized characteristics. A preliminary taxonomic determination was possible for organisms with

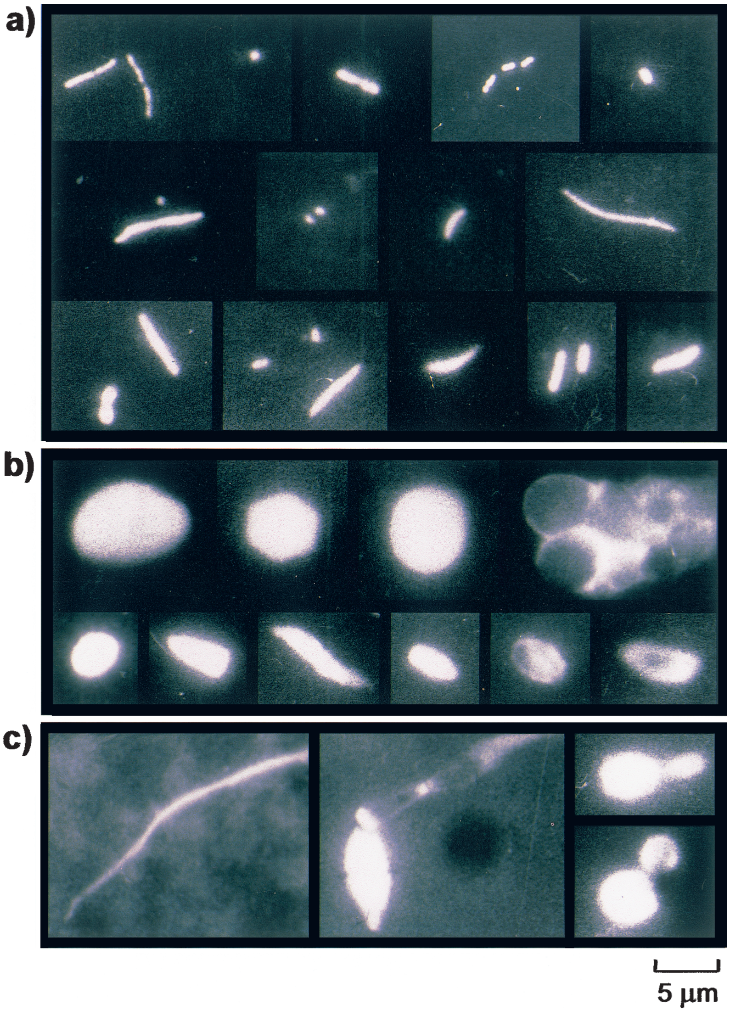

Fig. 1. The fluorescence microscopy of microorganisms detected in both the glacier and accreted ice. a): The most typical bacteria found in all ice horizons. b): Degraded and undegraded cyanobacterium-like cells. c): A fragment of an actinomycete filament, depth 3565 m (left), a fragment of a partially lysed fungal hypha with a conidium, depth 3685 m (center), and yeast cells, depth 3611 m (right).

rigid siliceous structures, such as diatomic and peridinia algae, radiolaria, and spores. The fact of long-term preservation of their shells and skeletons in ancient sedimentary rocks is well known to paleontologists and geologists who use them to establish the origin and age of these rocks. Although the content of such objects in samples was small, only several units per 100–150 ml^{-1} of thawed ice water, they were present at virtually all depths. At some depths, *e.g.* 2035 or 2400-m, up to 10^2–10^3 of these organisms per 1 m*l* of thawed water were observed. In view of the great dispersion of these objects in ice and of the small volumes of available samples, it is impossible to find any clear

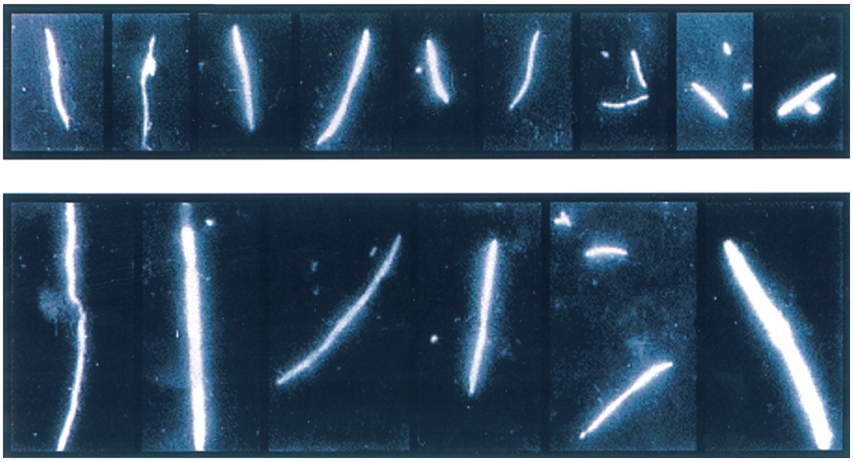

Fig. 2. Large number of bacterial cells, having the form of long rods and belonging to distinct morphological groups, depth 3299 m, magn. ×3000.

dependence of their distribution on the ages of ice layers. However, one may conclude at least that non-bacterial microscopic biological objects, as well as bacteria and dust particles, occur in large amounts in the layers of ice formed during global cooling on the Earth (Abyzov *et al*., 1998b).

Among remnants of unicellular eukaryotic organisms in the ice sheet, most of them (up to 80%) belonged to the diatomic algae, which were mainly represented only by single fragments of their shells. Evidently, these fragments can be attributed to dead cells that had already frozen in ice many thousands of years ago. However, almost all the less or more intact diatomic shells also showed signs of deformation. So, it is impossible to answer the question of whether these deformations are inherent to already dead and deformed cells that were brought with wind and then frozen in ice sheets. Or whether these cells remained intact and viable at this moment and likely inhabited the vicinity of the glacier, but were then deformed due to settlement of ice sheets and/or sampling of the cores (Fig. 3). The possibility of freezing into ice of live algae can be evidenced from our epifluorescence microscopy observations that revealed the presence of separate fluorescamine-stained luminous cells which were as bright by shining as living cells. Furthermore, notwithstanding the treatment of specimens with acetone during staining, some cells showed chlorophyll luminescence, similar to that normally observed for live cells after fixation. Unfortunately, because of very small amounts of available ice core samples and the very rare occurrence of intact algae in them, it was impossible to demonstrate the presence and preservation of chlorophyll in millennia-year-old ice. However, the presence of luminescent chlorophyll and numerous protein compounds in the algae cells, which were stored for at least 400000 years in the glacier, is a very interesting fact (Fig. 4, 5).

In a 2035 m deep ice layer that was mostly populated by algae, we observed a great diversity of these organisms; there were numerous fragments of shells having no obvious signs of leaching. These cells probably came from oceanic plankton, which were brought

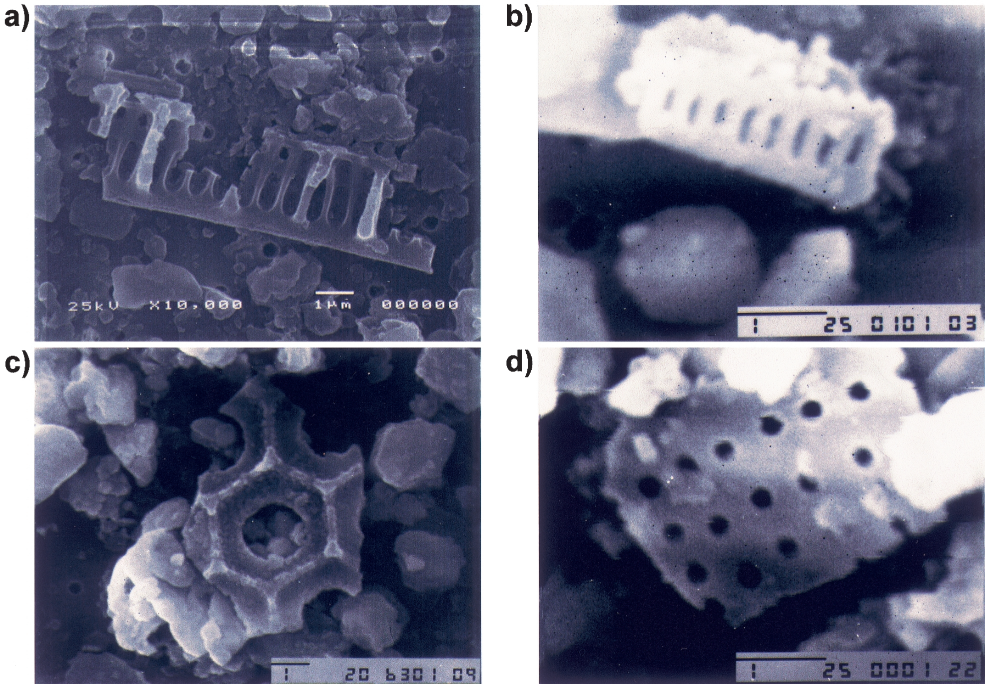

Fig. 3. Diatomic algae which were mainly represented only by single fragments of their shells. a): Depth 897 m. b): Depth 2972 m. c): Depth 3049 m. d): Depth 3225 m.

by wind, along with microscopic seawater drops. However, one cannot rule out the possibility that at least some of these cells originated from fresh-water reservoirs (Abyzov *et al*., 1999). As is known, complex microbial communities in which diatomic algae and aquatic mosses are abundant as well as bacteria and cyanobacteria are widespread in water and surface ice layers of lakes in the Dry Valleys region of Antarctica, where these microorganisms grow during summer thawing in microzones around eolic particles (Kanda and Iwatsuki, 1989; Kanda and Mochida, 1992; Imura *et al*., 1992, 1999; Gal'chenko, 1994; Gal'chenko *et al*., 1995; Priscu *et al*., 1999). The results of these works will be useful for further studies of Lake Vostok.

Well-preserved algae cells, which are likely to be representative of oceanic plankton, also occurred in the 2400-m deep ice layer. This layer was formed during global cooling, probably accompanied by highly intense wind activity; bubbles from the ocean surface were picked up by wind and instantaneously frozen to form small ice pieces which served as protecting capsules for live microorganisms including bacteria and microscopic algae among others.

Many ice layers, including the deepest ones, were found to contain remnants of coccolithophorides. Well preserved microscopic biological objects were found in ice sheet samples taken from the depth of 3225 m, the deepest point where a morphologically intact bio-object was observed, but its taxonomic position is still unclear. Beneath this point, down to the depth of 3344 m, only fragments of diatomic

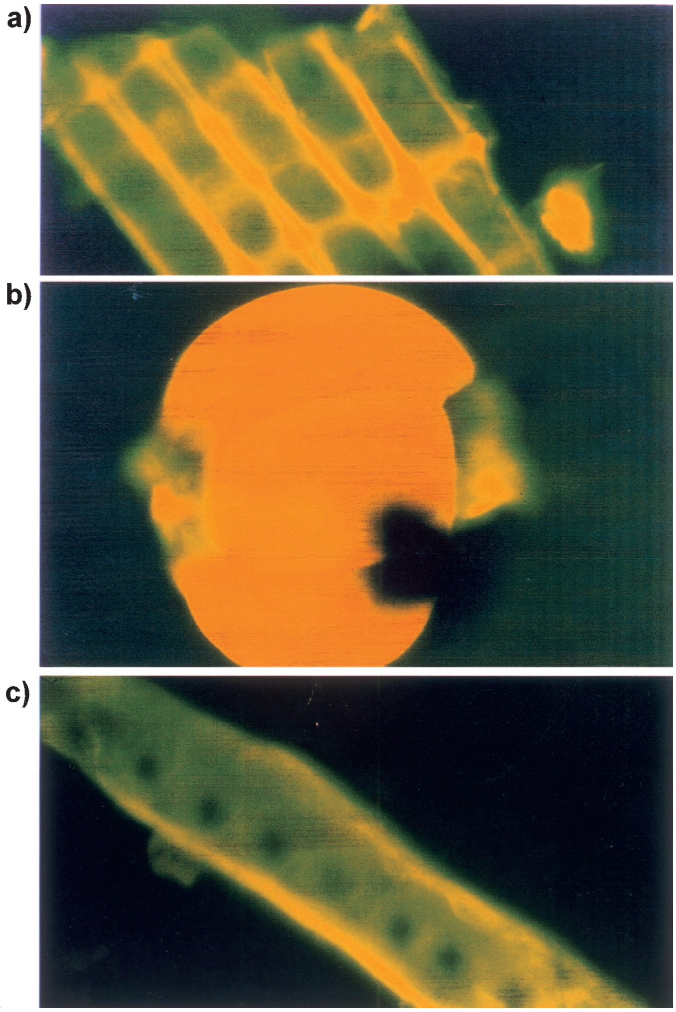

Fig. 4. Different object of biological origin revealed in glacier layers. a): Remnants of higher plants, depth 1097 m. b): Pollen of a higher plants, yellow-orange pigment, depth 1097 m. c): Remnants of diatomic shell, depth 2902 m.

algae occurred and they were increasingly destroyed with increasing depth (Abyzov *et al*., 1999).

Some future tasks in studies of Lake Vostok

Leading Russian scientists (Kapitsa *et al.,* 2002, personal commun.) believe that a significant new stage of investigation has begun in the study of basal ice layers. New techniques of deep drilling and scientific methodical approaches need to be developed for biological studies of these ancient layers.

In particular, classical microbiological studies should be supplemented by general

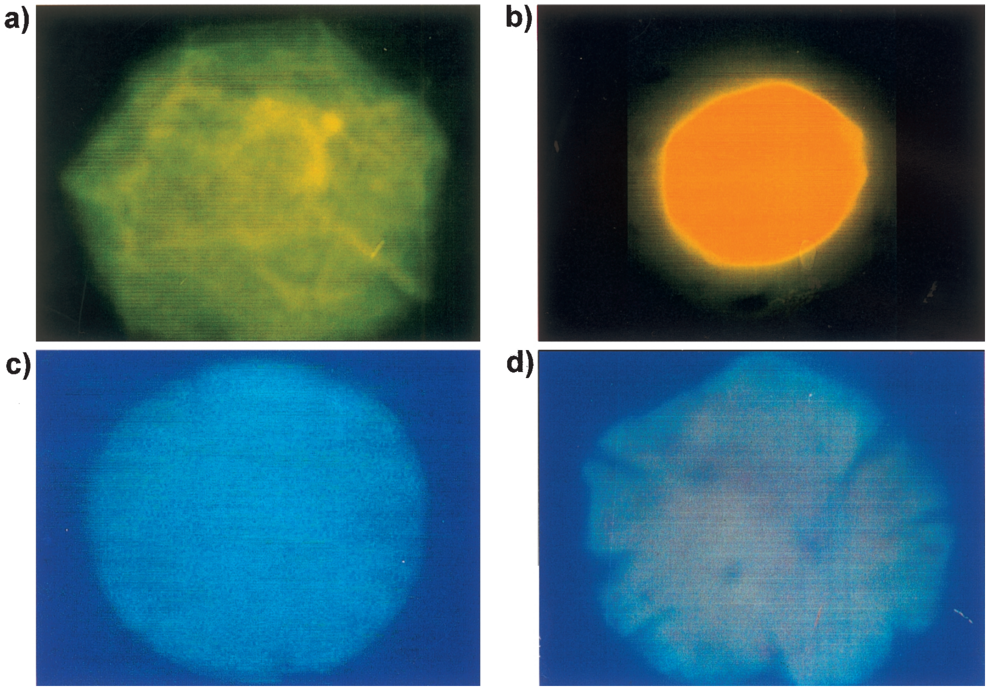

Fig. 5. Fluorescence of microalgae on filters stained by fluorescamine. a): In blue light, depth 3049 m. b): In blue light, depth 3025 m. c): In UV-light, depth 2950 m. d): In UV-light, depth 2902 m.

biological investigations. Since few of the microorganisms present in ancient Antarctic layers can be cultivated, as evident from previous experiments (Abyzov *et al.*, 1998b), it is very promising to use modern methods based on analysis of rRNA extracted directly from samples (*e.g.,* the review by Amann *et al.*, 1995). For samples of accretion ice (Lake Vostok, Antarctica), using the above mentioned analysis of bacterial 16S ribosomal DNA allowed Priscu *et al.* (1999) to reveal the members of the *Alpha*- and *Beta-Proteobacteria* and the actinomycetes.

Thus, for example, the well-known results of biological studies on Antarctic lakes (Kanda and Iwatsuki, 1989; Imura *et al.*, 1992; Gal'chenko, 1994) suggest a need to search for relict organisms which possibly were preserved in the form of spores in a subglacial lake since the Ice Age. Lake Vostok is a unique ecosystem which may harbor ancient forms of microorganisms that are not merely preserved, but actually function under such extreme conditions, for as we know the lowest temperature limit for some microorganisms' activities can be as low as -5°, -8° and even -12°C (Gounot, 1991; Russel, 1992; Gilichinsky *et al.*, 1995). In contrast to the majority of Antarctic lakes, Vostok is in perpetual darkness due to the great ice thickness above it, and as a consequence we may observe the absence of a photoautotrophic component of microbial biodiversity as one of its distinctive features. The ecosystem of this lake should be entirely microbial and the microorganisms inhabiting it should be heterotrophic, using allochtonous organic matter which penetrates into the water with mineral material during the glacier's horizontal flow. Nevertheless, in this stage of our investigations we cannot reject the possibility of presence of some remnants of ancient aquatic inhabitants (Poglazova *et al*., 2001). According to very interesting implications of Duxbury *et al*. (2001)'s model, "live organisms and/or their remnants in Vostok waters, could be much older than the age of the basal ice, and even older than the age of the Antarctic ice sheet, *i.e.* older than 5–30 Myrs. In terms of evolution of life on Earth, this time period is rather recent, and assuming that this lake was a productive ecosystem, remnants of its biota must certainly be preserved in its sediments." At the present stage of investigation it is not yet possible to obtain samples from the lake water and especially from the bottom deposits because intensive preliminary work is required to develop a method of aseptic penetration through the ice-water interface and to construct equipment which will operate in such a way that the ecological environment of the lake remains intact.

Conclusions

Since biological studies of the Antarctic ice sheet and the underglacial Lake Vostok have attracted great attention of the world-wide scientific community, international cooperation is very important in explorations of the Antarctic ice cover which are closely related to future astrobiological investigations.

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