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SHORT COMMUNICATIONS

Detection of Phage Infection in the Bacterial Population of Lake Untersee (Antarctica)

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It is presently generally accepted that phages are the most numerous life form on the planet [1, 2]. Phages play an ecologically significant role in aquatic ecosystems, where they are an important factor involved in the regulation of the structure and numbers of microbial communities [2]. The ecology of bacteriophages has been studied primarily in the ecosystems of temperate latitudes. Data on the distribution of bacteriophages in polar continental lakes are scarce; however, they indicate that phages play an important role in these ecosystems, where microbial communities prevail [3].

The studies of Antarctic lakes have demonstrated that photosynthetic production of organic matter by cyanobacteria is the key biogeochemical process determining the bacterial processes of sulfate reduction, methanogenesis, and methane oxidation [4]. The trophic structure of planktonic communities inhabiting these lakes is usually very simple and consists mainly of prokaryotes and one or two species of phytoplankton with an almost complete absence of zooplankton. Recent studies showed that representatives of numerous families of viruses are abundant in Antarctic lakes. Various species of bacteriophages, as well as viruses infecting algae and protozoa, were detected by means of transmission electron microscopy and metagenomic analysis [3, 5–8].

Phage lysis is known to promote the release of organic carbon, a source of nutrients and growth-stimulating compounds required for microbial production [3]. However, the process of bacteriophage infection does not always result in lysis of the host cell. The phenomenon of lysogeny, which is when the virus penetrates the cell so that its viral nucleic acid becomes integrated into the host cell chromosome (at this stage the virus is called a prophage), widely occurs among bacteria. Various factors, including ambient conditions, may induce the release of viral particles from infected host cells and subsequent cell lysis [1, 2]. It was demonstrated that most bacteria inhabiting polar lake ecosystems were lysogenic (unlike those from the lakes of temperate latitudes) [3]. According to the published data, the specificity level of viral infection in these ecosystems is quite low; that is, bacteriophages can infect a wide range of hosts [3]. Phages are involved in the transfer of genetic information between various species of bacteria, thereby affecting the evolution and ecological properties of bacterioplankton [5–8].

The goal of the present work was to reveal the phage component in the population of aerobic microorganisms from Lake Untersee samples.

Lake Untersee is located at 71°20′ S, 13°45′ E in the Otto-von-Gruber-Gebirge (Gruber Mountains) of central Dronning Maud Land. This ultra-oligotrophic lake is 563 m above sea level, with an area of 11.4 km^2 and is the largest surface lake in East Antarctica. Lake Untersee has two sub-basins; the larger one, 160 m deep, lies adjacent to the Anuchin Glacier and is separated by a sill at 50 m depth from the smaller, 100 m deep basin in the southwestern corner [9]. The deep basin is close to homothermal; the water temperature ranges from 0 to 5°C; the oxygen concentration in the aerobic part of the lake (0–70 m) is quite high and reaches 200% of saturation. In the intermediate zone (64–79 m), the oxygen content ranges from 0 to 160%; an anaerobic horizon was detected at a depth of 80–98 m. In contrast, the shallow basin is density stratified below the sill depth (~50 m), and, in the

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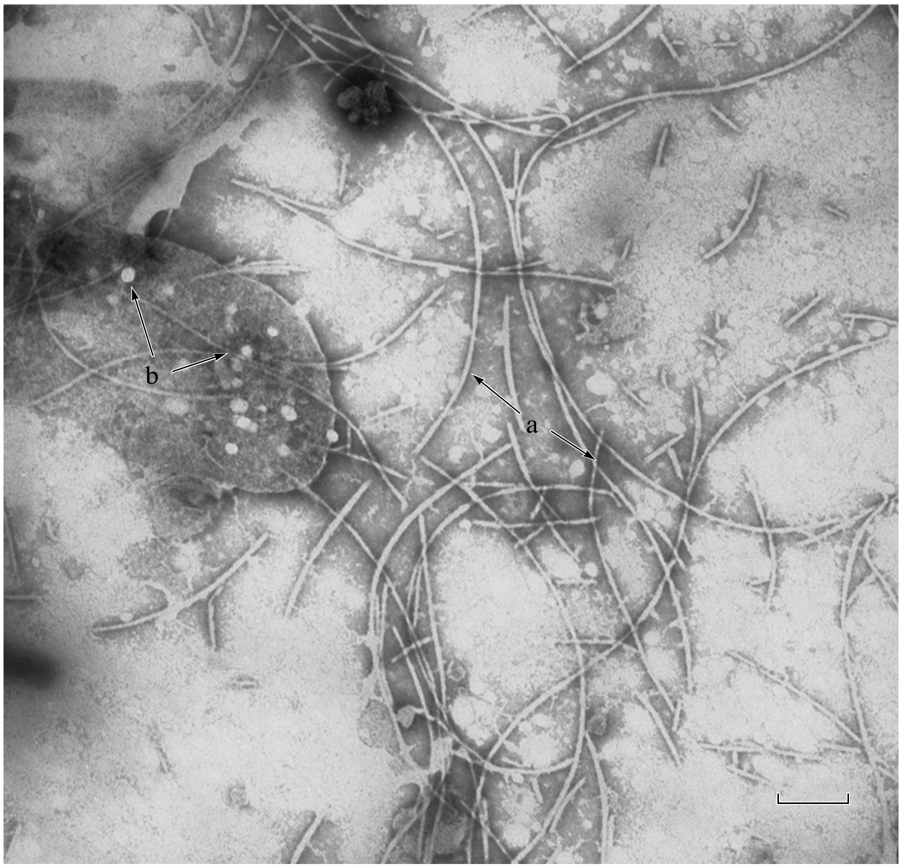
absence of oxygen at a depth of 80–90 m, the concentration of dissolved methane reaches 21 mM [10]. The high pH of this mixed water mass (pH 10.2–6.9) has been attributed to weathering of the predominant anorthosite rock in the lake's hollow [11, 12]. The benthic environment is dominated by cyanobacterial mats forming cuspate pinnacles dominated by *Leptolyngbya* spp. and laminated, conical stromatolites that rise up to 0.5 m above the lake floor, dominated by *Phormidium* spp. [12].

The subjects of our study were the colonies of aerobic heterotrophic bacteria obtained from the water sample collected at a depth of 70 m in the northern basin. At the sampling site, the water temperature was 5°C, pH was 7.5. The obtained water sample stored at 4°C was plated on standard nutrient media, including starvation agar (2% and 1.5% Difco agar), synthetic CP1 medium with glucose [13], agarized ISP1 medium (2% Difco agar), and ISP2 and ISP3 [14] with 0.25% yeast extract (Difco). Inoculated media were incubated at 16–20°C and at 28°C for 5–20 days. The number of colony-forming units (CFU) was then determined. On the agarized ISP1 and ISP3 media with yeast extract, phage plaques were detected in the zone of active growth of some bacterial colonies. The formation of phage plaques was observed visually or under a phase contrast microscope. Electron microscopic observations were carried out using a JEM-100CXII electron microscope (JEOL, Japan). The preparations were negatively stained with phosphotungstic acid for analysis [15]. Colonies of phage-sensitive bacteria were obtained and maintained on the agarized ISP1 and ISP3 media with yeast extract. Cell morphology was studied by phase contrast microscopy. To obtain virus material, the phage-sensitive cultures of the isolates were grown in liquid media at 28°C for 5–8 days on a shaker (180 rpm). The culture liquid was cleared by centrifugation at 7500 *g* (10 min) and filtered through a 0.2-μm membrane filter (Nuclepore Corp. Pleasanton Ca 94566 Polycarbonate). The presence of phage particles in the filtrate was determined by electron microscopy. To elucidate the lytic activity of the filtrate, the isolated strain of phage-sensitive bacteria, *Bacillus subtilis* ATCC 6633, as well as *B. mycoides, B. megatherium*, and *Streptomyces levoris* RIA 248 obtained from the strain collection of the Laboratory of Survival of Microorganisms, Winogradsky Institute of Microbiology, Russian Academy of Sciences, were used as the test cultures. These cultures were plated onto the agarized ISP1 and ISP3 media with yeast extract. Then, a drop of the filtrate (5 μL) was applied to the surface of the medium, and the cultures were incubated at 28°C for 1–3 days. Lytic activity was assessed by detecting the sterile zones on the surface of the media where the filtrate was applied.

In our experiments, we used the ISP1 and ISP3 media nutrient with yeast extract (0.2–0.3%). Under such high concentrations of yeast extract, the amount of released bacteriophages increases, which was demonstrated by Rautenshtein in [16]. In the studied water sample, the average number of bacteria grown on ISP1 and ISP3 media was 10^3 CFU/mL. Pale colonies consisting of motile rods prevailed, comprising up to 80–90% of the total number of identified prokaryotes. Spore-forming gram-positive rod-shaped bacteria identified as *Paenibacillus* sp. (data will be published later) grew as single colonies, their share not exceeding 1–2%. At the periphery, in the zone of active growth of one of these colonies, small negative colonies (0.15–2 mm) were detected after 3–5 day incubation. To elucidate where these colonies come from, samples taken from (1) the negative zones, (2) the filtrate obtained by filtering the culture liquid of the lysogenic isolate *Paenibacillus* sp., on the colonies of which these negative zones were detected, and (3) sterile zones on the surface of the media where the test cultures of *Bacillus subtilis* ATCC 6633 and *B. megatherium* grew and where the filtrate was applied, were studied under an electron microscope. As a result, phage particles of two morphologically distinct types, filamentous and spherical (figure), were detected in all three variants. It was demonstrated that the phage-containing filtrate exhibited low lytic activity both against the cells of the bacterial isolate in which they were detected and against the cells of the test cultures *Bacillus subtilis* ATCC 6633 and *B. megatherium*. A mixed infection of a bacterial cell with two unrelated phages, followed by reciprocal blocking of the phage production may be one of the causes of this low activity [16, 17]. This phenomenon widely occurs in nature. It is known from the literature that multiple phage infection of bacterioplankton is a common phenomenon in polar lake ecosystems [3]. While integrated into the bacterial genome, bacteriophages follow the lysogenic cycle and exist as prophages, thereby affecting the biology and ecological properties of bacteria. In this study, filamentous elongated viruses were detected among the phages infecting bacterial cells. It is well-known that filamentous phages are, for the most part, lysogenic or moderate phages [18]. Filamentous phage infection is a common phenomenon among gram-negative bacteria. So far, there are only two known occasions when gram-positive bacteria (propionic acid bacteria and clostridia) were infected by filamentous phages [2]. Recently, it was demonstrated that filamentous phages are capable of infecting cyanobacteria [19], which, as is well-known, play a key role in the ecology of aquatic ecosystems. In this work, filamentous phages were detected in the infected cells of an aerobic spore-forming bacterium belonging to the genus *Paenibacillus* and isolated from the water samples taken from an Antarctic lake. The presence of moderate phages indicates that lysogenic forms of bacteria are probably widespread among bacterioplankton species inhabiting Lake Untersee.

The fact that phage infection was detected in the bacterioplankton population of Lake Untersee confirms that phages interact with the bacteria inhabiting



Particles of the filamentous (a) and spherical (b) bacteriophages in the phage-sensitive culture of *Paenibacillus* sp. obtained from the water column of Lake Untersee. Scale bar, 80 nm.

Antarctic lakes, which indicates that bacteriophages may play an important role in the formation of microbiota, and affect many biogeochemical and ecological processes occurring in these ecosystems.

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