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EXPERIMENTAL ARTICLES

Response of Microbial Communities of Lake Baikal to Extreme Temperatures

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Abstract—The survival rate, metabolic activity, and ability for growth of microbial communities of Lake Baikal have been first studied after exposure to extremely low temperatures (freeze–thawing) for different lengths of time. It has been shown that short-term freezing (1–3 days) inhibits the growth and activity of microbial communities. The quantity of microorganisms increased after 7- and 15-day freezing. In the periods of maximums, the total number of microorganisms in the test samples was twice as high as in the control. It was established that after more prolonged freezing the microorganisms required more time after thawing to adapt to new conditions. In the variants with 7- and 15-day freezing, the activities of defrosted microbial communities were three or more times higher than in the control. The survival rate and activity of Baikal microorganisms after freeze–thawing confirms the fact that the Baikal microbial communities are highly resistant to this type of stress impact.

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Key words: microbial communities of Lake Baikal, total number of microorganisms, heterotrophic microorganisms, heterotrophic assimilation of carbon dioxide, freeze–thawing, extreme temperature, resistance.

Temperature is one of the most important factors that influences all life forms. Therefore, the study of the physiology of growth and development of microorganisms at extreme temperatures is significant for determination of the resistance of microbial populations to unfavorable environmental conditions. Aqueous environments have a tendency to develop obligate psychrophily as a result of the evolution of individual rare populations in microbiocenoses. Obligate psychrophiles develop more intensively than facultative ones at temperatures close to the minimum for their growth. This feature is extremely important for psychrophiles and may be a factor leading to their ecological dominance in loci with low temperatures [1, 2].

In Lake Baikal, low positive temperatures and annual freezing of surface water layers up to 1 m deep or more are the usual conditions of the existence of microbiocenoses. Research into microbial activity at stress temperatures is relevant both from the standpoint of studying the psychrophily of microorganisms and for evaluation of the role of Baikal microbial communities present under ice in the total cycle of matter in the Baikal ecosystem. The survival and activity of microbiocenoses at sustained negative temperatures has been studied insufficiently [3, 4]. There are no data on the response of Baikal microbial populations to stress impacts.

The goal of this work was to determine the character of the response and the measure of stability of microbial populations of the Baikal ecosystem under the action of stress temperatures which result in water freezing.

MATERIALS AND METHODS

Experiments on cultivation of heterotrophic microorganisms of Lake Baikal after exposure to natural and extremely low temperatures were performed during 2000–2004. The response of Baikal microorganisms to freeze–thawing and their further cultivation at different temperatures were studied in a batch mode. The objects of research were microbial communities from 200-m-deep pelagic waters of Lake Baikal. The water was poured into 10-l bottles (8 l per bottle). Control samples were left at 15°C without freezing and experimental samples were slowly frozen in the bottles to –15°C and stored for 1, 3, 7, and 15 days. After gentle defrosting (0 to + 4°C), water samples were exposed to 15°C for 35–60 days. Experiments were performed in two repeats; the results were averaged. Microorganisms were counted on Synpor 8 membrane ultrafilters, pore diameter 0.23 μm, using a MBB-1A microscope in 20 microscopic fields under 1350× magnification [5]. The survival rate and growth ability of microorganisms were determined as the number of colony-forming units

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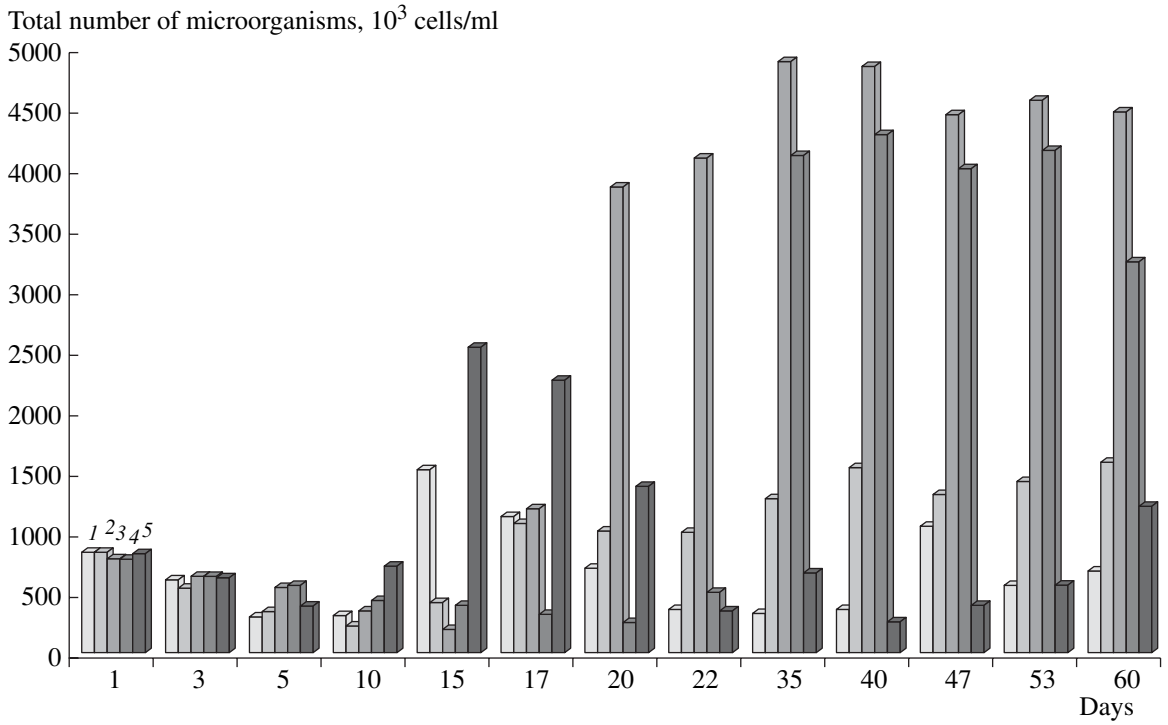

Fig. 1. The dynamics of total number of Baikal microorganisms after freezing for 1, 3, 7, and 15 days (*1–4*, respectively; *5*, control, no freezing).

(CFU) at inoculation of suspensions on Gorbenko medium (fish–peptone agar diluted tenfold, FPA : 10) in petri dishes by submerged inoculation. The microorganisms were identified to a genus according to Bergey's Manual of Systematic Bacteriology [6]. The functional activity of microbial populations was measured using the radiocarbon method [5] by the rate of carbon dioxide heterotrophic assimilation (HA). Production was counted with the HA coefficient equal to 0.06, a value which had been experimentally calculated for Lake Baikal [8].

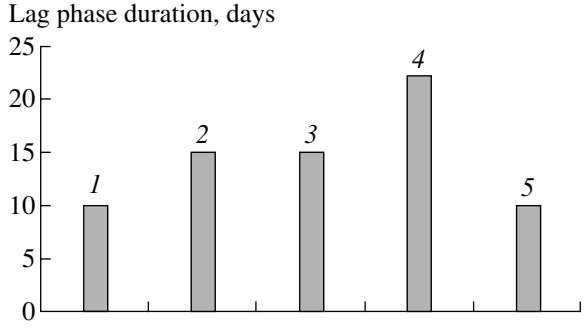

Fig. 2. The duration of the lag phase of developing microbial community of Lake Baikal, assessed by the total number of microorganisms, depending on the duration of sample freezing (designations as in Fig. 1).

RESULTS AND DISCUSSION

The responses of microbial communities to natural extreme factors (e.g., freeze–thawing) are diverse due to their different resistance, recovery of activity at a substantial decrease in quantity. The data on the dynamics of microbial development after freeze–thawing for different periods of time are given in Figures 1 and 3.

The analysis of the quantity of microorganisms after 24-h freezing showed that microbial populations in the experiment and control developed according to the classical growth curve (Fig. 1). The maximal values had been reached by day 15; however, the quantity of microorganisms in the control was 1.6-fold higher than in the experiment. Further development of microorganisms both in the experiment and in the control was of the same type and at the same quantitative level. Unlike one-day freezing, three-day freezing resulted in a shift of the quantity maximum from 15 to 40–60 days. In the control population, the maximum cell number was followed by a period of its drastic decrease, while the experimental population had a long stationary phase with cell number maintained at a level of 10^6 cells/ml (Fig. 1).

In the case of seven-day freezing of the experimental microbial population, the lag phase extended to 15 days. However, the population thawed after prolonged freezing gave a burst of growth up to 5×10^6 cells/ml, i.e., a 7.5 times the cell number in the control. Moreover, the experimental population reached

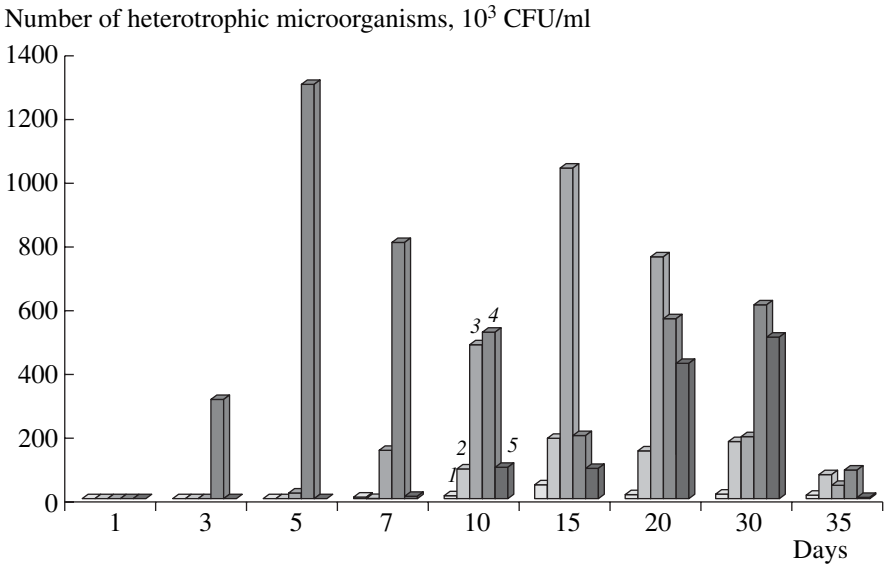

Fig. 3. The kinetics of development of heterotrophic microorganisms of Lake Baikal after freezing for 1, 3, 7, and 15 days (designations as in Fig. 1).

the maximal values of cell density on day 35 and maintained it at the same level until day 60 (Fig. 1).

A similar picture was observed in the experiment after 15-day freezing (Fig. 1). However, the lag phase reached 22 days; afterwards the microbial population increased in quantity to 4.5×10^6 cells/ml, which was sixfold higher than in the initial phase and in the control.

Thus, the lag phase of culture development became longer after more prolonged periods of freezing (Fig. 2). This fact suggests that the longer the freezing, the more time the microorganisms need to adapt to new temperature conditions after thawing. Finally, the quantity of microorganisms after long-term (more than seven days) freezing was sixfold higher than in the control.

The stress of freeze–thawing was expressed, first of all, in the lag phase increase. Vital activity inhibition is a response of any organism to any stress impact [2]. It is known that the recovery of quantity does not always correspond to the recovery of population activity after stress [9], although some published data demonstrate that negative temperatures are not a stress factor for oligotrophic bacteria [10, 11]. In the case of 7- and 15-day freezing, such stress impacts resulted in growth activation, evident as an increase of the number of microorganisms as compared with the control culture. The results are in agreement with the finding that cold shock changes bacterial physiology so that bacteria develop higher survival and growth rates as a result of the synthesis of cold shock proteins in response to cooling [2, 12].

The kinetics of microbial development subject to preservation of the colony-forming activity after one-day freezing is shown in Figure 3. The quantity of heterotrophic microorganisms in the lag phase was the same in the experiment and in the control: 20 CFU/ml on average. On day 15, the experimental population reached maximal values of 41 690 CFU/ml, which was 2.3 times lower than in the control at that moment. The pattern of development of heterotrophic microorganisms changed after three days of freezing (Fig. 3). The lag phase in the experimental population was as long as after one-day freezing (five days, Fig. 4). It was followed by active growth, and on day 10 the quantity of heterotrophic microorganisms was 89 000 CFU/ml. The die-off after the maximum in the control was more rapid than in the experiment. Preservation of the activity of heterotrophic microorganisms in the stationary phase is apparently due to accumulation of compounds necessary for maintenance of cell viability [13, 14].

Seven-day freezing stimulated the increase of CFU number in heterotrophic microorganisms: the lag phase reduced to three days, whereas in the control the period of adaptation was seven days. The experimental popu-

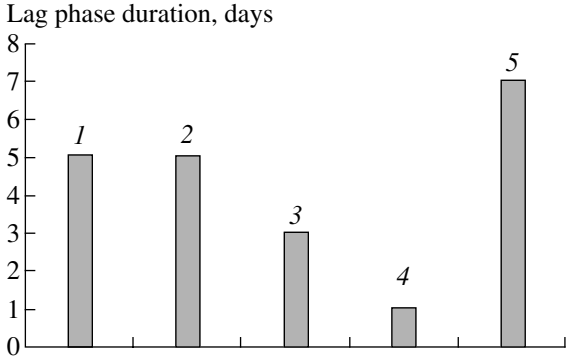

Fig. 4. The dependence of the lag phase of developing mixed cultures of heterotrophic microorganisms of Lake Baikal on the length of sample freezing (designations as in Fig. 1).

lation was characterized by rapid increase of CFU number in the logarithmic phase with the maximum of up to 1×10^6 CFU/ml (Fig. 3). The maximal CFU values in the experiment were double those in the control. During the subsequent period, the quantity decrease in experimental and control populations occurred at the same rate. On day 35, the CFU number in the experimental microbial population exceeded that in the control by two orders of magnitude (39000 and 2250 CFU/ml, respectively).

After 15-day freezing, the lag phase in the thawed microbial community was one day (Fig. 4). The number of heterotrophic microorganisms in this variant was 315 000 CFU/ml on day 3 and 1.3×10^6 CFU/ml on day 5 after thawing, which exceeded 4.6-fold the CFU maximum in the control (Fig. 3).

Our studies have shown that heterogeneous microbial cenoses of the Baikal pelagic waters contain bacterial populations in which the low-temperature freezing for 15 days induces the ability for rapid growth even during the first hours after thawing. Why was the same effect not observed after short 1- and 3-day freezing of the Baikal microbiocenoses? This fact has no satisfactory explanation as yet. Some data show that freeze–thawing damages the permeability barrier of both the outer and the cytoplasmic membrane of gram-negative bacteria, which results in the death of cells. Intracellular ice formation is considered as one of the most dangerous damaging factors arising upon the freezing of bacteria [15].

After different periods of freezing (7 and 15 days) in batch culture, the total number of microorganisms reached the maximum on day 35 and remained at this level for more than a month (Fig. 1). The evolutionary response of microorganisms to stress was manifested in their enhanced growth rate and stability.

The analysis of the morphological diversity of the microorganisms in batch culture at the beginning of the experiment revealed a great variety of pigmented colonies of microorganisms from the bacterial genera *Bacillus, Pseudomonas, Micrococcus*, and *Flavobacterium*, and yeasts *Torulopsis* and *Rhodotorula.* The pigmented forms were also predominant after 1- and 3-day freezing, whereas in the samples after 7- and 15-day freezing there were mainly gray–white colonies (for the most part, representatives of the genus *Pseudomonas*) and the dark–blue colonies formed by rods of the genus *Mycobacterium.*

Thus, mucoid white–gray *Pseudomonas* colonies were predominant in the samples after long freezing. The representatives of this genus possess more marked psychrophilic properties and competitiveness under changing temperatures, which is apparently associated with their capacity for slime formation [16, 17].

The functional activity of microbial populations after freeze–thawing was assessed by the values of heterotrophic carbon dioxide assimilation. The analysis of activity of microbial populations after freeze–thawing (at different periods of freezing) showed that microbiocenoses responded differently in each run. As is seen from Figure 5, the microbial population after one-day freezing

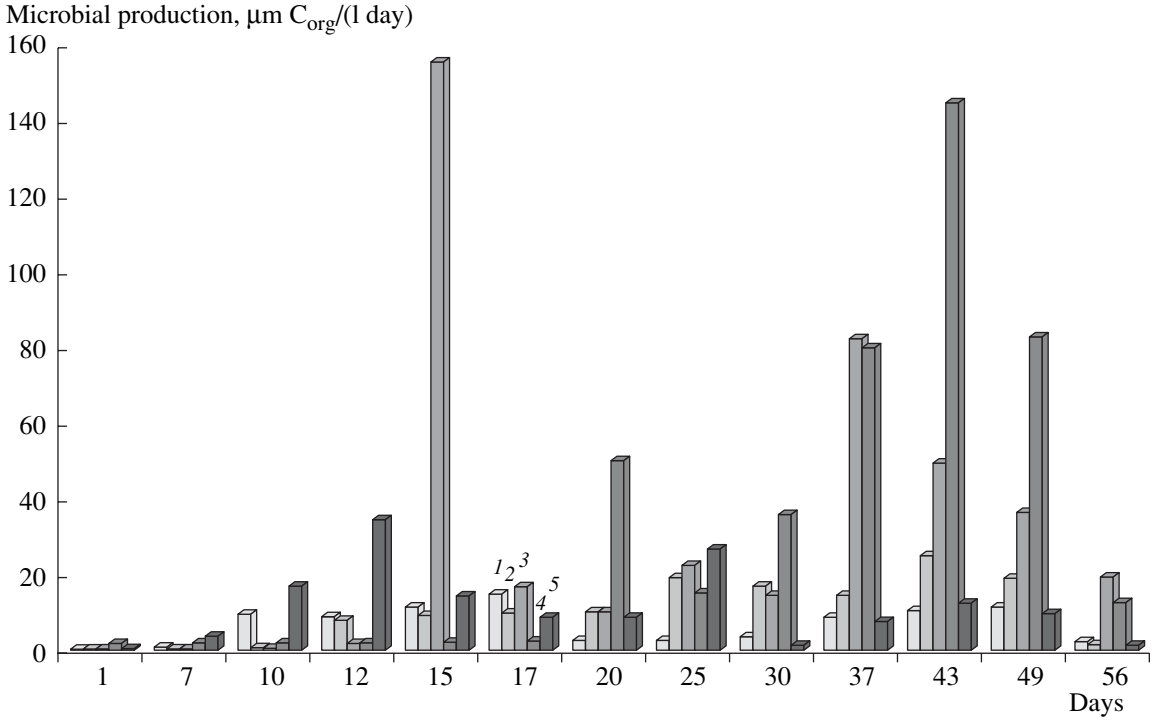

Fig. 5. The activity of Baikal microbiocenoses after freezing for 1, 3, 7, and 15 days (designations as in Fig. 1).

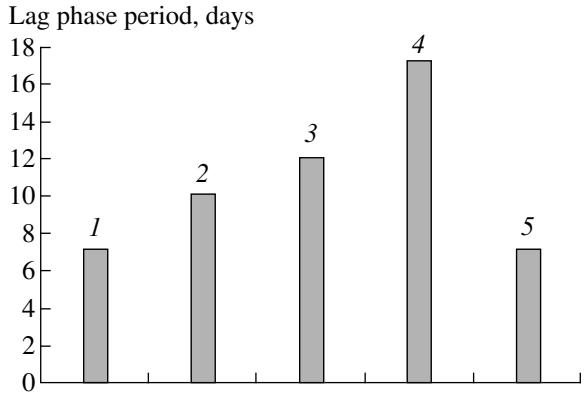

Fig. 6. The dependence of the lag phase of developing mixed microbial cultures from Lake Baikal after freezing on the length of sample freezing assessed by the value of carbon dioxide heterotrophic assimilation (designations as in Fig. 1).

had lower activity as compared with the control population, which had three peaks of activity in the course of development, indicating the succession of microbiocenoses in the Baikal pelagic waters. The experiments showed that developing microorganisms change from coccoid forms in the beginning of experiment over to the prevalence of rods on day 25 (the second peak) and their dominance on day 47 (the third peak of development). The maximal values of production in the control population were 2.5-fold higher than in the experiment. Freezing of the water with bacteria for three days (Fig. 5) increased the total production of microbiocenoses about twofold (as compared with one-day freezing).

The pattern of microbial activity drastically changed after 7- and 15-day freezing (Fig. 5). There were two distinct phases of the metabolic activity of microorganisms. The lag phase extended to 12 and 17 days (Fig. 6). Production in this period was 0.95 μg C_{org}/l day, which was 33 times less than in the control. A rapid burst of activity of experimental microorganisms after seven-day freezing in the maximal activity phase exceeded the activity of the control population by more than ten times. There were sharp amplitudes of oscillation in the experimental microbiocenoses with the maximums corresponding to 155 μg C_{org} /l day and 81.14 μg C_{org}/l day. The microbial population exhibited the same activity after 15-day freezing. After a long 17-day initial phase, there was a period of active assimilation of CO_2 and then, after a minor decline, an abrupt increase of activity to 143 μg C_{org}/l day, which exceeded the control values by 12 times. The total production of microbiocenoses frozen for 7 and 15 days during 56 days of observations was 2–3 times higher than in the control. Consequently, long freezing results in mobilization of the internal resources of water ecosystems, which remain untapped under normal conditions.

The findings give estimation of the stability of microbial communities of the Baikal water column, the flexibility and plasticity of their adaptation processes, and maintenance of their functional activity.

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